

Inter- Generic Relationship of *Ocimum* and *Origanum* Based on GC-MS Volatile Oils Data using Software NTSPSpc Version 2.0

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Abstract

To study of inter- generic relationships of *Origanum vulgare* L, *Origanum applii* (Domin) Borus, *Ocimum gratissimum* L. and *Ocimum basilicum* L essential oils peaks based on GC-MS methods. The result of the essential oils of *Origanum vulgare* L, *Origanum applii* (Domin) Borus, *Ocimum gratissimum* L. and *Ocimum basilicum* were constructed the phenogram formed in one cluster and two sub cluster. The inter- generic relationship of 26% formed in main clusters both genus of *Origanum* sp and *Ocimum* sp. The sub cluster I, *Ocimum gratissimum* L. and *O. basilicum* was very close relationship of 40% and 34% of sub cluster II *Origanum vulgare* L, and *O. applii* respectively.

Introduction

The storage of essential oils in higher plants is not restricted to specialized plant parts. Essential oils occur in both roots, stems, leaves, flowers and seeds, or in the plant as a whole. Both epidermal or mesophyll tissue can function as the site of terpene biosynthesis in general, whereas typical storage cells or cell structures characterize the taxonomic group of aromatic plants: Oil cells, secretory glands, ducts and canals and the *Labiatae*-typical glandular trichomes (*capitate* and *peltate glands*). Depending on morphological structures, varying secondary metabolism and thus, determined signalling and defence functions of essential oils in plant organs, the pronounced alteration gives the ability to obtain essential oil qualities that are quite different from one and the same plant.

Intraspecific and intervarietal differences can be observed in both morphology and chemical structures, which establish the basis for determining important chemically defined populations or *chemotypes* (Hay and Svoboda,1993). Despite aromatherapeutic demands, which require that the medicinal value of an essential oil be based on its complete composition rather than its constituent parts (Franchomme et al., 1990), one still has to consider the chemical specification of Essential oils with regard to toxic concentrations of single constituents (Tisserand and Balacs, 1995; Price and Price, 1999).

Knowledge about genetic diversity and population genetic structure is a good baseline for formulating effective conservation plans, and can often provide novel, conservation-relevant insights. An effective conservation strategy for a species can be made only after detailed population genetic information becomes available. Estimates of genetic similarity using genetic fingerprinting data are a useful tool in plant breeding, allowing breeders to make informed decisions regarding the selection of germplasm to be used in crossing schemes. Fingerprints themselves are also

useful to breeders for every day for protection of their own varieties and to seed producers, growers and end users for checking the identity and purity of their produce (Milbourne *et al.*, 1997).

Materials and Methods

Genetic materials

Origanum vulgare L. (UEC 121.409), *O. applii* (Domin) Borus (UEC 121.410), *Ocimum gratissimum* L. (UEC 121.407) and *O. basilicum* L. (UEC 121.408) were chosen to the present study. The aromatic plants were collected from CPQBA/UNICAMP experimental field, between 9:00 and 10:00 am, in the first week on March, in full flowering, except to *O. vulgare* L. in vegetative stage (Sartoratto *et al.*, 2004).

Essential oil extraction

The oil extraction was obtained from 40g fresh plants by steam distillation using Clevenger system, during 3h. The aqueous phase was extracted with dichloromethane (3x50mL). The organic phase was dried with sodium sulphate, filtered and the solvent evaporated until dryness. The oil was solubilized in ethyl acetate for gas chromatography and mass spectrometry analysis (Sartoratto *et al.*, 2004).

Chromatography conditions

Sartoratto *et al.*, (2004) work done on the identification of essential oil constituents was conducted by gas-chromatography in Hewlett-Packard 5890 Series II (Palo Alto, CA, USA) equipment, with selective mass detector HP-5971 in the electron impact (EI) ionization mode (70 eV), injector *split/splitless*, capillary column HP-5 (25 m x 0.2 mm x 0.33 µm). Temperature: injector = 220°C, column = 60°C, 3°C.min⁻¹, 240°C (7 min). Carrier gas (He) = 1.0 mL.min⁻¹. Retention indices (RI) have been obtained according to the method (Van den Dool, and Kratz, 1963).

Data analysis

The chromatogram peaks were converted into a “1” and “0” matrix, to indicate the presence or absence of a peak, respectively. Genetic similarities (GS) were estimated for all comparisons of each samples according to Nei (1972) as $GS = 2n_{xy} / (n_x + n_y)$ in which n_x and n_y are the total numbers of peaks in the chromatograms of the samples x and y , respectively, and n_{xy} is the number of peaks shared by the two samples. To examine the inter- genetic relationships between four species populations, a dendrogram was constructed by an unweighted paired group method of cluster analysis using arithmetic averages (UPGMA) option of the NTSYSpc-2.0 software.

Results and Discussion

Table-1 identified with 60 peaks, with the assumption that peaks with the same retention time on different chromatograms was the same compounds. The data on these 60 peaks for four plant samples such as *Origanum vulgare* L., *O. applii* (Domin) Borus, *Ocimum gratissimum* L. and *O. basilicum* L. (Sartoratto *et al.*, (2004). The phenogram constructed based on common peaks of essential oils identification from the *Ocimum gratissimum*, *O. basilicum*, *Origanum vulgare* L., and *O. applii* shown in fig-1. The inter generic relationship of 26% formed in main clusters both genus of *Origanum* sp and *Ocimum* sp. The graphic phenogram (Fig. 1) distantly placed *Origanum vulgare* L., and *O. applii* was 34% similarity. And *Ocimum gratissimum* L. and *O. basilicum* was 40% similarity. According to literature reported the distantly placed *C. martinii* (var. *motia* and var. *so.a*) from the rest of the taxa sharing 54% similarity. *C. pendulus* was distantly placed from rest of the accessions but was closer to the subclusters containing *C. nardus* var. *nardus*, *C. nardus* var. *Java II* and *C. winterianus* (49, 44, 52% similarity, respectively) on one hand and to the subcluster containing *C. exuosus* and *C. citratus* (52, 43% similarity, respectively (Khanuja *et al.*, 2005). In conclusion of the present study, essential oils constituents can be used as an additional tool to assist in

identification of similar and closely related species of *Ocimum* sp and *Origanum* sp.

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Table 1. Inter- generic chemotypical variation of *Ocimum* and *Origanum* species (Sartoratto et al., 2004).

Composition	RRI	<i>Ocimum gratissimum</i>	<i>O. basilicum</i>	<i>Origanum.</i> <i>vulgare</i>	<i>O. applii</i>
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1-octen-3-ol	978	-	-	0.68	-
3-octanol	998	-	-	-	-
<i>p</i> -cimene	1024	-	-	1.50	-
limonene	1028	-	-	-	-
1,8-cineol	1031	-	1.05	-	-
<i>trans</i> - β -ocimene	1035	-	-	0.39	-
γ -terpinene	1057	-	-	1.99	-
isopentyl n-butyrate	1068	-	-	-	-
α -terpinolene	1083	-	-	0.31	-
fenchone	1088	-	0.53	-	-
linalool	1093	-	32.06	-	4.92
<i>cis-p</i> -menth-2-en-1-ol	1122	-	-	1.47	-
<i>trans-p</i> -menth-2-en-1-ol	1140	-	-	0.86	-
camphor	1150	-	10.1	-	-
borneol	1169	-	-	2.52	-
Terpin-4-ol	1176	0.26	0.99	33.3	3.08
cimen-8-ol	1182	-	-	-	0.64
α -terpineol	1188	-	3.90	4.25	-
<i>trans</i> -piperitol	1208	-	-	0.16	-
thymol methyl ether	1231	-	-	0.24	1.47
neral	1238	-	-	-	-
carvacrol methyl ether	1241	-	-	1.33	5.91
carvone	1241	-	-	-	-
geranial	1270	-	-	-	-
thymol	1294	-	-	38.0	64.5
carvacrol	1298	-	-	-	-
eugenol	1358	93.9	28.1	-	-
piperitenone oxide	1373	-	-	-	-
Geranyl acetate	1379	-	-	0.33	-
β -bourbonene	1385	-	0.23	-	1.35
β -elemene	1393	-	1.92	-	-
Cedrene <1,7-di-epi- α >	1397	-	-	-	-
<i>trans</i> -caryophyllene	1422	1.08	2.00	2.66	-
β -gurjunene	1430	-	-	-	0.36
β -bergamotene	1435	-	1.66	-	-
α -guaiene	1438	-	0.29	-	-
β -farnesene	1454	-	-	-	-
α -humulene	1455	-	0.77	-	-
<i>allo</i> -aromadendrene	1461	-	-	-	0.28
Germacrene D	1482	4.23	5.49	1.47	4.79
Curcumene <AR>	1483	-	-	-	-
γ -muurolene	1484	-	-	-	-
zingiberene	1495	-	-	-	-
δ -guaiene	1506	-	0.68	-	-
β -bisabolene	1508	-	-	1.05	1.98
γ -cadinene	1516	-	1.27	-	-
δ -cadinene	1524	-	0.29	-	-
espatulenol	1580	-	-	1.44	1.62
caryophyllene oxide	1586	-	-	1.07	-
<i>epi</i> - α -muurolol	1643	-	5.81	-	0.72
α -eudesmol	1655	-	0.24	-	-
α -cadinol	1656	0.16	0.38	0.29	1.53

Table 2. Volatile data converted on inter-generic chemotypical variation of *Ocimum* and *Origanum* species.

Composition	RRI	<i>Ocimum gratissimum</i>	<i>O. basilicum</i>	<i>Origanum vulgare</i>	<i>O. applii</i>
1-octen-3-ol	978	0	0	1	0
3-octanol	998	0	0	0	0
<i>p</i> -cimene	1024	0	0	1	0
limonene	1028	0	0	0	0
1,8-cineol	1031	0	1	0	0
<i>trans</i> - β -ocimene	1035	0	0	1	0
γ -terpinene	1057	0	0	1	0
isopentyl n-butyrate	1068	0	0	0	0
α -terpinolene	1083	0	0	1	0
fenchone	1088	0	1	0	0
linalool	1093	0	1	0	1
<i>cis-p</i> -menth-2-en-1-ol	1122	0	0	1	0
<i>trans-p</i> -menth-2-en-1-ol	1140	0	0	1	0
camphor	1150	0	1	0	0
borneol	1169	0	0	1	0
Terpin-4-ol	1176	1	1	1	1
cimen-8-ol	1182	0	0	0	1
α -terpineol	1188	0	1	1	0
<i>trans</i> -piperitol	1208	0	0	1	0
thymol methyl ether	1231	0	0	1	1
neral	1238	0	0	0	0
carvacrol methyl ether	1241	0	0	1	1
carvone	1241	0	0	0	0
geranial	1270	0	0	0	0
thymol	1294	0	0	1	1
carvacrol	1298	0	0	0	0
eugenol	1358	1	1	0	0
piperitenone oxide	1373	0	0	0	0
Geranyl acetate	1379	0	0	1	0
β -bourbonene	1385	0	1	0	1
β -elemene	1393	0	1	0	0
Cedrene <1,7-di-epi- α >	1397	0	0	0	0
<i>trans</i> -caryophyllene	1422	1	1	1	0
β -gurjunene	1430	0	0	0	1
β -bergamotene	1435	0	1	0	0
α -guaiene	1438	0	1	0	0
β -farnesene	1454	0	0	0	0
α -humulene	1455	0	1	0	-
<i>allo</i> -aromadendrene	1461	0	0	0	1
Germacrene D	1482	1	1	1	1
Curcumene <AR>	1483	0	0	0	0
γ -muurolene	1484	0	0	0	0
zingiberene	1495	0	0	0	0
δ -guaiene	1506	0	1	0	0
β -bisabolene	1508	0	0	1	1
γ -cadinene	1516	0	1	0	0
δ -cadinene	1524	0	1	0	0

espatulenol	1580	0	0	1	1
caryophyllene oxide	1586	0	0	1	0
<i>epi</i> - α -muurolol	1643	0	1	0	1
α -eudesmol	1655	0	1	0	0
α -cadinol	1656	1	1	1	1

Table 3. The average of taxonomical similarities of *Ocimum* sp. and *Origanum* sp.

Population	<i>Ocimum</i>	<i>Origanum</i>		
	<i>gratissimum</i>	<i>O. basilicum</i>	<i>vulgare</i>	<i>O. applii</i>
<i>Ocimum gratissimum</i>	0.0000			
<i>O. basilicum</i>	0.4	0.0000		
<i>Origanum vulgare</i>	0.31	0.2	0.0000	
<i>O. applii</i>	0.32	0.24	0.34	0.0000

Fig.1. Phenogram based on 60 essential oils peaks on *Ocimum* sp and *Origanum* sp.



