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Chemical variability of the leaf oil of 113 hybrids from *Citrus clementina* (Commun) × *Citrus deliciosa* (Willow Leaf)

Félix Tomi,[1]* Marina Barzalona,[1] Joseph Casanova[1] and François Luro[2]

[1] Université de Corse–CNRS, UMR 6134 SPE, Equipe Chimie et Biomasse, Route des Sanguinaires, 20000 Ajaccio, France
[2] Institut National de la Recherche Agronomique, Centre de Corse, Unité de Recherche GEQA, 20230 San Ghjulianu, France

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ABSTRACT: Essential oils of 113 *Citrus clementina* (Commun) × *Citrus deliciosa* (Willow Leaf) hybrids were obtained from leaves collected on trees located in the same orchard. Their chemical composition was investigated by capillary GC, GC–MS and ^{13}C-NMR and the results were submitted to statistical analysis. Three chemical compositions were characterized. The crossing between clementine and mandarin produces mainly leaf oils characterized by a composition similar to those of the parents (sabinene/linalool or methyl N-methylanthranylate). However, several samples exhibited an original composition (γ-terpinene/linalool). Copyright © 2008 John Wiley & Sons, Ltd.

KEY WORDS: *Citrus*; hybrid; leaf oil; essential oil composition; GC; GC–MS; ^{13}C-NMR

Introduction

For centuries citrus breeding methodologies were based on the singling-out of natural wild varieties and selection of spontaneous mutations occurring in cultivated citrus. As an example, clementine trees present a panel of different dates for fruit maturity, covering 6 months from September to March in the Mediterranean basin, based only on selected variants originated from mutations. Other species, such as mandarins, pommelos and sweet oranges, were obtained according to the same model of genetic variation. Controlled hybridizations have been carried out since the end of the nineteenth century to improve agronomical performances of rootstocks and cultivars. The first reported work concerned the introduction of the cold resistance character of trifoliate orange (*Poncirus trifoliata*) into sweet orange (*Citrus sinensis*), with the objective of producing new cold-resistant rootstocks.[1] The hybridization to create new genotypes in citrus was limited by the long non-productive juvenile phase, which prevents the selection of seedlings before they are 5–8 years old. Furthermore, the general high heterozygosity of many citrus cultivars causes a high variability in progeny. In addition, nucellar polyembryony, highly frequent in citrus, represents a strong impediment to the production of sexual progeny by producing a competition for germination between sexual and nucellar embryos. In spite of those limitations, breeders have released hybrids producing fruits with acceptable quality, usually combining mandarins (*Citrus reticulata*) with their hybrids, clementines, tangors, tangelos and oranges.[2] Nevertheless, only a few of them are successfully cultivated and diffused, due to unfavourable characters or to their poor adaptation for different climate and soil conditions. To solve those problems, breeders look for predictive tools which could inform, at an early stage of plant development, on the future fruit characters or the tree comportment for biotic and non-biotic stresses.

Chemical compositions of peel and leaf essential oils have previously been studied for their application in differentiating taxa at intra- or interspecific levels[3–5] and to evaluate new citrus hybrids by comparison with their parents. In the literature the chemical composition of a hybrid is usually closer to one of the hybrid progeny.[5,6]

This study is part of a global work carried out for the genetic improvement of the clementine (*C. clementina*) by creating hybrids with Willow Leaf mandarin (*C. deliciosa*), New hybrids combining qualitative specific characters of mandarin (fragrance) and clementine (sugar, acidity) are expected. Although there are no previous studies allowing use of leaf chemical compositions to predict the future fruit characters, we report the first characterization of leaf oil composition of >100 hybrids. The oil compositions of hybrids are partitioned into groups using principal components analysis (PCA) and compared to those of both parents.

Materials and Methods

Plant Material

Hybrids were obtained by seeding seeds extracted from clementine fruits originated from hand pollinations by

*Correspondence to: F. Tomi, Université de Corse–CNRS, UMR 6134 SPE, Equipe Chimie et Biomasse, Route des Sanguinaires, 20000 Ajaccio, France.
E-mail: felix.tomi@univ-corse.fr
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Willow Leaf mandarin pollen. After germination, young plants were grafted onto a Carrizo citrange (*Poncirus trifoliata* × *Citrus sinensis*) rootstock. At 18 months old, 113 grafted hybrids and their parents were planted in the same orchard and submitted to homogenous growth cultural conditions. The experimental field is located at the Station de Recherches Agronomiques of INRA-CIRAD, San Ghjulianu, Corsica, France (latitude 42°17′N, longitude 9°32′E); Mediterranean climate; average rainfall and temperature, 840 mm and 15.2 °C per annum, respectively; soil derived from alluvial deposits and classified as fersiallitic, pH range 6.0–6.6. The sanitation and physiological development of these trees have been controlled every year. At 6 years old, these trees have not yet produced any fruits but were sufficiently developed to permit us a large leaf sampling.

Sampling and Extraction of Essential Oils

For each hybrid and both parents, about 300 g leaves from the last autumn leaf flush were picked all around the same tree, early in the morning and in dry weather, from March to April 2004. The parents clementine (*C. clementina*) and mandarin (*C. deliciosa*) were sampled in triplicate.

Fresh leaves were submitted to water distillation for 3 h, using a Clevenger-type apparatus. To avoid any damage, the storage of leaves before distillation was limited to 1 day. Only one sample did not produced essential oil in our experimental conditions.

Analytical GC

GC analysis was carried out using a Perkin-Elmer Autosystem apparatus equipped with FID and fused-silica capillary columns (50 m × 0.22 mm i.d., film thickness 0.25 μm), BP-1 (dimethyl siloxane) and BP-20 (polyethylene glycol). Oven temperature was programmed from 60 °C to 220 °C at 2 °C/min, then held isothermal at 220 °C for 20 min; injector temperature, 250 °C; detector temperature, 250 °C; carrier gas, helium at a flow rate of 0.8 ml/min; split, 1:60. The relative proportions of the essential oil constituents were expressed as percentages obtained by peak area normalization, without using correcting factors.

GC–MS Analysis

Samples were analysed on a Perkin-Elmer quadrupole MS system (Model 910) coupled with the above gas chromatograph, equipped with a BP-1 capillary column and operating under the same conditions described above, except for the carrier gas flow rate (1 ml/min.). The MS operating parameters were: ionization voltage, 70 eV; ion source temperature, 230 °C; scan mass range, 35–450 Da.

^{13}C-NMR Analysis

All NMR spectra were recorded on a Bruker AC 200 Fourier Transform spectrometer operating at 50.323 MHz for ^{13}C-NMR, equipped with a 5 mm probe, in deuterated chloroform, with all shifts referred to internal tetramethylsilane (TMS). ^{13}C-NMR spectra were recorded with the following parameters: pulse width, 3 μs (flip angle 45°); acquisition time, 1.3 s for 32 K data table with a spectral width of 12 500 Hz (250 p.p.m.); CPD mode decoupling; digital resolution, 0.763 Hz/pt. The number of accumulated scans was 3000 for each sample (70 mg of the oil in 0.5 ml $CDCl_3$). Exponential line broadening multiplication (1 Hz) of the free induction decay was applied before Fourier transformation.

Identification of Components

Identification of the individual components was based on: (a) comparison of their GC retention indices (RI) on apolar and polar columns, determined relative to the retention time of a series of *n*-alkanes with linear interpolation (Target Compounds software of Perkin-Elmer) with those of authentic compounds and literature data (see Table 1); (b) computer matching with mass spectral libraries[7–9] and comparison with spectra of authentic samples or literature data;[10,11] (c) comparison of the chemical shifts of carbons in the ^{13}C-NMR spectrum of the mixture with those of reference spectra compiled in our spectral library, with the help of laboratory-produced software.[12] This technique allows the identification of individual components to a content of 0.5% without any previous purification.

All the leaf oils were investigated by GC, in combination with retention indices (RI), on apolar and polar columns. Twenty-four leaf oils (Nos 2, 3, 9, 11, 12, 19, 21, 24, 26, 31, 33, 45, 51–54, 76, 81, 88, 90, 97, 104, 113, 114) were analysed by ^{13}C-NMR, while six leaf oils (Nos 11, 12, 31, 46, 54, 104) were analysed by GC–MS.

Chemometric Analysis

Principal component analysis (PCA) was performed using XLSTAT (Addinsoft, France). Clustering was also achieved using the K-means partitioning program (Pierre Legendre, Canada).[13]

Results and Discussion

Essential oils isolated by hydrodistillation of leaves of 113 hybrids of *Citrus clementina* (Commun) × *Citrus deliciosa* (Willow Leaf) were analysed by GC in combination with retention indices. Several oil samples, selected on the basis of their chromatographic profiles, were also analysed by GC–MS and/or ^{13}C-NMR, which ensured the identification of individual components.

Table 1a. Chemical composition of citrus hybrid leaf oils (group I, subgroup IA)

Constituents	RI P	RI A	RI[#]	1 H5	2 N3	3 J2	4 B1	5 B10	6 H9	7 F2	8 L4	9 B5	10 E1	11 N4	12 L5	13 E7	14 N6
α-Thujene*	1024	923	925[a]	0.4	0.1		0.8	0.4	1.2	0.7	0.4	0.3	0.4	0.6	0.7	0.6	0.3
α-Pinene*	1024	931	932[a]	1.5	0.3	0.2	2.2	1.7	3.1	2.0	1.5	1.3	1.5	1.9	2.1	2.0	1.2
Camphene	1068	944	948[a]	tr			0.1	tr	0.1	0.1	tr	tr	tr	tr	tr	tr	tr
β-Pinene	1111	971	981[b]	2.1	0.1	tr	3.0	2.3	3.6	2.8	2.0	1.9	1.9	2.4	2.5	2.6	1.5
Sabinene	1122	965	976[b]	45.4	0.1	0.1	43.6	43.8	43.3	43.2	41.3	39.2	38.4	38.3	35.9	33.6	33.2
Δ-3-Carene	1148	1006	1009[d]	3.4	10.7	11.1	tr	tr	tr	tr	tr	tr	4.7	4.3	5.1	tr	5.7
Myrcene	1160	980	983[a]	3.0	2.3	2.3	2.8	2.5	3.4	2.5	2.5	2.6	3.1	3.6	3.2	2.2	2.8
α-Phellandrene	1165	997	1000[a]	0.4	0.5	0.6	0.1	0.1	0.1	0.1	0.1		0.5	0.4	0.5	0.1	0.5
α-Terpinene	1180	1009	1015[a]	0.9	0.2	0.3	0.9	1.3	1.2	1.0	1.1	1.2	1.2	1.3	1.2	1.5	0.7
Limonene	1202	1022	1022[a]	2.9	18.1	16.4	3.2	3.2	4.9	2.7	1.7	3.3	7.4	12.0	4.8	4.7	10.0
β-Phellandrene	1210	1021	1025[b]	0.8	0.4	0.5	0.6	0.6	0.6	0.6	0.6	0.5	0.8	0.7	0.7	0.6	0.7
(*Z*)-*β*-Ocimene	1232	1025	1026[b]	0.2	1.0	1.0	0.1	0.2	0.2	0.1	0.2	0.1	0.1	0.1	0.2	0.1	0.1
γ-Terpinene	1245	1049	1053[a]	1.4	1.9	0.1	13.5	3.6	12.7	12.2	1.7	2.0	1.8	8.0	6.8	9.9	1.0
(*E*)-*β*-Ocimene	1249	1036	1038[b]	5.2	20.6	19.3	3.7	4.2	3.0	1.7	5.7	3.4	2.3	1.8	3.9	1.7	3.4
p-Cymene	1270	1012	1016[b]	0.1	0.4	0.2	0.5	0.4	1.3	0.3	tr	tr	0.1	0.5	0.7	0.8	0.1
Terpinolene	1282	1078	1074[d]	0.8	1.7	1.9	0.7	0.5	1.0	0.8	0.4	0.5	1.3	1.5	1.6	0.8	1.0
Octanal	1289	979	982[c]														
6-Methylhept-5-en-2-one	1336	960	968[b]		tr	0.1									0.2		0.2
Nonanal	1388	1082	1082[c]														
p-Cymenene	1435	1072	1076[g]		0.1	0.2			0.2					0.2	0.1		tr
trans-Sabinene hydrate	1459	1053	1060[b]	0.8	tr	tr	0.9	0.6	0.5	0.8	0.7	1.0	0.7	0.4	0.7	0.3	1.0
Citronellal	1476	1130	1137[d]	1.2	1.5	3.8	tr			0.1	tr		1.6	0.1	5.4		2.0
Decanal	1495	1183	1191[c]	tr								tr		tr			tr
Linalool	1544	1084	1085[c]	21.3	24.8	21.4	16.6	25.4	10.7	22.1	32.6	31.7	14.4	9.5	6.8	28.6	22.4
Linalyl acetate	1549	1239	1246[d]			0.2											
cis-p-Menth-2-en-1-ol	1558	1107	1111[d]	0.2			0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.2	0.3	0.2
β-Elemene	1586	1388	1389[e]														
Thymyl methyl oxide	1589	1214	1215[h]						0.1					0.5			
(*E*)-*β*-Caryophyllene	1592	1418	1421[e]	0.1	0.6	0.3	0.1	0.2	0.1	0.1	0.2	0.1	tr	0.8	0.2	0.2	0.1
Terpinen-4-ol	1598	1162	1170[d]	3.0	tr	0.1	2.7	4.6	3.9	3.1	3.9	3.7	3.9	3.8	3.5	5.6	2.3
trans-p-Menth-2-en-1-ol	1623	1123	1128[d]	0.1	tr	tr	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1
Citronellyl acetate*	1654	1332	1335[d]			0.3								0.4	tr		tr
(*E*)-*β*-Farnesene*	1654	1448	1446[e]												tr		tr
Neral	1678	1214	1218[d]	tr	0.2	0.6							1.0		0.9		1.3
α-Terpinyl acetate	1681	1332	1333[d]											tr			
α-Humulene	1660	1453	1455[e]	tr		tr								0.1		0.2	
α-Terpineol	1690	1172	1180[d]	0.3	0.2	0.1	0.4	1.0	0.2	0.5		0.4	0.9	0.3	0.6	1.5	0.8
Germacrene D	1704	1480	1475[e]														
Neryl acetate	1722	1340	1345[d]		0.6	0.7						tr	0.8	0.1	0.1		0.2
α-Bisabolene	1724	1496	1494[e]	tr													
Bicyclogermacrene	1727	1492	1495[e]	tr	0.3				0.1	0.1	0.2			0.1			
Geranial	1732	1242	1242[d]		0.3										1.2		1.7
(*E,E*)-*α*-Farnesene	1744	1500	1498[e]											0.1	tr		
δ-Cadinene	1746	1516	1520[e]														
Geranyl acetate	1754	1358	1363[d]	0.1	0.1	0.8							3.0	0.1	0.8		0.9
Citronellol	1761	1207	1215[d]	0.5	1.4	2.8	tr			tr		tr	0.5	0.1	2.2		0.3
Nerol	1797	1207	1218[d]	0.1	0.8	2.7							1.6	tr	0.9		0.7
trans-Carveol	1834	1199	1209[d]														
Geraniol	1844	1232	1243[d]	tr	0.1	0.2							0.5	tr	0.2		0.3
Caryophyllene oxide	1980	1576	1576[d]														
(*E*)-Nerolidol	2038	1547	1553[d]		0.2	0.1		tr	0.1		tr			0.2	tr	0.1	
Methyl N-methylanthranylate	2083	1382	1389[b]	tr	3.0	0.1	0.1	1.0	tr	tr	0.1	tr	0.1	0.1	0.4	0.1	tr
Spathulenol*	2115	1557	1565[f]														
Ethyl N-methylanthranylate*	2115	1450	—					0.4									
Thymol	2182	1266	1270[d]						2.4					0.5	2.4		
α-Cadinol	2228	1641	1644[f]														
β-Sinensal	2229	1673	1671[c]	1.8	0.1	4.1	tr	0.5	tr		0.1	tr	1.9	0.4	1.3	0.9	1.2
α-Sinensal	2330	1725	1730[c]	0.9	4.5	2.7	2.2	0.7	1.0	1.2	1.0	2.6	0.4	2.9	0.2	0.4	0.2
Total				98.0	92.7	92.6	96.9	98.8	98.3	97.9	97.3	93.6	96.7	95.5	98.1	99.2	97.9

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Table 1a. (*Continued*)

Constituents	RI P	RI A	RI[#]	15 C2	16 M7	17 K8	18 B9	19 H1	20 D6	21 M6	22 F9	23 L7	24 E9	25 J7	26 C3	27 I5	28 G8
α-Thujene*	1024	923	925[a]	0.8	0.6	0.5	0.7	0.2	0.5	0.5	0.8	0.4	0.7	tr	tr	tr	0.2
α-Pinene*	1024	931	932[a]	2.3	1.7	1.4	1.8	0.9	1.3	1.7	2.3	1.6	2.0	0.1	0.1	0.1	0.7
Camphene	1068	944	948[a]	tr	tr	tr	tr	tr	tr	0.1	0.1	tr	0.1				tr
β-Pinene	1111	971	981[b]	2.6	2.5	2.0	2.2	1.3	1.7	2.7	3.2	2.2	2.9	0.1	tr		1.2
Sabinene	1122	965	976[b]	32.6	32.2	31.7	29.3	27.0	25.8	57.2	51.5	47.5	47.3	0.3	0.1	0.1	25.3
Δ-3-Carene	1148	1006	1009[d]	tr	tr	3.4	5.3	5.8	4.2	tr	tr	tr	tr		5.8	6.3	4.7
Myrcene	1160	980	983[a]	2.4	2.4	2.5	2.7	2.6	2.3	3.3	3.1	2.7	2.7	0.2	1.1	1.3	2.3
α-Phellandrene	1165	997	1000[a]	0.1	0.1	0.4	0.5	0.5	0.4	0.1	0.1	0.1	0.1		0.4	0.5	0.4
α-Terpinene	1180	1009	1015[a]	1.1	1.2	0.8	1.1	0.8	0.8	1.4	1.3	0.9	1.2		0.1	0.1	0.8
Limonene	1202	1022	1022[a]	6.9	2.7	5.9	4.5	7.7	5.9	2.2	3.8	4.0	2.4	4.8	7.4	19.9	2.2
β-Phellandrene	1210	1021	1025[b]	0.5	0.5	0.6	0.7	0.6	0.6	0.8	0.6	0.6	0.5	tr	0.3	0.4	0.7
(*Z*)-β-Ocimene	1232	1025	1026[b]	0.2	0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.1	0.1	0.3	0.3	0.2	0.2
γ-Terpinene	1245	1049	1053[a]	12.4	14.2	6.5	9.9	1.2	6.6	2.3	7.6	1.5	6.7	1.1	0.3	0.1	1.4
(*E*)-β-Ocimene	1249	1036	1038[b]	1.9	1.8	2.8	3.7	1.6	3.4	1.3	2.4	2.0	2.8	6.7	7.1	5.2	4.6
p-Cymene	1270	1012	1016[b]	1.4	0.5	0.8	0.6	0.1	0.6	0.1	1.0	0.1	0.8	0.5	0.1	0.1	0.1
Terpinolene	1282	1078	1074[d]	0.7	0.9	1.1	1.5	1.2	1.2	0.6	0.8	0.3	0.7	0.1	0.9	0.9	1.0
Octanal	1289	979	982[c]														
6-Methylhept-5-en-2-one	1336	960	968[b]			0.1	0.1	tr	0.3						0.5	0.1	tr
Nonanal	1388	1082	1082[c]														
p-Cymenene	1435	1072	1076[g]			0.1	tr	tr			0.2		0.2	0.6			
trans-Sabinene hydrate	1459	1053	1060[b]	0.5	0.8	0.8	0.6	0.8	0.7	1.0	0.9	1.0	0.9		0.1	0.1	0.6
Citronellal	1476	1130	1137[d]			1.3	1.2	2.8	3.0			tr			3.2	3.7	3.0
Decanal	1495	1183	1191[c]														
Linalool	1544	1084	1085[c]	25.9	30.9	22.2	23.4	28.3	25.9	13.6	9.9	29.0	17.0	76.4	57.7	45.3	40.8
Linalyl acetate	1549	1239	1246[d]														
cis-p-Menth-2-en-1-ol	1558	1107	1111[d]	0.2	0.3	0.1	0.2	0.2	0.2	0.3	0.3	0.2	0.3				0.2
β-Elemene	1586	1388	1389[e]														
Thymyl methyl oxide	1589	1214	1215[h]			0.3							0.4				
(*E*)-β-Caryophyllene	1592	1418	1421[e]	0.2	tr	0.1	0.2	tr	0.1	tr		0.1	tr	0.8	0.1	0.2	0.1
Terpinen-4-ol	1598	1162	1170[d]	3.5	4.3	2.6	3.1	3.0	2.4	4.7	4.4	3.1	4.5	0.1	0.1	0.1	3.7
trans-p-Menth-2-en-1-ol	1623	1123	1128[d]	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.2				0.1
Citronellyl acetate*	1654	1332	1335[d]					0.1	0.2							0.2	
(*E*)-β-Farnesene*	1654	1448	1446[e]														
Neral	1678	1214	1218[d]			0.5	0.3	0.5	1.9						2.1	1.1	
α-Terpinyl acetate	1681	1332	1333[d]														
α-Humulene	1660	1453	1455[e]												tr		
α-Terpineol	1690	1172	1180[d]	0.4	0.6	0.9	0.6	0.6	0.8	0.4	0.6	0.5	0.7	0.2	0.2	0.2	0.4
Germacrene D	1704	1480	1475[e]														
Neryl acetate	1722	1340	1345[d]						0.6					0.4	0.2	1.1	0.2
α-Bisabolene	1724	1496	1494[e]			0.8	0.4			tr							
Bicyclogermacrene	1727	1492	1495[e]	0.2	tr					tr		tr		0.2			
Geranial	1732	1242	1242[d]			0.8	0.4	0.6	2.6						2.7	1.5	
(*E,E*)-α-Farnesene	1744	1500	1498[e]														
δ-Cadinene	1746	1516	1520[e]														
Geranyl acetate	1754	1358	1363[d]			0.4	0.2	1.8	1.4						0.3	1.1	0.2
Citronellol	1761	1207	1215[d]			0.7	1.2	1.7	0.6	tr	tr	tr	0.1		2.0	2.9	1.7
Nerol	1797	1207	1218[d]			3.7	1.6	2.1	0.7						2.7	3.0	1.2
trans-Carveol	1834	1199	1209[d]														
Geraniol	1844	1232	1243[d]	tr		0.1	0.2		0.3					0.1	0.7	0.6	tr
Caryophyllene oxide	1980	1576	1576[d]														
(*E*)-Nerolidol	2038	1547	1553[d]	0.1	0.1	tr	0.1					tr		0.2	tr	0.1	0.1
Methyl N-methylanthranylate	2083	1382	1389[b]	1.4		0.7	0.5	0.1	0.4	tr	0.4	tr	tr	2.3	0.7	1.9	tr
Spathulenol*	2115	1557	1565[f]	tr													
Ethyl N-methylanthranylate*	2115	1450	—			0.1					tr			0.6			
Thymol	2182	1266	1270[d]			1.9		tr			2.3		2.3				0.1
α-Cadinol	2228	1641	1644[f]														
β-Sinensal	2229	1673	1671[c]					2.2	tr	2.5	0.8	0.9	0.9				tr
α-Sinensal	2330	1725	1730[c]	0.8	0.9	0.6	0.8	0.7	0.6	1.6	0.5	0.6	0.5	2.3	0.7	0.8	0.5
Total				98.4	98.5	98.8	99.1	96.5	97.7	97.1	98.6	98.9	98.5	96.1	97.3	98.4	98.2

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Table 1a. (*Continued*)

Constituents	RI P	RI A	29 G9	30 G10	31 G3	32 E5	33 G2	34 L2	35 K5	36 D1	37 B7	38 D7	39 I9
α-Thujene*	1024	923	0.2	0.7		0.6	tr	0.4	0.7	0.6	0.7	0.6	0.4
α-Pinene*	1024	931	0.7	2.1		1.8	0.2	1.4	2.3	1.7	2.1	1.9	1.7
Camphene	1068	944	tr	tr		tr		0.1	0.1	0.1	tr	tr	tr
β-Pinene	1111	971	1.4	2.4		2.3		2.1	2.9	2.6	2.8	2.7	2.2
Sabinene	1122	965	35.0	26.3		35.0	tr	46.6	46.6	46.1	45.3	45.3	44.4
Δ-3-Carene	1148	1006		tr	0.1		10.1	tr	tr	tr	2.0	0.1	tr
Myrcene	1160	980	1.9	1.8	0.4	2.1	1.7	2.5	3.0	2.7	2.8	2.5	2.7
α-Phellandrene	1165	997	0.1	0.1		0.1	0.7	0.1	0.1	0.1	0.3	0.1	0.1
α-Terpinene	1180	1009	0.6	1.1		0.8	0.2	0.9	1.3	1.1	1.1	0.9	1.3
Limonene	1202	1022	6.4	2.8	8.3	3.4	10.5	3.0	3.1	2.7	4.8	8.1	1.7
β-Phellandrene	1210	1021	0.5	0.4		0.4	0.6		0.6		0.7	0.5	0.7
(*Z*)-β-Ocimene	1232	1025	0.1	0.1	1.3	0.1	0.2	0.1	0.1	0.2	0.2	0.2	0.2
γ-Terpinene	1245	1049	1.0	11.8	tr	7.0	0.1	1.4	6.5	8.4	10.2	8.1	2.1
(*E*)-β-Ocimene	1249	1036	1.9	2.5	19.1	1.2	3.3	2.0	1.6	4.2	4.1	3.7	3.2
p-Cymene	1270	1012	0.1	0.9		0.5	0.1	tr	0.7	0.4	0.7	1.2	0.1
Terpinolene	1282	1078	0.2	0.7	tr	0.6	1.4	0.3	0.7	0.6	1.0	0.6	0.5
Octanal	1289	979											
6-Methylhept-5-en-2-one	1336	960					0.3						
Nonanal	1388	1082											
p-Cymenene	1435	1072				0.1			0.1			0.1	
trans-Sabinene hydrate	1459	1053	0.8	0.3		0.9	0.3	1.2	0.8	0.7	0.4	0.4	0.6
Citronellal	1476	1130	tr		tr	tr	11.7	0.1			0.4		tr
Decanal	1495	1183			0.1			tr					
Linalool	1544	1084	39.7	39.0	37.7	36.3	35.3	31.6	17.6	19.6	12.4	14.5	29.9
Linalyl acetate	1549	1239											
cis-*p*-Menth-2-en-1-ol	1558	1107	0.1	0.2		0.2		0.2	0.3		0.2	0.2	0.2
β-Elemene	1586	1388			0.1								
(*E*)-β-Caryophyllene	1592	1418		0.1	1.1	0.1	tr	tr	0.2	tr	0.1	0.1	0.1
Thymyl methyl oxide	1589	1214	0.3										
Terpinen-4-ol	1598	1162	1.7	3.8		2.6	0.1	3.1	4.8	3.8	3.2	2.8	4.0
trans-*p*-Menth-2-en-1-ol	1623	1123	tr	0.1		0.1		0.1	0.2	0.1	0.1	0.1	0.1
Citronellyl acetate*	1654	1332					0.1						
(*E*)-β-Farnesene*	1654	1448											
α-Humulene	1660	1453			0.2							0.1	
Neral	1678	1214					2.1						
α-Terpinyl acetate	1681	1332											
α-Terpineol	1690	1172	0.2	1.3	tr	0.8	0.4	0.6	0.5	0.3	0.7	0.3	0.4
Germacrene D	1704	1480											
Neryl acetate	1722	1340											
α-Bisabolene	1724	1496					0.7	tr			tr		
Bicyclogermacrene	1727	1492			1.6				0.1	tr			0.1
Geranial	1732	1242					2.8				0.1		
(*E*,*E*)-α-Farnesene	1744	1500							0.1				
δ-Cadinene	1746	1516											
Geranyl acetate	1754	1358					3.8				tr		
Citronellol	1761	1207			0.1		3.7	tr	tr	tr	1.0		tr
Nerol	1797	1207			tr		2.9	tr			0.3		tr
trans-Carveol	1834	1199			0.2								
Geraniol	1844	1232		tr	tr		0.8				0.1		tr
Caryophyllene oxide	1980	1576			0.9								
(*E*)-Nerolidol	2038	1547		tr	0.6	tr	0.1		0.1		tr	tr	0.1
Methyl N-methylanthranylate	2083	1382	5.6	0.6	0.3	tr	0.3	tr	tr	0.1	0.4	1.4	0.2
Ethyl N-methylanthranylate*	2115	1450		0.1							0.2	0.8	
Spathulenol*	2115	1557	0.4		0.5								
Thymol	2182	1266				2.2			1.9			1.0	
α-Cadinol	2228	1641			0.8								
β-Sinensal	2229	1673	0.5		tr		2.5	0.8	1.2	0.9	0.7	0.6	tr
α-Sinensal	2330	1725	0.2	0.3	22.1	0.7	0.7	0.6	0.5	1.1	0.4	0.3	2.3
Total			99.4	99.2	95.0	99.2	97.0	98.6	98.2	97.0	99.1	98.9	97.0

The 55 identified compounds accounted for 93.6–99.9% of the total amount of the oil. A tremendous chemical variability was observed in the composition of the 113 samples. Indeed, the major components exhibited a wide range of concentrations: methyl N-methylanthranylate (0.0–85.2%), linalool (0.0–76.4%), γ-terpinene (0.0–63.4%), sabinene (0.0–57.2%), limonene (1.7–55.8%), (*E*)-β-ocimene (0.1–20.6%), ethyl N-methylanthranylate (0.0–16.2%), thymol (0.0–13.0%), Δ-3-carene (0.0–11.1%), citronellal (0.0–11.7%). The sesquiterpenes were detected in very low amounts except for α-sinensal (up to 22.1%).

The analyses of 115 samples, 113 hybrids (Nos 2–114) and the parents (*C. clementina*, No. 1, and *C. deliciosa*, No. 115) were submitted to PCA analysis and a K-means

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Table 1b. Chemical composition of citrus hybrid leaf oils (group I, subgroup IB)

Constituents	40 D10	41 B4	42 J9	43 C8	44 K4	45 F10	46 I2	47 F4	48 H7	49 N9	50 I1	51 J6	52 C5	53 B3	54 F8	55 K7	56 L9
α-Thujene*	0.9	2.5	2.5	2.5	2.3	2.1	2.1	2.0	1.3	1.9	1.7	1.9	1.7	2.0	1.8	1.2	1.4
α-Pinene*	2.0	4.7	4.8	4.9	4.5	4.2	3.8	3.8	2.6	3.3	3.3	3.6	3.1	3.8	3.5	2.8	2.7
Camphene	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
β-Pinene	3.2	5.4	5.3	4.8	4.9	5.3	4.7	4.2	3.4	4.4	3.6	4.3	3.6	4.6	4.5	3.2	3.3
Sabinene	0.8	0.6	0.5	0.5	0.5	2.3	0.5	0.4	0.7	0.5	0.3	0.4	0.4	0.5	0.5	5.3	0.4
Δ-3-Carene	0.1			2.7	2.5	2.3		3.5	2.2	0.5	3.2	2.1	3.3	tr		tr	3.3
Myrcene	1.1	1.2	1.2	1.5	1.6	1.4	1.1	1.5	1.2	1.0	1.4	1.3	1.4	1.1	1.1	1.0	1.3
α-Phellandrene	0.1	0.1	0.1	0.3	0.3	0.3	0.1	0.4	0.3	0.1	0.3	0.3	0.4	0.1	0.1	0.1	0.3
α-Terpinene	1.1	1.3	1.3	1.4	1.2	1.3	1.2	1.1	1.0	1.0	0.8	0.9	1.0	1.0	0.7	0.9	0.7
Limonene	5.6	4.9	4.9	7.9	9.3	7.1	4.0	4.8	7.0	4.8	6.2	6.3	6.0	4.2	4.2	8.8	6.8
β-Phellandrene	0.1	tr	tr	0.2	0.2	0.2		0.2	0.2	tr	0.2	0.1	0.2	0.1		0.1	0.2
(*Z*)-β-Ocimene	0.1	0.1	tr	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
γ-Terpinene	63.4	62.1	61.9	59.7	55.3	54.1	51.8	47.4	46.0	44.0	41.4	40.9	40.4	39.8	39.7	35.7	27.3
(*E*)-β-Ocimene	2.6	1.6	0.8	3.2	2.3	1.1	2.5	1.0	1.5	1.9	3.2	1.0	2.0	1.6	2.0	1.1	1.9
p-Cymene	4.0	2.3	2.2	1.3	3.4	4.4	1.8	2.3	2.8	4.5	2.0	4.4	1.3	5.2	6.2	3.0	3.9
Terpinolene	2.6	2.4	2.4	2.7	2.6	2.5	2.1	2.5	2.2	1.8	2.2	2.4	2.2	2.1	2.0	1.5	2.0
Octanal																	
6-Methylhept-5-en-2-one					tr				tr		tr	0.1	0.2				
Nonanal																	
p-Cymenene			tr		tr					4.5		0.3		0.6	0.8		0.7
trans-Sabinene hydrate	tr	0.1	0.1		0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Citronellal				0.3	0.9	0.3		3.0	0.6		2.1	0.1	1.9	tr			3.7
Decanal							tr				0.1	0.1		tr			
Linalool	6.4	7.6	9.3	1.3	3.5	5.4	16.7	16.9	19.9	27.6	22.5	14.2	23.4	18.8	14.6	30.8	23.7
Linalyl acetate																	
cis-p-Menth-2-en-1-ol	tr	tr				tr	tr		tr		tr			tr	tr	tr	
β-Elemene																	
(*E*)-β-Caryophyllene	0.2	0.1	0.1	0.1	0.1	0.1	tr	tr	0.2	tr	0.1	tr	0.1	tr	0.1	0.3	0.1
Thymyl methyl oxide						tr					0.2	0.5			1.6		0.5
Terpinen-4-ol	0.5	0.3	0.2	0.2	0.2	0.7	0.3	0.2	0.4	0.3	0.2	0.3	0.2	0.3	0.3	0.8	0.3
trans-p-Menth-2-en-1-ol		tr				tr	tr					tr		tr			
Citronellyl acetate*					0.1												
(*E*)-β-Farnesene*																	
α-Humulene																	
Neral					0.1			0.1	0.1		0.2	0.3	1.3				0.4
α-Terpinyl acetate							tr										
α-Terpineol	0.4	0.2	0.3	0.2	0.2	0.4	0.3	0.2	0.4	0.5	0.2	0.4	0.3	0.4	0.6	0.5	0.3
Germacrene D																	
Neryl acetate				0.1	0.4	0.1					0.1	tr	0.1			0.1	tr
α-Bisabolene				0.1		0.1			0.1								
Bicyclogermacrene			tr	0.1		tr	tr		0.1						tr		
Geranial				0.1		tr		0.1	0.2				1.7				0.5
(*E*,*E*)-α-Farnesene																	
δ-Cadinene																	
Geranyl acetate				0.3	0.4	0.1		0.3	0.1		0.3	0.1	0.5				0.2
Citronellol				0.1	0.3	0.4	tr	1.3	0.5		0.6	0.1	0.5				1.0
Nerol				0.1	0.3	0.2		1.0	0.4		0.2	0.2	0.8				0.2
trans-Carveol																	
Geraniol	tr			tr	tr	tr					0.1	0.1	0.1				0.1
Caryophyllene oxide																	
(*E*)-Nerolidol	0.1	tr	tr	tr	tr	tr			0.1		0.1	0.1				0.1	tr
Methyl N-methylanthranylate	1.0	1.1	0.1	0.4	0.2	1.2		0.8	2.4	1.1	0.1	tr		tr		1.2	2.0
Ethyl N-methylanthranylate*	0.6	tr		0.1		0.9		0.1	0.7	tr						0.3	tr
Spathulenol*																	
Thymol	tr	tr	tr			tr	tr				tr	9.6	0.1	12.0	13.0		8.4
α-Cadinol																	
β-Sinensal	1.4	0.9	0.9	1.8	1.5	0.6						0.9	tr		0.8		1.0
α-Sinensal	0.7	0.3	0.6	0.6	0.3	0.3	0.4	0.6	0.9	0.3	2.0	0.4	0.4		0.7	0.6	0.2
Total	98.3	99.5	98.9	99.0	99.6	99.3	93.2	99.3	98.8	98.1	97.1	97.2	98.4	98.4	98.3	99.0	98.8

partition method. PCA (Figure 1) and K-means partition suggested the existence of two groups of equal importance with respect to the content of methyl N-methylanthranylate, representing 68% of total variability detected in this progeny (axis 1). The variability along axis 2 (18%) was assigned to the content of γ-terpinene.

The leaf oil from the clementine parent (*C. clementina*) (No. 1), which presents sabinene and linalool as the major components (45.4% and 21.3%, respectively; others ≤3.4% each), is located in group I, also containing samples 2–56; Tables 1a and 1b). The leaf oil from the second parent, Willow Leaf mandarin, was characterized by a high percentage

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of methyl N-methylanthranylate (74.3%) and belonged to group II together with samples 57–114 (Table 2).

With respect to the amount of sabinene, linalool and γ-terpinene, group I could be divided into two subgroups. Subgroup IA (samples 1–39) is characterized by the sabinene–linalol association (0.0–57.2%/6.8–76.4%; Table 1a). Subgroup IB (samples 40–56) is characterized by the γ-terpinene–linalool association (7.3–63.4%/1.3–30.8%; Table 1b). α-Thujene, α- and β-pinenes, myrcene, β-phellandrene, (*Z*)- and (*E*)-β-ocimenes, limonene, *p*-cymene, terpinolene, terpinen-4-ol, α-terpineol and sinensal isomers were present in moderate amounts in almost all the samples.

Group II (Nos 57–115) is dominated by methyl N-methylanthranylate (36.8–85.2%; Table 2). Variable amounts of a few compounds were also observed: limonene (3.0–55.8%; it should be pointed out that limonene is a major component in samples 113 and 114), γ-terpinene (0.0–22.0%) and ethyl N-methylanthranylate (0.0–16.2%). It is also pointed out that sabinene and linalool, the main components of cluster I, were present at a very low ratios in all the samples of the second group, ≤3.8% and ≤2.3%, respectively.

The chemical compositions of samples 2–39 (subgroup IA) were close to that of the clementine parent (*C. clementina*, No. 1), whereas those of samples 57–114 (group II) were close to that of mandarin parent (*C. deliciosa*, No. 115). Conversely, several samples, belonging to the subgroup IB, present a different composition, characterized by a high content of γ-terpinene (27.3–63.4%), associated to linalool in variable amounts (1.3–30.8%). This result showed that citrus hybrids may produce an essential oil whose chemical composition could be differentiated from those of the parents. It should be pointed out that this composition was previously found in leaf oils of mandarin species.[3,4,14]

Considering the repartition of methyl N-methylanthranylate into the progeny, an equal distribution (half of the samples containing low amounts of this compound and the rest with high amounts, such as Willow Leaf mandarin) suggests that the genetic control of its quantity is based on a major gene present into the mandarin genome and absent in the clementine genome. As we have suspected previously in the chemical analysis of leaf oils from somatic hybrids,[15] we can certify with the results of the present study that this gene is heterozygous with a dominant allele. Samples present in group II will be more favourable to produce fruit developing mandarin aroma. It will be interesting also to know which kind of fruit will be produced by the hybrids from atypical subgroup IB and by some others characterized by particular amounts of compounds such as α-sinensal, citronellal or thymol.

In conclusion, we have demonstrated that the crossing between two citrus varieties (*C. clementina* and *C. deliciosa*) produces mainly leaf oils characterized by a composition

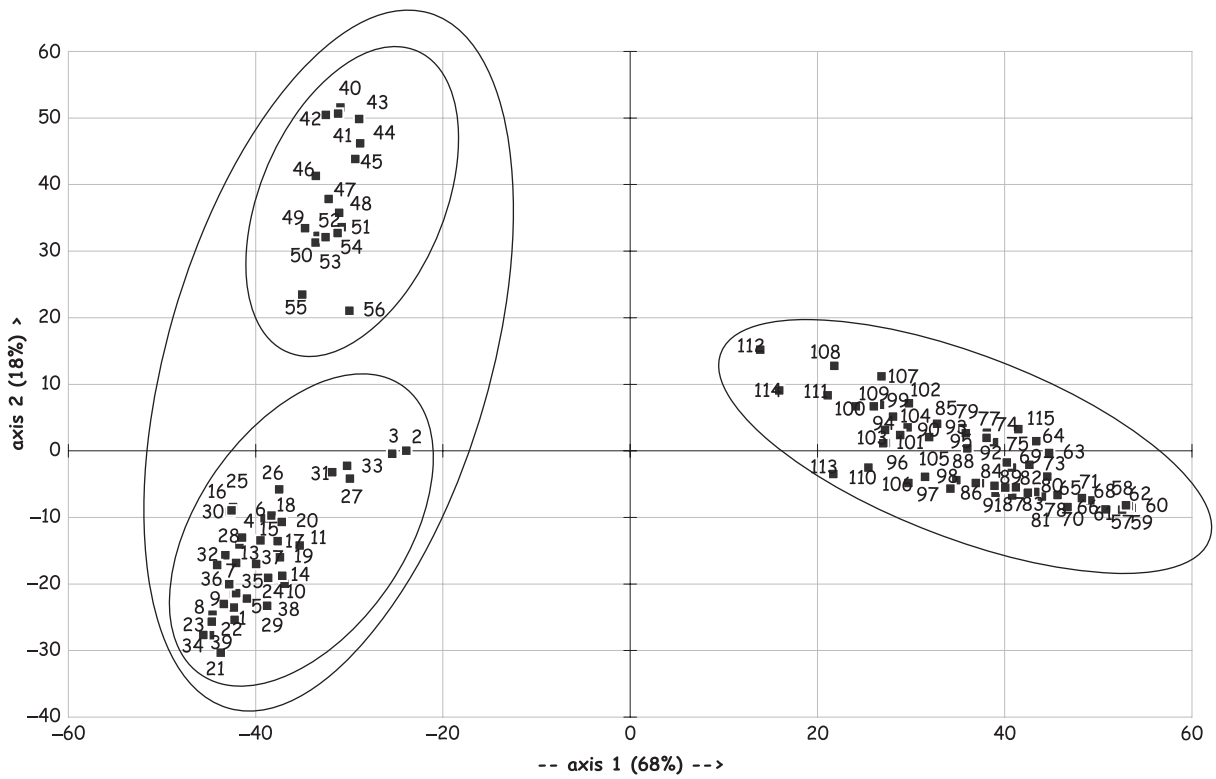

Figure 1. PCA scatterplot of *Citrus* leaf oils

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Table 2. Chemical composition of citrus hybrid leaf oils (group II)

Constituents	RI P	RI A	57 G1	58 H4	59 C1	60 N2	61 K1	62 D9	63 F3	64 J1	65 F7	66 L6	67 J4	68 N5	69 I7	70 D8	71 G6
α-Thujene*	1024	923							0.3	0.4	0.2				0.3		
α-Pinene*	1024	931	tr	tr	0.1		0.1	0.1	0.6	0.8	0.6	0.1	0.2	0.1	0.7	0.1	0.1
Camphene	1068	944								tr							
β-Pinene	1111	971			0.1		0.1		0.8	1.1	0.6	0.1	0.1		0.7	0.1	
Sabinene	1122	965	tr	tr	1.2	tr	1.5	0.1	1.0	0.1	0.3	2.0	2.4	tr	1.5	0.9	tr
Δ-3-Carene	1148	1006										tr					
Myrcene	1160	980	0.2	0.2	0.3	0.3	0.4	0.3	0.3	0.3	0.1	0.4	0.4	0.3	0.3	0.3	0.5
α-Phellandrene	1165	997							tr	tr							
α-Terpinene	1180	1009			tr		tr		0.1	tr	0.1	tr	0.1		0.2		
Limonene	1202	1022	10.1	11.8	13.0	13.1	13.7	17.5	5.7	3.0	3.5	16.9	17.3	22.9	6.1	15.9	25.4
β-Phellandrene	1210	1021	tr		tr			tr		tr							
(Z)-β-Ocimene	1232	1025	0.7	0.4	0.1	0.3	0.2	0.5	0.1	0.3	0.1	0.4	0.1	0.3	0.2	0.2	
γ-Terpinene	1245	1049	tr	0.1	0.1		0.1	0.1	9.6	10.9	4.4	0.1	0.3		6.4	0.5	
(E)-β-Ocimene	1249	1036	2.0	1.5	0.4	1.5	1.2	1.8	0.3	0.7	0.3	1.8	0.4	0.4	0.7	0.4	1.3
p-Cymene	1270	1012		tr				tr	1.6	3.7	1.9		tr		0.9	0.2	
Terpinolene	1282	1078					tr		0.2	0.2	0.1	tr	0.1		0.2	tr	
Octanal	1289	979							tr	tr			tr				
6-Methylhept-5-en-2-one	1336	960											tr				
Nonanal	1388	1082															
p-Cymenene	1435	1072									tr				0.1		
trans-Sabinene hydrate	1459	1053			tr		tr		0.1	tr		tr	0.1				
Citronellal	1476	1130															
Decanal	1495	1183			0.1		0.1		0.1	0.1				tr			
Linalol	1544	1084	1.0	0.7	0.4	0.1	0.7	0.2	0.3	0.6	0.5	1.0	0.3	0.1	0.6	0.5	0.1
Linalyl acetate	1549	1239															
cis-p-Menth-2-en-1-ol	1558	1107											tr				
β-Elemene	1586	1388															
(E)-β-Caryophyllene	1592	1418	0.1	0.1	0.1	0.1	tr	0.1	0.1	tr	0.7	0.1	0.1	0.1	0.6	0.1	0.1
Thymyl methyl oxide	1589	1214			0.1				0.2	tr	0.1				0.2		
Terpinen-4-ol	1598	1162			0.1		0.2	tr	0.1	0.1	0.1	0.2	0.7		0.3		
trans-p-Menth-2-en-1-ol	1623	1123											tr				
Citronellyl acetate*	1654	1332															
(E)-β-Farnesene*	1654	1448															
α-Humulene	1660	1453										tr					
Neral	1678	1214															
α-Terpinyl acetate	1681	1332															
α-Terpineol	1690	1172				tr	tr		0.1	0.1	tr		tr		0.1		
Germacrene D	1704	1480															
Neryl acetate	1722	1340															
α-Bisabolene	1724	1496															
Bicyclogermacrene	1727	1492					tr				tr	tr	tr			tr	
Geranial	1732	1242															
(E,E)-α-Farnesene	1744	1500															
δ-Cadinene	1746	1516															
Geranyl acetate	1754	1358															
Citronellol	1761	1207															
Nerol	1797	1207											tr				
trans-Carveol	1834	1199															
Geraniol	1844	1232															
Caryophyllene oxide	1980	1576															
(E)-Nerolidol	2038	1547															
Methyl N-methylanthranylate	2083	1382	85.2	84.3	83.5	83.4	81.5	78.0	77.7	76.8	76.7	76.3	75.6	75.5	72.6	72.6	71.9
Ethyl N-methylanthranylate*	2115	1450		0.5				0.8	0.3		9.0				6.2	7.0	
Spathulenol*	2115	1557															
Thymol	2182	1266							0.2	0.2	0.1				0.8		
α-Cadinol	2228	1641															
β-Sinensal	2229	1673				0.4	tr	0.3	tr	tr	tr		0.4			0.3	0.2
α-Sinensal	2330	1725	tr		tr							tr	0.1				
Total			99.3	99.6	99.6	99.2	99.8	99.8	99.8	99.4	99.4	99.4	98.6	99.7	99.7	99.1	99.6

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Table 2. (*Continued*)

Constituents	RI P	RI A	72 F1	73 N8	74 L3	75 F5	76 M1	77 K6	78 E3	79 E10	80 M2	81 N7	82 N1	83 G4	84 B8	85 E2	86 J8
α-Thujene*	1024	923	tr	0.2	0.4	0.5	0.5	0.4		0.4		tr	0.2		tr	0.5	
α-Pinene*	1024	931	0.2	0.5	0.9	0.9	0.9	1.1	0.1	1.0	0.1	0.2	0.5	0.1	0.1	1.0	0.2
Camphene	1068	944			tr			tr								tr	
β-Pinene	1111	971	0.2	0.5	1.1	1.1	1.2	1.1		1.0	tr	0.1	0.5	tr	0.1	1.0	0.1
Sabinene	1122	965	3.8	0.3	1.5	1.4	0.2	0.6	tr	0.8	0.6	1.6	0.1	tr	0.2	1.2	1.4
Δ-3-Carene	1148	1006		0.1	0.1					tr	tr			tr	tr	0.1	
Myrcene	1160	980	0.6	0.3	0.4	0.4	0.4	0.4	0.4	0.3	0.4	0.6	0.4	0.4	0.4	0.6	0.6
α-Phellandrene	1165	997		tr	tr		tr			tr						tr	
α-Terpinene	1180	1009	0.1	tr	0.1	0.1	0.2	tr		0.2	tr	0.1	tr		tr	0.2	tr
Limonene	1202	1022	19.5	19.4	8.7	8.9	10.1	11.8	28.3	6.5	31.0	24.9	24.1	26.9	22.8	14.7	28.8
β-Phellandrene	1210	1021	0.1		tr						0.1					0.1	0.1
(*Z*)-*β*-Ocimene	1232	1025	0.2	0.1	0.3	0.1	0.1	0.2	0.5	0.4	0.1	0.3	0.1	0.2	0.2	0.1	0.2
γ-Terpinene	1245	1049	0.2	5.7	10.7	12.4	12.9	10.6		12.5	tr	0.2	5.3	0.5	0.9	11.5	0.1
(*E*)-*β*-Ocimene	1249	1036	1.6	0.2	0.8	0.4	0.2	0.2	0.8	1.2	0.1	1.1	0.2	0.3	1.0	0.5	1.0
p-Cymene	1270	1012		0.5	2.7	1.5	2.6	2.5		1.0		tr	0.4	tr	0.2	0.5	
Terpinolene	1282	1078	tr	0.1	0.2	0.3	0.4	0.1		0.3		tr	0.1	tr	tr	0.4	tr
Octanal	1289	979			tr			tr								tr	
6-Methylhept-5-en-2-one	1336	960															
Nonanal	1388	1082															
p-Cymenene	1435	1072			0.1			tr									
trans-Sabinene hydrate	1459	1053	0.1		0.1	tr	tr	tr			tr	tr				tr	tr
Citronellal	1476	1130															
Decanal	1495	1183	0.1		0.1			0.1			tr	0.1	0.1				
Linalool	1544	1084	0.1	0.1	0.2	0.3	0.3	0.6	0.2	0.7	0.1	0.1	0.1	0.2	0.8	0.4	0.1
Linalyl acetate	1549	1239															
cis-*p*-Menth-2-en-1-ol	1558	1107										tr					
β-Elemene	1586	1388															
(*E*)-*β*-Caryophyllene	1592	1418			tr	0.1	0.1		tr	0.2	0.1	0.1		0.4	0.4	0.1	0.1
Thymyl methyl oxide	1589	1214	0.1		tr	tr		0.1					0.1				
Terpinen-4-ol	1598	1162	0.4	0.1	0.2	0.2	0.1	0.1		0.2	0.1	0.2	tr		tr	0.2	0.2
trans-*p*-Menth-2-en-1-ol	1623	1123															
Citronellyl acetate*	1654	1332															
(*E*)-*β*-Farnesene*	1654	1448															
α-Humulene	1660	1453	tr														
Neral	1678	1214															
α-Terpinyl acetate	1681	1332															
α-Terpineol	1690	1172	tr		0.1	0.1	0.2	0.1								tr	
Germacrene D	1704	1480															
Neryl acetate	1722	1340											tr	tr			
α-Bisabolene	1724	1496															
Bicyclogermacrene	1727	1492	tr								tr						
Geranial	1732	1242															
(*E*,*E*)-*α*-Farnesene	1744	1500															
δ-Cadinene	1746	1516															
Geranyl acetate	1754	1358															
Citronellol	1761	1207															
Nerol	1797	1207															
trans-Carveol	1834	1199															
Geraniol	1844	1232															
Caryophyllene oxide	1980	1576															
(*E*)-Nerolidol	2038	1547															
Methyl N-methylanthranylate	2083	1382	71.3	70.9	70.5	69.8	69.0	68.6	68.4	67.2	67.0	66.9	66.8	66.3	66.2	65.6	63.8
Ethyl N-methylanthranylate*	2115	1450		tr		0.4			0.7	5.4		2.8	0.5	3.8	6.2	0.2	3.0
Spathulenol*	2115	1557															
Thymol	2182	1266		0.2		0.1	0.1	0.2									
α-Cadinol	2228	1641															
β-Sinensal	2229	1673	0.8	0.2	0.2	0.1	0.1			0.1	0.2	tr	0.1	0.4		0.4	0.1
α-Sinensal	2330	1725			0.1							tr		tr		0.1	
Total			99.4	99.4	99.4	99.1	99.6	98.8	99.4	99.4	99.9	99.3	99.6	99.5	99.5	99.3	99.8

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Table 2. (*Continued*)

Constituents	RI P	RI A	87 D4	88 H6	89 K3	90 B6	91 I3	92 M3	93 H3	94 K9	95 D5	96 C9	97 E8	98 E6	99 C10	100 M9
α-Thujene*	1024	923		0.3		0.5			0.8	0.5	0.4	0.4			0.3	0.7
α-Pinene*	1024	931	0.1	0.6	0.1	1.1	0.1	0.1	1.6	1.1	0.8	1.0	0.1	0.2	0.6	1.5
Camphene	1068	944		tr		tr			tr		tr				tr	tr
β-Pinene	1111	971		0.7		1.1	tr		1.8	1.2	0.8	1.0	tr	0.2	0.8	1.5
Sabinene	1122	965	tr	0.6	tr	0.1	tr	tr	1.4	0.3	0.6	3.4	0.1	2.0	1.0	1.8
Δ-3-Carene	1148	1006	tr	tr	tr		tr	tr	tr		tr	tr		tr		0.2
Myrcene	1160	980	0.5	0.5	0.6	0.6	0.5	0.6	0.7	0.3	0.6	0.5	0.4	0.8	0.6	0.8
α-Phellandrene	1165	997		tr		tr			tr		tr	tr			tr	tr
α-Terpinene	1180	1009		0.1		0.1			0.2		0.1	0.2		0.1	0.2	0.2
Limonene	1202	1022	33.6	24.9	35.3	20.5	34.4	39.1	10.7	20.3	27.4	14.6	37.8	38.7	23.9	18.3
β-Phellandrene	1210	1021			0.1				0.1							
(*Z*)-β-Ocimene	1232	1025	0.2	0.2	0.2	0.1	0.2	0.1	0.1		0.1	0.1	0.2	tr	0.3	0.1
γ-Terpinene	1245	1049	tr	7.6		11.4	0.3		21.1	15.0	8.9	10.1	0.2	0.1	10.8	15.4
(*E*)-β-Ocimene	1249	1036	1.0	0.4	1.0	0.5	0.8	0.3	0.6	0.3	0.2	0.5	0.4	0.5	1.1	0.4
p-Cymene	1270	1012	tr	0.4		2.1	0.1		0.8	1.7	0.5	1.3			1.5	1.4
Terpinolene	1282	1078		0.2		0.4			0.6			0.3		tr	0.4	0.5
Octanal	1289	979		tr												
6-Methylhept-5-en-2-one	1336	960														
Nonanal	1388	1082														
p-Cymenene	1435	1072				0.2									0.1	tr
trans-Sabinene hydrate	1459	1053		tr					0.1			tr		tr	tr	tr
Citronellal	1476	1130														
Decanal	1495	1183		0.1	tr				0.2					0.1		
Linalool	1544	1084	0.2	0.1	0.1	0.1	0.4	0.3	0.3		0.3	2.3	0.1	0.5	0.3	0.2
Linalyl acetate	1549	1239														
cis-p-Menth-2-en-1-ol	1558	1107							tr			tr		tr		tr
β-Elemene	1586	1388														
(*E*)-β-Caryophyllene	1592	1418	0.1	0.1	0.1	0.1	0.1	0.2			0.2	0.1	0.3	0.1	0.2	tr
Thymyl methyl oxide	1589	1214				0.1						0.1				0.3
Terpinen-4-ol	1598	1162		0.1		tr			0.2		0.1	0.6		0.2	0.2	0.4
trans-p-Menth-2-en-1-ol	1623	1123	tr					tr				tr				
Citronellyl acetate*	1654	1332														
(*E*)-β-Farnesene*	1654	1448														
α-Humulene	1660	1453									tr					
Neral	1678	1214														
α-Terpinyl acetate	1681	1332														
α-Terpineol	1690	1172		tr					0.1			0.2		tr	0.1	
Germacrene D	1704	1480														
Neryl acetate	1722	1340														
α-Bisabolene	1724	1496									tr					
Bicyclogermacrene	1727	1492			tr	tr							tr	tr	0.1	tr
Geranial	1732	1242														
(*E*,*E*)-α-Farnesene	1744	1500														
δ-Cadinene	1746	1516														
Geranyl acetate	1754	1358														tr
Citronellol	1761	1207														
Nerol	1797	1207														0.1
trans-Carveol	1834	1199														
Geraniol	1844	1232														
Caryophyllene oxide	1980	1576														
(*E*)-Nerolidol	2038	1547														
Methyl N-methylanthranylate	2083	1382	63.4	62.3	61.6	60.1	60.1	58.4	57.8	57.2	57.2	56.8	56.2	56.2	55.9	54.9
Ethyl N-methylanthranylate*	2115	1450	0.1	0.1	0.1		2.6	0.2		2.0	0.2	5.4	3.7		0.3	0.1
Spathulenol*	2115	1557														
Thymol	2182	1266				tr						0.2			0.7	0.3
α-Cadinol	2228	1641														
β-Sinensal	2229	1673	0.3	0.4	0.5	0.2			0.4							
α-Sinensal	2330	1725		tr							0.1					0.1
Total			99.5	99.7	99.7	99.3	99.6	99.3	99.6	99.9	98.4	99.1	99.5	99.7	99.4	99.1

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Table 2. (*Continued*)

Constituents	RI P	RI A	101 L8	102 C4	103 C7	104 M8	105 J5	106 M4	107 D2	108 I8	109 I6	110 G7	111 H8	112 F6	113 K2	114 L1	115 G5
α-Thujene*	1024	923	0.4	0.5	0.2	0.2		tr	0.5	0.9	0.5	tr	0.6	0.8		0.6	0.5
α-Pinene*	1024	931	0.8	1.0	0.6	0.5	0.2	0.2	1.1	1.8	1.1	0.1	1.3	1.6	0.3	1.3	0.9
Camphene	1068	944			tr					tr				tr		tr	tr
β-Pinene	1111	971	1.0	1.1	0.6	0.7	tr	0.1	1.1	1.9	1.2	0.1	1.4	1.9	0.1	1.4	1.2
Sabinene	1122	965	1.2	0.3	0.3	0.2	0.2	2.2	0.1	0.2	0.2	0.2	1.4	0.2	2.7	1.2	0.1
Δ-3-Carene	1148	1006	0.3		tr		tr	0.1			tr	tr		tr	tr	tr	tr
Myrcene	1160	980	0.7	0.6	0.3	0.6	0.8	0.9	0.4	0.8	0.8	0.5	0.8	0.9	0.8	0.9	0.3
α-Phellandrene	1165	997	tr	tr		tr		tr		tr	tr		tr	tr		tr	tr
α-Terpinene	1180	1009		0.1	0.1	0.1		0.1	tr	0.3	0.2	tr	0.3	0.4	0.1	0.1	0.1
Limonene	1202	1022	23.7	28.3	19.7	28.3	46.8	43.6	32.0	18.4	32.2	31.4	29.1	29.8	55.8	39.5	4.9
β-Phellandrene	1210	1021								0.1			0.1	0.1	0.1	0.1	tr
(*Z*)-*β*-Ocimene	1232	1025	0.2	0.2	0.2	0.1	0.2	0.1	0.2	0.1	0.1	0.2	tr	0.1	0.3	0.1	0.2
γ-Terpinene	1245	1049	9.3	11.8	6.7	8.9	tr	0.2	13.0	20.7	12.8	1.1	15.5	22.0	0.1	14.7	12.8
(*E*)-*β*-Ocimene	1249	1036	1.0	0.5	0.5	0.3	0.9	1.2	0.2	1.0	0.3	1.1	0.5	0.7	0.6	0.2	0.3
p-Cymene	1270	1012	1.2	1.3	1.2	1.0		tr	0.9	2.3	1.4	0.2	0.9	1.2	tr	1.3	2.9
Terpinolene	1282	1078	0.4	0.3	0.1	0.3			0.2	0.7	0.4	0.1	0.6	0.8	tr	0.3	0.3
Octanal	1289	979											0.1	tr			
6-Methylhept-5-en-2-one	1336	960															
Nonanal	1388	1082															
p-Cymenene	1435	1072	tr			tr				0.1	tr		tr				0.1
trans-Sabinene hydrate	1459	1053	tr	tr				tr		tr	tr		tr	tr	tr	tr	tr
Citronellal	1476	1130						tr									
Decanal	1495	1183								0.1	tr			0.1		0.1	
Linalool	1544	1084	0.3	0.3	0.4	0.1	0.3	0.3	tr	0.2	0.4	0.2	0.5	0.7	tr	0.2	0.3
Linalyl acetate	1549	1239															
cis-*p*-Menth-2-en-1-ol	1558	1107	tr					tr							tr		
β-Elemene	1586	1388															
(*E*)-*β*-Caryophyllene	1592	1418	0.1	0.1	0.4	0.1	0.1	tr	0.2	0.1	0.1	0.5	tr	0.1	0.1	0.1	0.1
Thymyl methyl oxide	1589	1214		tr	tr					tr						0.1	
Terpinen-4-ol	1598	1162	0.5	0.2	0.1	0.1	0.1	0.4	tr	0.1	0.1	0.1	0.2	0.1	0.2	0.2	0.1
trans-*p*-Menth-2-en-1-ol	1623	1123	tr					tr		tr							
Citronellyl acetate*	1654	1332															
(*E*)-*β*-Farnesene*	1654	1448						tr									
α-Humulene	1660	1453															
Neral	1678	1214															tr
α-Terpinyl acetate	1681	1332				0.1											
α-Terpineol	1690	1172	0.1	tr				tr		0.1	0.1	tr	0.1	0.1		0.1	0.1
Germacrene D	1704	1480															
Neryl acetate	1722	1340														tr	
α-Bisabolene	1724	1496															
Bicyclogermacrene	1727	1492		tr	tr		tr	tr				tr		tr	tr		
Geranial	1732	1242															
(*E*,*E*)-*α*-Farnesene	1744	1500															
δ-Cadinene	1746	1516															
Geranyl acetate	1754	1358						tr									
Citronellol	1761	1207						tr									
Nerol	1797	1207															
trans-Carveol	1834	1199															
Geraniol	1844	1232															
Caryophyllene oxide	1980	1576															
(*E*)-Nerolidol	2038	1547															
Methyl N-methylanthranylate	2083	1382	54.8	52.9	52.5	51.1	50.3	49.8	49.6	49.5	47.5	46.7	45.7	37.9	37.5	36.8	74.3
Ethyl N-methylanthranylate*	2115	1450	1.7		15.2	7.2				0.4		16.2	0.3			0.1	0.2
Spathulenol*	2115	1557															
Thymol	2182	1266						tr		tr	tr		0.1			0.3	0.1
α-Cadinol	2228	1641															
β-Sinensal	2229	1673	0.2	0.1			tr	0.3		0.1	0.2	0.6		tr	0.4	tr	
α-Sinensal	2330	1725	0.1	0.1													
Total			97.9	99.6	99.1	99.9	99.9	99.5	99.5	99.9	99.6	99.3	99.5	99.5	99.1	99.7	99.8

Order of elution and percentages (%) of individual components are given on polar column, except for compounds with an asterisk, for which percentages are given on apolar column.
RI P, RI A, retention indices on polar and apolar column, respectively; tr, trace (≤0.05%).
RI[#], literature data: [a]ref 16, [b]ref 11, [c]ref 17, [d]ref 18, [e]ref 10, [f]ref 19, [g]ref 20, [h]ref 21.

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similar to those of parents (96 of 113 samples). However, 17 samples, belonging to subgroup IB, exhibited an original composition. These results associated with the chemical composition of peel oils and DNA fingerprints may permit predictive data for selection for a breeding programme at early stage of citrus development.

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