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Composition of volatile in micropropagated and field grown aromatic plants from Tuscany Islands

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Aromatic plant species present in the natural Park of Tuscany Archipelago are used as flavoring agents and spices, as dietary supplements and in cosmetics and aromatherapy. The plants are usually collected from wild stands, inducing a depletion of the natural habitat. Therefore, micropropagation of these aromatic plants can play a role in the protection of the natural ecosystem, can guarantee a massive sustainable production and can provide standardized plant materials for diverse economical purposes. The aim of this study is to compare the volatile organic compounds produced by the wild plants with those from *in vitro* plantlets using headspace solid phase micro-extraction (HS-SPME) followed by capillary gas-chromatography coupled to mass spectrometry (GC-MS). Typical plants of this natural area selected for this work were *Calamintha nepeta* L., *Crithmum maritimum* L., *Lavandula angustifolia* L., *Myrtus communis* L., *Rosmarinus officinalis* L., *Salvia officinalis* L. and *Satureja hortensis* L. Different explants were used: microcuttings with vegetative apical parts, axillary buds and internodes. Sterilization percentage, multiplication rate and shoot length, as well as root formation were measured. The volatile aromatic profiles produced from *in vitro* plantlets were compared with those of the wild plants, in particular for *C. maritimum*, *R. officinalis*, *S. officinalis* and *S. hortensis*. This study indicated that the micropropagation technique can represent a valid alternative to produce massive and sterile plant material characterised by the same aromatic flavour as in the wild grown plants.

Key words: VOC, *in vitro* shoot cultures, aromatic plants, HS-SPME, GC-MS, biodiversity

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INTRODUCTION

The Tuscany Archipelago is the largest marine (and land) Park in Europe, with more than 50000 hectares of sea and about 20000 hectares of land. The Archipelago includes seven main islands as well as some minor islands and rocks (Landi, 1989). The vegetation, influenced by the Mediterranean climate and by the insularity, is characterised by several aromatic species. Typical plants of the Mediterranean that can be observed for example on the Elba Island are adapted to grow near the sea, tolerating salt stress, drought, and rock side soils include *Crithmum maritimum* L*., Rosmarinus officinalis* L*., Myrtus* sp*., Spartium* sp*., Cistus* sp*., Lavandula* sp*., Helichrysum* spp. and *Juniperus* spp. (Rinaldi, 2001). These plants constitute an ecological niche for their typical aromatic characteristics and represent an ecological population to exploit and to preserve (Hamilton, 2004; Abraham, 2010). The aromatic plants are usually collected on their natural stands and used as spices in traditional food and cosmetics, in phytotherapy and aromatherapy. This practice harms the natural habitat, so the development of efficient protocols for rapid clonal propagation and the conservation of germplasm of selected species typical of these islands is badly needed. *In vitro* culture of aromatic and medicinal plants is a useful technique to produce rapidly and in small spaces a large amount of plant material, avoiding the damage and the extinction of natural field grown plants (Debnath *et al*., 2006; Lucchesini & Mensuali-Sodi, 2010; Ruffoni *et al*., 2010). Moreover, micropropagation represents a valid alternative for rapid clonal propagation and an improvement of the production and marketing of the selected plants. The micropropagation protocols are based on regeneration of plants starting from different organs (Lucchesini & Mensuali-Sodi, 2010). The *in vitro* culture technique can be also useful for the production of active compounds naturally present in aromatic plants and in particular enhancing the levels of some metabolites (Pistelli *et al*., 2010).

The plants are able to emit organic volatile compounds (VOC), but the intensity and profile of emission is dependent on the genetic variability and plasticity of phenotypes (Dicke & Loreto, 2010). VOC are mainly produced by plants for three main reasons: plants-plants interactions, signals for symbiotic organisms, and as insect attractants or repellents. Many VOCs produced by plants are constituents of aromatic essential oils, often used in the food and perfume industries as flavors and/or fragrances (Maffei *et al.,* 2011). Moreover, it is known that essential oils can have antibacterial, anti-inflammatory and other pharmaceutical uses (Maffei *et al.,* 2011).

Headspace solid phase micro-extraction (HS-SPME) followed by capillary gas-chromatography mass-spectrometry (GC-MS) is currently a widely used technique for the characterization of the composition of plant volatile fraction, due to its easy and fast management (Belliardo *et al*., 2006).

The aim of the present work was to investigate, for the first time, the chemical composition of the aroma scent emitted by *in vitro* plantlets (ivP) of several aromatic species, in comparison with the field-grown mother-plants (fgP), using HS-SPME. Seven typical species grown in the Tuscany Archipleago were chosen as plant

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Abbreviations: BA, benzyladenine; GC-MS, capillary gas-chromatography mass spectrometry; HS-SPME, headspace solid phase micro-extraction; IAA, indole-3-acetic acid; IBA, indolebutiric acid; MS0, basal Murashige and Skoog medium; NAA, naphtalene acetic acid; PAR, photosynthetically active radiation

material from different families: *Calamintha nepeta* (L.) Savi, *Lavandula angustifolia* Mill., *Rosmarinus officinalis* L., *Salvia officinalis* L. and *Satureja hortensis* L. belonging to Labiatae, *Crithmum maritimum* L. (Umbelliferae family) and *Myrtus communis* L. (Myrtaceae family).

MATERIALS AND METHODS

Plant Materials. *Calamintha nepeta* L., *Crithmum maritimum* L., *Lavandula angustifolia* Mill., *Myrtus communis* L., *Rosmarinus officinalis* L., *Salvia officinalis* L., *Satureja hortensis* L., representatives of the plant species typical of the Tuscan herbs, were collected in spring 2011 in the Elba Island of the Tuscan Archipelago, and kept in pots in a greenhouse for acclimatization. A voucher specimen was authenticated by S. Maccioni (Università di Pisa, Dipartimento di Biologia) and deposited at the Botanical Garden of the University of Pisa.

Micropropagation. Different types of explants were used: apical portions of *R. officinalis* and *S. hortensis,* internodes with lateral buds for *C. nepeta, M. communis, L. angustifolia*, and *S. officinalis,* basal shoots of *C. maritimum.* The explants from the selected species were differently sterilised. *C. nepeta* internodes with lateral buds, 10 mm length, were submerged in 0.05% (v/v) Tween-20® for 20 min, followed by washing with sterile water for 5 min. The explants were then transferred in 50% (v/v) commercial hypochlorite for 10 min, and then washed with sterile water for 5 min (sterilization method called S1) (Grigoriadou & Maloupa, 2008)**.** Ten-millimetre long internodes of *M. communis, L. officinalis*, and *S. officinalis* and basal shoots of *C. maritimum* were treated with detergent 0.05% (v/v) Tween-20® for 20 min, then dipped in a 70% (v/v) ethanol solution for 1 min before surface sterilized in 25%(v/v) commercial sodium hypochlorite for 10 min, and washed with sterile water for 5 min (S2) (Ruffoni & Mascarello, 2009). The S3 method, adopted for the apical portions of *R. officinalis* and *S. hortensis*, comprised a preliminary incubation of the explants in 2% (w/v) sucrose and 0.01% (v/v) commercial sodium hypochlorite for 15 days in the dark (Ruffoni & Mascarello, 2009). After washing with water for 5 min, the explants were then sterilized with the S2 treatment. Shoot proliferation was obtained by culturing the sterile explants in different media. The constituents of the basal medium (MS0) were MS macro- and micronutrients, vitamins (Murashige & Skoog, 1962), 3% (w/v) sucrose, 0.8% (w/v) agar and 0.05% (v/v) "Plant preservative mixture" (PPM); the above medium with the addition of 0.5 mg/L benzyladenine (BA) was called M1. The shoot proliferation medium for *R. officinalis* (M2) contained an extra 0.017 mg/L indole-3-acetic acid (IAA). The shoot culture multiplication medium for *C. maritimum* (M3) contained MS0 medium with the addition of 0.5 mg/L BA and 0.46 mg/L NAA. The rooting phase was induced *in vitro* by transferring the shoots to rooting media according to the literature. The following rooting media were utilized: R1, composed of MS0 and 0.2 mg/L NAA for *C. nepeta, R. officinalis* and *L. angustifolia* (Andrade *et al.*, 1999); R2 (MS0 with 1 mg/L IAA + 1.5% sucrose) was used for *M. communis* (Scarpa *et al.*, 2000), R3 (MS0 + 0.1 mg/L IBA) for *C. maritimum* (Grigoriadou & Maloupa, 2008), and medium R4 (MS0 + 0.5 mg/L IBA) for *S. officinalis* and *S. hortensis* (Arikat *et al.,* 2004). All media were adjusted to pH 5.7 with 0.1 M KOH before autoclaving for 20 min at 120°C. Cultures were maintained in a growthchamber at 22 ± 1°C under 16 h light and 8 h dark photoperiod provided by cool white fluorescent tubes (Philips TLM 40W/33RS) with 80 μmoles m^{-2} s^{-1} PAR. Cultures were monitored regularly for shoot production. Vitality was recorded after one week; after four weeks, the number and length of shoots, and percentage of root formation were recorded and the plantlets were transferred to fresh medium.

SPME analyses. Emitted volatiles were analyzed using a Supelco SPME device coated with polydimethylsiloxane (PDMS, 100 μm) in order to sample the headspace of 10 g of fresh plant mass comprising either field grown plants or aerial parts without flowers of *in vitro* plantlets collected at the end of 4-week subculture. Each sample was introduced into a 30 ml glass conical flask and allowed to equilibrate for 30 min. After the equilibration time, the fiber was exposed to the headspace for 15 min at room temperature; once sampling was finished, the fiber was withdrawn into the needle and transferred to the injector of the GC and GC-MS system, where the fiber was desorbed.

Gas Chromatography-FID. GC analyses were performed using an HP-5890 Series II instrument equipped with HP-WAX and HP-5 capillary columns (30 m × 0.25 mm, 0.25 μm film thickness), working with the following temperature program: 60°C for 10 min, rising at 5°C/min to 220°C; splitless injection mode, injector temperature, 250°C; carrier gas, nitrogen (2 ml/min); detector, dual FID. The identification of the components was performed for the both columns by comparison of their retention time with those of pure authentic samples and by means of their linear retention indices (l*RI*) relative to a series of *n*-hydrocarbons. The percentage of the volatile constituents was obtained by FID peak-area normalization.

Gas Chromatography-Mass Spectrometry. GC-MS analyses were performed with a Varian CP-3800 gas chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm; coating thickness 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: splitless injection mode, injector temperature, 250°C; oven temperature programmed from 60 °C to 240°C at 3°C/min; carrier gas, helium at 1 ml/min; injection, 0.2 μl (10% hexane solution). Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to a series of *n*-hydrocarbons, and on computer matching against commercial (NIST 98 and ADAMS) and home-made library mass spectra built up from pure substances, components of known oils and MS literature data (Adams, 1995; Connolly & Hill, 1991; Jennings & Shibamoto, 1980; Massada, 1976; Stenhagen *et al.,* 1974; Swigar & Silverstein, 1981).

RESULTS AND DISCUSSION

Calamintha nepeta L., *Crithmum maritimum* L. *Lavandula angustifolia* Mill., *Myrtus communis* L., *Rosmarinus officinalis* L., *Salvia officinalis* L. and *Satureja hortensis* L., typical species of the Tuscan Archipelago, were selected for micropropagation with the aim to preserve the environment and to collect the germplasm of typical insular Mediterranean plants. The main type of explants used for the sterilization process were microcutting (vegetative apical portions), in some cases axillary buds or internodes. Various sterilization methods were used to select the best method for each explants. Table 1 shows the sterilization efficiency expressed as the explants'vitality after 7 days of culture. The explants of *C. maritimum* and *M. commu-*

Table 1. Vitality percentage, multiplication rate, shoot length, and root formation of micropropagated plants.
Mean values from five independent replicates ± S.D. are shown. Abbreviations represent respectively: S1, S2 and S3 (sterilization method), M1, M2 and M3 (proliferation media), R1, R2, R3 and R4 (rooting media). For details see the material and methods section.

Species	Explants	Vitality (%)	Shoot n°/explant	Shoot height (cm)	Roots (%)
Calaminta nepeta L.	internodal segments	50 (S1)	4 ± 0.5 (M1)	2.80 ± 0.43	100 (R1)
Crithmum maritimum L.	basal shoots	100 (S2)	3 ± 0.3 (M3)	2.72 ± 0.33	40 (R3)
Lavandula angustifolia Mill.	internodal segments	80 (S2)	3 ± 0.4 (M1)	1.84 ± 0.49	40 (R1)
Myrtus communis L.	internodal segments	100 (S2)	3 ± 0.5 (M1)	3.27 ± 0.51	79 (R2)
Rosmarinus officinalis L.	apical portions	80 (S3)	1 ± 0.1 (M2)	1.50 ± 0.50	0 (R1)
Salvia officinalis L.	internodal segments	10 (S2)	2 ± 0.3 (M1)	4.29 ± 0.41	100 (R4)
Satureja hortensis L.	apical portions	80 (S3)	1 ± 0.2 (M1)	2.90 ± 1.17	5 (R4)

nis gave the best results of sterilization, reaching 100% vitality, not contaminated, as indicated in the literature (Grigoriadou & Maloupa, 2008; Ruffoni & Mascarello, 2009). A good percentage of success, 80%, was also observed for apical portions of *R. officinalis* and *S. hortensis* as well as for internodes of *L. angustifolia*. The method S3 included a pre-treatment with sucrose for 15 days, already successfully demonstrated in other Mediterranean explants (Ruffoni & Mascarello, 2009). A lower percentage of vitality was observed for explants of *C. nepeta* (50%) and for *S. officinalis*, as known from the literature (Avato *et al.,* 2005). The best explants were propagated using different media, chosen for simplicity of use, and subcultured every 4 weeks. The multiplication of shoots and their length were evaluated at the end of the cycle of subculture (Table 1). M1 medium, with addition of only BA as growth regulator, exhibited good results for *M. communis, C. nepeta, L. angustifolia* and *S. officinalis*; this confirms that proliferation can be achieved also with a minimum addition of growth regulators, as often reported in the literature for some species (Scarpa *et al.*, 2000; Avato *et al.,* 2005; Ruffoni & Mascarello, 2009). However, for *C. maritimum* the highest number of shoots was obtained adding both auxin (NAA) and cytokinin (BA) to the MS0 medium (Grigoriadou & Maloupa, 2008). *S. hortensis* produced modest results of propagation when apical portions were used as explants, confirming the literature data for *S. obovata* (Arrebola, 1997). *R. officina-*

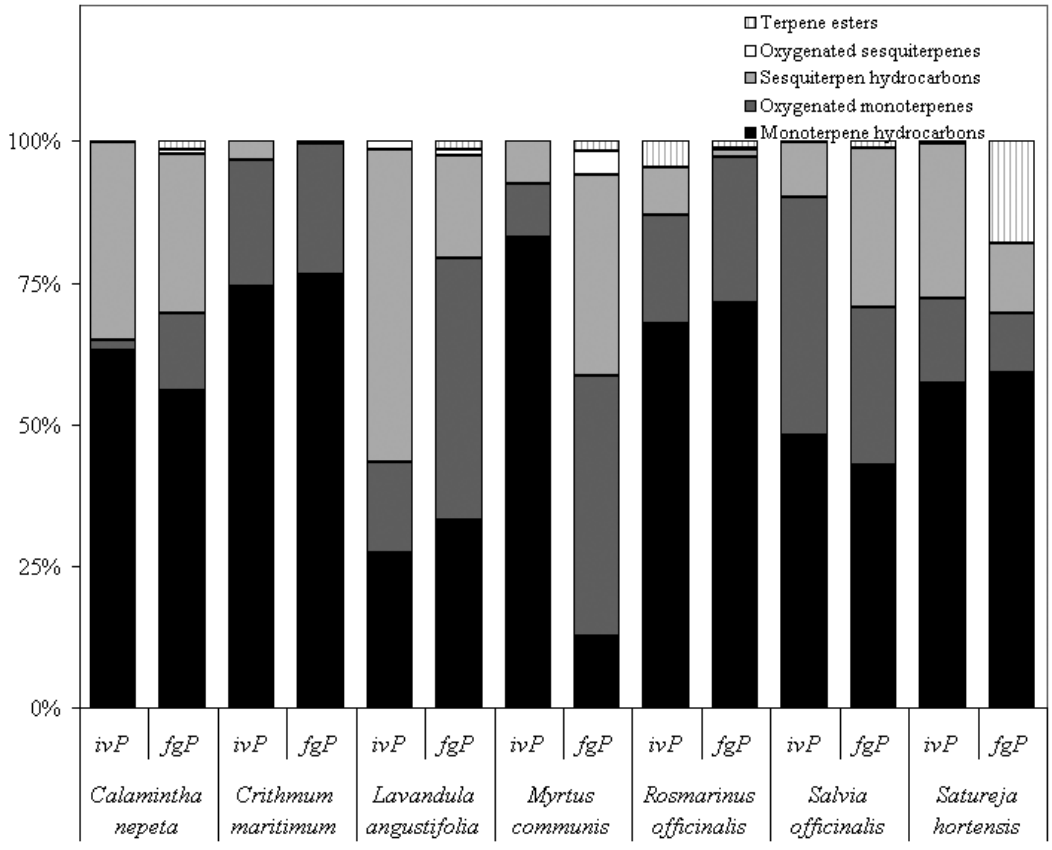

Figure 1. Terpene composition of the headspace of plants grown in the field (fgP) and *in vitro* (ivP).

Table 2. Main constituents identified in HS-SPME of examinated species

Compound			*C. nepeta*		*C. maritimum*		*L. angustifolia*		*M. communis*		*R. officinalis*		*S. officinalis*		*S. hortensis*	
		I*RI*	% ivP	% fgP	% ivP	% fgP	% ivP	% fgP	% ivP	% fgP	% ivP	% fgP	% ivP	% fgP	% ivP	% fgP
α-thujene	MH	932			1.2	0.3		0.4			0.3	0.9	0.3	0.8	1.5	1.4
tricyclene	MH	938							65.0							
α-pinene	MH	940	1.8	6.3	10.3	11.9	1.4	4.6	4.2	4.8	24.7	34.4	14.6	5.3	1.5	3.0
camphene	MH	955	1.8		0.4		0.6	1.6			13.4	19.7	8.5	1.9	0.1	3.3
β-thujene	MH	971			1.0											
sabinene	MH	978	1.2	1.4	15.0	1.5	0.5	0.4							0.2	0.2
β-pinene	MH	981	42.5	8.4			2.4	2.3			9.6	10.5	19.4	10.1	1.0	1.8
myrcene	MH	993		2.1	1.7	0.9	1.6	1.9			10.8	3.2	1.4	11.0	1.0	1.7
α-phellandrene	MH	1006					0.7	1.4			0.1				0.3	
δ-3-carene	MH	1012		0.3			6.9	11.3							0.1	
α-terpinene	MH	1019			0.3	0.4					0.4		0.1	0.3	3.4	2.5
o-cymene	MH	1026									0.5		0.1	1.1		
p-cymene	MH	1028			9.6	10.1	1.0	0.6		2.1		0.6			15.6	30.3
β-phellandrene	MH	1031					6.2									
limonene	MH	1032	15.0	34.8						2.5	4.0		2.3	4.3	1.2	
1,8-cineol	MH	1036					11.9	22.2		32.5	10.7	19.2	7.2	5.8		1.2
(*Z*)-β-ocimene	MH	1042			0.6			5.3						4.4		
(*E*)-β-ocimene	MH	1053						0.4						0.4		
γ-terpinene	MH	1062		0.4	30.9	49.3	0.4	0.1	2.9		1.5	0.2	0.4	1.1	29.0	11.7
p-mentha-3,8-diene	MH	1072							2.0							
cis-sabinene hydrate	MH	1072						0.5				0.2				
p-cymenene	MH	1080													0.4	0.8
isoterpinolene	MH	1086					0.5	0.8								
terpinolene	MH	1090	0.1	0.3	1.3		1.4	0.7			1.7	0.3	0.9	0.6		
linalool	MO	1102										0.5		0.4		0.5
nonanal	ALD	1104								1.7						
α-thujone	MO	1109											20.7	9.2		0.4
β-thujone	MO	1120											4.5	3.1		
cis-limonene oxide	MO	1137	0.2	0.4												
neo-alloocimene	MH	1142												0.6		
camphor	MO	1148						21.5			7.9	1.4	8.8	6.9		0.3
menthone	MO	1154	0.5	0.4												
menthofuran	MO	1164	0.5	0.7												
trans-pinocamphone	MO	1165											0.4			
borneol	MO	1169					1.8	1.1		0.9		3.5		1.4		2.0
cis-pinocamphone	MO	1177										0.3	0.2	0.3		
myrtenal	MO	1184		1.8												
α-terpineol	MO	1192					0.2	0.2		0.9		0.2				
1-dodecene	ALK	1192					1.8									
decanal	ALD	1206								1.4						
verbenone	MO	1214						0.2			0.3	0.1				
methyl thymol	MO	1235			0.4	0.3			7.2						0.2	11.9
pulegone	MO	1237		0.5												
methyl carvacrol	MO	1244			21.1	22.0			1.2						0.3	4.1
piperitone oxide	MO	1257		4.9												

bornyl acetate	TE	1287						1.2			4.5	1.2	0.2	0.8		1.4
thymol	MO	1293														5.5
lavandulyl acetate	TE	1289						0.2								
n-tridecane	ALK	1300					2.0									
carvacrol	MO	1301	0.5												14.4	
myrtenyl acetate	TE	1327								1.3						
δ-elemene	SH	1340												0.4		
piperitenone	MO	1342		0.4												
piperitenone oxide	MO	1363		4.1												
cyclosativene	SH	1371												0.7		
α-copaene	SH	1376	0.1	0.7										0.6		
β-bourbonene	SH	1383		1.1												
β-cubebene	SH	1390	0.9	0.3												
7-*epi*-sesquithujene	SH	1391					0.9									
β-elemene	SH	1392		0.5				0.3		6.9						
n-tetradecane	ALK	1400					0.5									
isocaryophyllene	SH	1406					0.4								0.2	
α-cedrene	SH	1409	0.6													
α-gurjunene	SH	1410					2.1	3.0						0.3		
caryophyllene	SH	1418	2.7	10.3		0.1	19.3	6.5	6.8	8.1	5.0	1.1	1.5	3.3	20.0	5.0
β-gurjunene	SH	1428	1.8	0.5			0.3							0.2		
trans-α-bergamotene	SH	1437		0.5	0.6		3.3								0.1	
cis-muurola-3,5-diene	SH	1448	13.9				0.9	0.7								
aromadendrene	SH	1445		0.4			0.3							2.0		
α-himachalene	SH	1453	0.2	0.4												
α-humulene	SH	1456		0.7			0.8	0.2		3.0	3.5	0.1	8.1	11.3	1.1	0.6
(*E*)-β-farnesene	SH	1460					1.7									
cis-muurola-4(14),5--diene	SH	1463	9.1				1.1	0.7								
γ-muurolene	SH	1477												1.4		
germacrene D	SH	1481	2.0	10.7	0.7	0.3	8.5	2.1						2.0		2.2
β-selinene	SH	1485	1.3				0.4			3.8						
α-zingiberene	SH	1495			0.6											
bicyclogermacrene	SH	1495	0.1				1.1	0.1						3.2	1.0	0.5
α-selinene	SH	1497								4.7						
n-pentadecane	ALK	1500					1.9									
(*E*,*E*)-α-farnesene	SH	1508					0.8									
β-bisabolene	SH	1509	0.1	0.5	0.3										4.0	3.5
trans-γ-cadinene	SH	1513	0.2				3.8	3.8						0.4		
trans-calamenene	SH	1522	1.1					0.1								
δ-cadinene	SH	1523		0.3			1.5							1.0		
β-sesquiphellandrene	SH	1524			0.5											
caryophyllene oxide	SO	1582						0.5		3.2		0.3				
1,10-*di*-*epi*-cubenol	SO	1614	0.2	0.4			0.4									
germacrene B	SH	1556			0.4	0.1										
diilapiol	PP	1623			0.6	2.3			3.4							
epi-α-cadinol	SO	1642					0.9	0.5								
pentyl decanoate	AE									13.6						
α-bisabolol	SO	1684		0.4												

n-heptadecane	ALK	1700					0.6			0.9						
n-octadecane	ALK	1800								0.9						
Monoterpene hydrocarbons	MH		62.4	54.0	72.3	74.4	23.6	32.8	74.1	9.4	67.2	70.0	48.0	41.9	55.3	56.7
Oxygenated monoterpenes	MO		1.7	13.2	21.5	22.3	13.9	45.5	8.4	34.3	18.9	25.4	41.8	27.3	14.4	9.9
Sesquiterpen hydrocarbons	SH		34.6	26.9	3.3	0.5	47.6	18	6.8	26.5	8.5	1.2	9.6	27.7	26.4	11.8
Oxygenated sesquiterpenes	SO		0.2	0.8			1.3	1.0		3.2		0.3				
Terpene esters	TE			1.5				1.5		1.3	4.5	1.2	0.2	1.2	0.5	17.4
Phenylpropanoids	PP				0.6	2.3			3.4							
Alkanes	ALK						7.4			1.8						0.2
Adeydes	ALD						0.2			3.1						0.5
Acids/esters	AE			0.3						13.6						
Total			98.4	94.9	97.5	99.5	92.8	97.4	92.7	93.2	98.9	97.9	99.6	96.6	96.6	95.8

I*RI*, Linear retention index. The components are listed in order of their elution on the DB-5 column. IvP, *in vitro* plants; fgP, field-grown plants. MH, monoterpene hydrocarbons; MO, oxygenated monoterpenes; SH, sesquiterpene hydrocarbons; SO, oxygenated sesquiterpenes; TE, terpene esters; ALD, aldehydes; ALK, alkanes/alkenes; PP, phenyl propanoids; AE, esters. Compounds present at less than 0.1% were excluded from the table and from analysis.

lis exhibited the lowest number of shoot proliferation even in a specific medium (M2), and no root formation was observed (R1 medium), confirming the refractivity of this species to proliferation (Misra & Chaturvedi, 1984). The shoot length is also shown in Table 1, and the varied growth ability of the explants confirmed the data already known from the literature. Notably, we devised a good method for the *in vitro* propagation of *C. nepeta*, not considered for micropropagation until now (Misra & Chaturvedi, 1984, Arrebola, 1997; Avato *et al.,* 2005; Grigoriadou & Maloupa, 2008). Root formation was then induced in order to produce aseptically whole plants to be easily transferred in diverse environments. Best results were obtained with *C. nepeta* and *S. officinalis*, whereas the percentage of produced roots was lower for other species.

The volatiles emitted from the analysed species and identified by GC-MS are reported in Table 2. In total, 71 and 78 compounds were identified in micropropagated (ivP) and field-grown (fgP) plants. The headspace analyses of these two growth conditions accounted for 92.7–99.6% and 93.2–99.5% of the total compositions, respectively. The volatile fractions were characterized mainly by hydrocarbons and oxygenated monoterpenes together with sesquiterpene hydrocarbons (Fig. 1). The SPME analysis of the two samples of *C. nepeta* showed a similar composition in monoterpenes (64.1 and 67.2% in ivP and fgP, respectively) and sesquiterpenes (34.1 and 26.9% in ivP and fgP, respectively), even if fgP exhibited higher percentages of oxygenated monoterpenes (13.2%) than ivP (1.7%). In fgP typical compounds of *Calamintha* spp. were detected, as limonene (34.8%), piperitone oxide and piperitenone oxide (4.9 and 4.1%, respectively), together with low amounts of pulegone and menthone, (0.5 and 0.4%, respectively), in accordance with literature data (De Pooter *et al.,* 1986). In contrast, ivP showed the presence of *cis*-muurola 3,5-diene (13.9%) and cis-muurola-4(14),5-diene (9.1%), not detected in fgP, and a high percentage of β-pinene (42.5%, against 8.4% in fgP). The headspaces of *C. maritimum* samples showed a similar composition, characterised by monoterpene hydrocarbons (72.3% in ivP and 74.4% in fgP) and oxygenated monoterpenes (21.5% in ivP and 22.3% in fgP), differing only by the percentages of sabinene (15.0% in ivP and 1.5% in fgP) and γ-terpinene (30.9% in ivP and 49.3 in fgP). The identified constituents are in agreement also with the composition of the essential oils reported in the literature (Özcan *et al.,* 2006), even if many reports on the essential oil composition of sea fennel grown in different parts of Mediterranean area showed differences in chemical constituents, suggesting different chemotypes of this species (Kulisic-Bilusic *et al.,* 2010). The two *L. angustifolia* samples showed qualitative differences in the composition of the volatiles emitted: the ivP headspace was characterised especially by sesquiterpene hydrocarbons (47.2%), while fgP exhibited high percentages of oxygenated and hydrocarbon monoterpenes (45.2% and 32.3%, respectively). 1,8-cineol, the compound that gives the particular flavour of the lavender fields, was detected in both samples (11.9% in ivP and 22.2% in fgP), while camphor was identified only in the headspace of fgP (21.5%). These results represent new data regarding the head space composition of the vegetative aerial part of *L. angustifolia*, since earlier reports focused on the flower aroma and/or the reproductive aerial parts (Kim *et al.,* 2002, Da Porto & Decorti 2008). SPME analyses of micropropagated *L. viridis* plants and field-grown mother-plant exhibited a different composition of the emitted volatiles (Gonçalves *et al.*, 2008). The headspace analysis obtained from field-grown *M. communis* showed a similar composition with the literature data (Flamini *et al.,* 2004), with the presence of 1,8-cineol (32.5%), α-pinene (4.8%), limonene (2.5%) and mirtenyl acetate (1.3%). In contrast, in the SPME analysis of ivP, tricyclene was revealed as the main constituent (65.0%), together with methyl thymol (7.2%), showing a very different composition from the field-grown plant. The SPME analysis of *R. officinalis* samples showed the presence of similar percentages of monoterpene hydrocarbons (67.0% in ivP and 70.0% in fgP), but quantitative differences in the percentages of oxygenated monoterpenes (18.9% in ivP and 25.2% in fgP) and of sesquiterpene hydrocarbons (8.5% in ivP and 1.2% in fgP). α-pinene (24.7% and 34.4% in ivP and fgP, respectively). 1,8-cineol (10.7% in ivP and 19.2% in fgP) and camphor (7.9% in ivP and 1.4% in fgP) represent the most important constituents of the essential

oil and aroma of *R. officinalis* (Katerinopoulos *et al.,* 2005; Zawirska-Wojtasiak & Wasowicz, 2009). The main compounds identified in the headspace of both samples of *S. officinalis* were α-thujone (20.7% and 9.2% in ivP and fgP, respectively), β-pinene (19.4% in ivP and 10.1% in fgP) and α-pinene (14.6% and 5.3% in ivP and fgP, respectively), together with camphor (8.8% in ivP and 6.9% in fgP) and α-humulene (8.1% in ivP and 11.3% in fgP), in agreement with the quali-quantitative composition reported for *S. officinalis* essential oil (Longaray Delamare *et al*., 2007; Santos-Gomes *et al.,* 2001). Despite the presence of compounds characteristic for *S. officinalis* in both analysed samples, the micropropagated plants showed higher content of oxygenated monoterpenes (41.8%) then the field-grown plants (27.1%), while the sesquiterpene hydrocarbons were present especially in the field-grown samples (26.8% and 9.6% in ivP and fgP, respectively). The headspace of the *S. hortensis* two samples was characterised mainly by γ-terpinene (29.0% in ivP and 11.7% in fgP), *p*-cymene (15.6% in ivP and 30.3% in fgP), carvacrol (14.4%, identified only in ivP) and methylthymol and thymol (11.9 and 5.5%, respectively, the both detected only in the field-grown plants), characteristic constituents of the essential oil from aerial parts. γ-terpinene and *p*-cymene are biogenetic precursors (*via* enzymatic hydroxylation) of the phenolic terpenes thymol and carvacrol, so there is a correlation between these compounds and their percentages are affected by harvesting time and location (Azaz *et al.,* 2005; Sefidkon *et al.,* 2006; Güllüce *et al.,* 2003; Abu-Lafi *et al.,* 2008).

Many VOCs produced by officinal and medicinal plants are commercialized as flavors and/or fragrances, and their use in the food and perfume industries has a long tradition. This work showed that the headspace composition of micropropagated plantlets of *C. maritimum*, *R. officinalis*, *S. officinalis* and *S. hortensis* is very similar to the corresponding field-grown plants, containing the same main constituents, although further investigations are requested for *C. nepeta*, *L. angustifolia* and *M. communis* in order to obtain *in vitro* plant material with aromatic profile more similar to the wild plants. These results indicated that micropropagation can represent a valid alternative to produce rapidly large amounts of plant material characterised by the same aromatic flavor as the wild grown plants. Other advantages of this technique are the protection of the natural ecosystem avoiding the damage of endemic plants. The emission of VOC by the micropropagated plants can represent a good market actractant for baby plants. Customers may use them as spices or as steril ornamental plants to be transported everywhere.

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