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Research Paper

Morphological and biochemical intraspecific characterization of *Ocimum basilicum* L.

Filip Varga[a], Klaudija Carović-Stanko[a, b, *], Mihailo Ristić[c], Martina Grdiša[a, b], Zlatko Liber[b, d], Zlatko Šatović[a, b]

[a] University of Zagreb, Faculty of Agriculture, Department of Seed Science and Technology, Svetošimunska cesta 25, 10000 Zagreb, Croatia
[b] Centre of Excellence for Biodiversity and Molecular Plant Breeding, Svetošimunska cesta 25, HR-10000 Zagreb, Croatia
[c] Institute for Medicinal Plant Research "Dr Josif Pančić", Tadeuša Košćuška 1, 11000 Belgrade, Serbia
[d] University of Zagreb, Faculty of Science, Division of Biology, Department of Botany, Marulićev trg 9a, 10000 Zagreb, Croatia

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ABSTRACT

Ocimum basilicum L. exhibits a great variety of cultivars grown for various purposes. The aim of this research was to analyse the chemical composition of 85 *O. basilicum* accessions cultivated throughout the world using gas chromatography (GC/FID and GC/MS). All accessions were previously categorized into six morphotypes based on 24 morphological traits: True basil, Small-leaf, Lettuce-leaf, Purple basil A, Purple basil B and Purple basil C morphotype. A total of 77 volatiles were observed, with seven of them being in concentrations higher than 10% in at least one accession (1,8-cineole, linalool, linalool acetate, methyl chavicol, eugenol, *trans*-methyl cinnamate, *trans*-α-bergamotene). Based on the essential oil composition of 85 *O. basilicum* accessions, we propose the intraspecific characterization of *O. basilicum* into five chemotypes: (A) High-linalool, (B) Linalool/*trans*-α-bergamotene, (C) Linalool/methyl chavicol, (D) Linalool/*trans*-methyl cinnamate and (E) High-methyl chavicol chemotype. Groups based on morphological and biochemical traits do not necessarily coincide with one another. Due to great morphological and biochemical diversity, both morphological and chemical characterizations are crucial for the effective management of germplasm collections to facilitate the use of the accessions in future breeding programs.

1. Introduction

Ocimum basilicum L. is a widely known member of Lamiaceae family. At present, this annual aromatic plant, native to Southeast Asia, is globally cultivated and has significant economic value. The plants are ornamental, the leaves are used in cooking, and its essential oil is often an ingredient used in the personal care, and household cleaning products industry (Putievsky and Galambosi, 1999). Basil essential oil has been recognized as an antioxidant (Politeo et al., 2007) and local anaesthetic, and some of the compounds found in it are antibacterial, fungistatic and insecticidal (Carovic-Stanko et al., 2010). There is also a long tradition of using basil as a medicinal plant in treating coughs, diarrhoea, worm infestations and kidney malfunctions (Holm, 1999). Recent studies even suggest that basil oil displays great potential as a stress repressor (Nakamura et al., 2009), and it is also used as a component in drugs for leukaemia treatment (Moteki et al., 2002). A long tradition of cultivation for different market requirements led to great intraspecific variation at both a morphological and a biochemical level. A long tradition of cultivation for different market requirements led to great intraspecific variation at both a morphological and a biochemical level.

Darrah (1980) offered classification of basil cultivars into seven types based on morphology: tall slender type, large-leafed robust type, dwarf type, compact type, purpurascens type, purple type and citriodorum type. Differences among basil cultivars can be observed through leaf size and colour (from green to dark purple), flower colour (white, red, lavender or purple), shape and height of plants as well as flowering time and aroma (Morales et al., 1993). For identification of cultivars, a standardized descriptor list based on morphological traits was developed by the International Union for the Protection of New Varieties of Plants (UPOV). This has led to the recent characterization of *O. basilicum* into six distinct morphotypes: Lettuce-leaf, Small-leaf, True basil, Purple basil (A), Purple basil (B) and Purple basil (C) (Carovic-Stanko et al., 2011b).

The chemical composition of the essential oils in basil has been the subject of many studies since the 1930s (Carovic-Stanko et al., 2011a; De Masi et al., 2006; Kwee and Niemeyer, 2011; Labra et al., 2004;

* Corresponding author at: University of Zagreb, Faculty of Agriculture, Department of Seed Science and Technology, Svetošimunska cesta 25, 10000 Zagreb, Croatia.
Email address: kcarovic@agr.hr (K. Carović-Stanko)

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Wesolowska et al., 2012). As analytical techniques developed throughout the years, the number of successfully identified compounds increased, and today over 140 compounds found in basil oil are known. The ratio of these compounds determines the physical properties of the oil. Monoterpenes and phenylpropanoids are the prevalent groups present in the essential oils of *O. basilicum.* Morphologically similar accessions could be differentiated based on the presence or high concentration of specific compounds (Hiltunen and Holm, 1999). Based solely on chemical composition, many characterizations have been proposed (Bernhardt et al., 2014; da Costa et al., 2015; Grayer et al., 1996; Marotti et al., 1996; Telci et al., 2006). These characterizations are based on the prevalence of one or more chemical compounds in the oil above a fixed threshold. The majority of researchers take into account compounds present in concentrations higher than 10% (Carovic-Stanko et al., 2010; Liber et al., 2011) or 20% of the essential oil (Grayer et al., 1996; Labra et al., 2004). Different chemotypes have also been classified on the basis of their geographical origin, each with a specific chemical composition. The European chemotype (Italy, France, Bulgaria) is characterized by linalool and methyl chavicol (estragole), as its main compounds. The Tropical chemotype is rich in *trans*-methyl cinnamate and originates from India, Guatemala and Pakistan. The Reunion chemotype from Thailand, Madagascar and Vietnam has high concentrations of methyl chavicol, while the chemotype from Russia and North Africa is eugenol-rich (Vernin and Metzger, 1984).

A large proportion of similar studies have concentrated on cultivars grown nationally (Beatovic et al., 2015; Dambolena et al., 2010; Hadipanah et al., 2015; Marotti et al., 1996; Telci et al., 2006; Zheljazkov et al., 2008), or on the elucidation of the taxonomic position of *O. basilicum* L., within the genus Ocimum (Carovic-Stanko et al., 2011a; Trevisan et al., 2006; Vieira and Simon, 2006). In our research, we included some of the most widely grown cultivars and varieties (*O. basilicum* Genovese, *O. basilicum* Sweet Basil, *O. basilicum* var. *difforme*, *O. basilicum* var. *purpurascens*) as well as other, less-known types, which are cultivated throughout the world. Our goal was to determine if there is a total correspondence between certain morphotypes and chemotypes. This information could be utilized as a screening method for determining the chemical composition of certain genotypes based on the morphological traits they exhibit. The aims of this study were: (1) to examine the biochemical diversity of *O. basilicum,* based on the chemical properties of essential oils, (2) to propose intraspecific characterization into chemotypes, and (3) to compare characterizations based on morphological and biochemical traits.

2. Materials and methods

2.1. Plant material

This research was carried out on 85 accessions of *O. basilicum* (Table 1). Seed material was obtained from the Collection of Medicinal and Aromatic Plants of the Department of Seed Science and Technology, Faculty of Agriculture, University of Zagreb, Croatia (http://cpgrd.hcphs.hr). Accessions were grown in a greenhouse in sterilized soil mix, and the seedlings were transplanted in the field. The samples were collected from five plants per accession during the flowering phase. Characterization of accessions into six morphoytpes was previously conducted based on 24 morphological traits. The typical green accessions, including cultivars 'Genovese' and 'Sweet basil', were grouped together in True basil morphotype. Small-leaf basil morphotype was composed of accessions with a rounded plant habit and a short plant height. Lettuce-leaf basil morphotype was composed of accessions, which were discriminated by the shape, width and blistering of the leaf blade. Purple basil A morphotype, grouped accessions displaying anthocyanin coloration on stems, but having green leaves, while Purple basil B morphotype included purple-leaf accessions, 'Dark Opal' being the most typical cultivar. Purple basil C morphotype was composed of a single accession of cultivar 'Purple Ruffles' (Carovic-Stanko et al., 2011b,c).

2.2. Extraction of essential oil and oil compounds identification

A single essential oil sample was isolated from the leaves of five plants per accession by hydro-distillation, for three hours in a Clevenger-type apparatus. Analytical gas chromatography (GC/FID) analysis of essential oils was carried out on an Agilent Technologies, model 7890A gas chromatograph (Agilent Technologies Co. Ltd., Shanghai Branch Company, Shanghai, China), equipped with a split-splitless injector and an automatic liquid sampler (ALS), attached to an HP-5 silica capillary column (30 m × 0.32 mm, 0.25 μm film thickness), and fitted to a flame ionization detector (FID). Carrier gas flow rate (H_2) was 1 ml/min at 210 °C (constant pressure mode), injector temperature was 250 °C, detector temperature 260 °C, while column temperature was linearly programmed from 60 to 240 °C (at rate of 4°/min), and held isothermally at 260 °C for the next 10 min. Solutions of essential oils in ethanol (~1%) were consecutively injected by ALS (1 μl, split mode, 1:30). Area percent reports, obtained as a result of the standard processing of chromatograms, were used as a basis for the quantification purposes.

The same chromatographic analytical conditions, as those mentioned for GC/FID, were employed for gas chromatography/mass spectrometry (GC/MS) analysis, along with capillary column HP-5MS (30 m × 0.25 mm, 0.25 μm film thickness), using HP G 1800C GCD Series II Electron Ionization Detector (EID) system (Hewlett-Packard, Palo Alto, CA, USA). Instead of hydrogen, helium was used as a carrier gas. The transfer line was heated at 240 °C. Mass spectra were acquired in EI mode (70 eV) in *m/z* range 40–400. Sample solutions in ethanol (~1%) were injected by ALS (1 μl, split mode: 1:30). The compounds were identified by comparing their mass spectra to those from Wiley275 and NIST/NBS libraries, using different search engines. Probability Merge Search (PBM) was included in instruments G1701DA. ver. D.00.00.38 data station software and NIST 2.0 search program. The experimental values for retention indices were determined by the use of calibrated Automated Mass Spectral Deconvolution and Identification System software (AMDIS ver.2.64), compared to those from the available literature (Adams, 2007), and used as an additional tool to approve MS findings. Compound contents were expressed as percentage of said compounds in total oil samples.

2.3. Data analysis

Correlations among the seven major compounds were calculated by the PROC CORR procedure in SAS 9.3 (SAS Institute, Cary NC). Principal Components Analysis (PCA) was performed using the PROC PRINCOMP procedure. The pairwise Euclidean distances between accessions were calculated, and the distance matrix was used in the Cluster Analysis (CA). The average linkage method (i.e., UPGMA) of PROC CLUSTER was applied, and the accessions were classified in groups representing distinct chemotypes. The optimal number of clusters was assessed by calculating Pseudo F (PSF) statistics.

3. Results and discussion

Using GC/FID and GC/MS, a total of 77 volatiles were identified and quantified as essential oil compounds in 85 *O. basilicum* L. accessions (Table 1).

The highest number of identified compounds was found in the essential oils of accessions S14 and S54 (71 compounds), while the lowest numbers were found in accessions S46 and S55 (47 compounds).

Table 1
Accession number, taxon, country of origin and morphotype of *O. basilicum* accessions included in the analysis.

No.	Accession no.[a]	Taxon/cultivar	Country of origin	Morphotype	Chemotype (A–E)[b]
S01	MAP02297	'Albahaca Grande Verde'	Spain	True basil	A
S02	MAP02291	–	Azerbaijan	True basil	A
S03	MAP02293	'Bavires'	Germany	True basil	A
S04	MAP02328	Genovese Basil – Compatto FT	Canada	True basil	A
S05	MAP01645	'Envigor'	Canada	True basil	A
S06	MAP02336	Gecofure Basil	Canada	True basil	A
S07	MAP00294	Genovese Basil	Croatia	True basil	A
S08	MAP00558	Genovese Basil	Italy	True basil	A
S09	MAP00331	Genovese Basil	Macedonia	True basil	A
S10	MAP00391	Genovese Basil	Croatia	True basil	A
S11	MAP00298	Genovese Basil	Italy	True basil	A
S12	MAP00005	Genovese Basil	Slovakia	True basil	A
S13	MAP02331	Genovese Basil – 'Superbo'	Canada	True basil	A
S14	MAP00145	Genoveser	Austria	True basil	A
S15	MAP02301	Genoveser grossblättrig	Germany	True basil	A
S16	MAP02303	'Gigante'	Germany	True basil	A
S17	MAP02282	'Grand Vert de Genes'	Germany	True basil	A
S18	MAP01642	'Green Gate'	Canada	True basil	A
S19	MAP00576	Grosses gruenes	Austria	True basil	A
S20	MAP02290	Italian Large-Leafed	Italy	True basil	C
S21	MAP02287	–	Italy	True basil	A
S22	MAP02295	–	Japan	True basil	A
S23	MAP02315	Japan A	Germany	True basil	B
S24	MAP02320	–	Madagascar	True basil	A
S25	MAP02314	Mittelgrossblättriges Grünes	Germany	True basil	A
S26	MAP02337	Genovese Basil – Nufar F1	Canada	True basil	C
S27	MAP02288	Genovese Basil – Nufar F1	Germany	True basil	C
S28	MAP01622	Ohre	Germany	True basil	A
S29	MAP02283	–	Russia	True basil	C
S30	MAP02338	'Stella'	Canada	True basil	A
S31	MAP00186	Sweet basil	Croatia	True basil	A
S32	MAP00232	Sweet basil	Croatia	True basil	A
S33	MAP00414	Sweet basil	USA	True basil	C
S34	MAP02296	–	Togo	True basil	A
S35	MAP01640	Bush Basil	Canada	Small-leaf basil	B
S36	MAP01632	Comune a Foglia Piccola	Germany	Small-leaf basil	A
S37	MAP00560	'Fine verde'	Italy	Small-leaf basil	B
S38	MAP02334	Greek Bush Basil	Canada	Small-leaf basil	A
S39	MAP01648	'Green Globe'	Canada	Small-leaf basil	B
S40	MAP02327	'Marseilles'	Canada	Small-leaf basil	A
S41	MAP02298	'Massilia'	Germany	Small-leaf basil	A
S42	MAP02326	'Medinette'	Canada	Small-leaf basil	A
S43	MAP02300	'Piccolo'	Italy	Small-leaf basil	A
S44	MAP01641	'Spicy Globe'	Canada	Small-leaf basil	B
S45	MAP00559	Blistered lettuce-leaf basil	Italy	Lettuce-leaf basil	C
S46	MAP02286	–	Italy	Lettuce-leaf basil	C
S47	MAP02281	Lactucaefolium	Germany	Lettuce-leaf basil	C
S48	MAP01654	var. *difforme*/Napoletano Basil	Canada	Lettuce-leaf basil	B
S49	MAP01623	var. *difforme*	Germany	Lettuce-leaf basil	C
S50	MAP00375	var. *difforme*/'A foglie di lattuga'	Italy	Lettuce-leaf basil	C
S51	MAP02307	var. *difforme*	Uzbekistan	Lettuce-leaf basil	C
S52	MAP02321	var. *purpurescens*	Jemen	Purple basil A	C
S53	MAP02333	'Magical Michael'	Canada	Purple basil A	A
S54	MAP01658	'Oriental Breeze'	Canada	Purple basil A	A
S55	MAP02285	'Petit anis blanc'	Germany	Purple basil A	E
S56	MAP02311	–	Romania	Purple basil A	A
S57	MAP02312	'Sasklavi'	Georgia	Purple basil A	D
S58	MAP02299	Tetri Rechani (Weisser Rechani)	Georgia	Purple basil A	D
S59	MAP02313	var. *purpurascens*	Uzbekistan	Purple basil A	A
S60	MAP00334	var. *purpurascens*	Russia	Purple basil A	E
S61	MAP02292	var. *purpurascens*	China	Purple basil A	C
S62	MAP02302	var. *purpurascens*	Irak	Purple basil A	D
S63	MAP00335	var. *purpurascens*/Erevanskii	Russia	Purple basil A	E
S64	MAP01629	var. *purpurascens*/Mexican Basil	Germany	Purple basil A	D
S65	MAP00146	var. *purpurascens*/no. 3193	Austria	Purple basil A	D
S66	MAP02305	var. *purpurascens*/Persian Anise-scented Basil	Germany	Purple basil A	D
S67	MAP01644	Anise Basil	Canada	Purple basil B	C
S68	MAP01639	Ararat	Canada	Purple basil B	C
S69	MAP00333	'Dark Opal'	Russia	Purple basil B	A
S70	MAP00284	'Dark Opal'	Russia	Purple basil B	A
S71	MAP00004	'Opal'	Slovakia	Purple basil B	A
S72	MAP00283	Opal-ZS98	Slovakia	Purple basil B	A
S73	MAP02308	'Licorice'	Germany	Purple basil B	A
S74	MAP01630	'Metalica'	Germany	Purple basil B	A

Table 1 (*Continued*)

No.	Accession no.[a]	Taxon/cultivar	Country of origin	Morphotype	Chemotype (A–E)[b]
S75	MAP01650	'Osmin'	Canada	Purple basil B	A
S76	MAP02330	'Purple Delight'	Canada	Purple basil B	A
S77	MAP02306	'Susambari'	Georgia	Purple basil B	B
S78	MAP02335	'Sweet Salad'	Canada	Purple basil B	C
S79	MAP01657	Thai Basil 'Queenette'	Canada	Purple basil B	D
S80	MAP02294	var. *purpurascens*	Armenia	Purple basil B	C
S81	MAP02289	var. *purpurascens*	Armenia	Purple basil B	C
S82	MAP02319	var. *purpurascens*/'Kardinal'	Germany	Purple basil B	C
S83	MAP02309	var. *purpurascens*/'Rothaut'	Germany	Purple basil B	A
S84	MAP02317	var. *thyrsiflorum*/'Siam Queen'	Thailand	Purple basil B	E
S85	MAP01643	'Purple Ruffles'	Canada	Purple basil C	B

[a] Accession number from The Collection of Medicinal and Aromatic Plants, Zagreb, Croatia available at: cpgrd.hcphs.hr.
[b] Chemotypes: (A) Linalool, (B) Linalool/*trans*-α-bergamotene, (C) Linalool/methyl chavicol, (D) Linalool/*trans*-methyl cinnamate, (E) Methyl chavicol.

Seven compounds were present in concentrations higher than 10% in at least one accession and were considered as major compounds (Fig. 1). These compounds were oxygenated monoterpenes (1,8-cineole, linalool, linalool acetate), phenylpropane derivatives (methyl chavicol, eugenol, *trans*-methyl cinnamate) and sesquiterpene hydrocarbon (*trans*-α-bergamotene). The complete chemical composition of accessions in this research is shown in Supplementary Table S1.

Linalool was the dominant constituent in most samples, ranging from 27.6% to 89.7%. Methyl chavicol was the dominant constituent in eight samples, ranging from 35% to 93.8%. In an additional 27 samples, methyl chavicol was the second or third major compound, while in the rest of the samples it was present in very low concentrations or even absent. Additionally, 1,8-cineole was present in all of the accessions with an average of 6.3%, except in accessions S56, S57 and S60, where it was found in traces (less than 0.1%). Similar to 1,8-cineole, eugenol was present in almost all accessions in an even lower concentration (an average of 1.8%). It was not found in S51, S60 and S80, while in S62 its concentration was considerably high (13.9%). *Trans*-methyl cinnamate was absent or found in small quantities in most accessions, except for nine accessions in which it was the first or second major compound, ranging from 7.3% to 36.7%. *Trans*-α-bergamotene was also present in all accessions in small quantities (average of 2.2%), except for S37 (16.4%) and S85 (15.2%). There was a substantial amount of linalool acetate in S40 (6.3%), S41 (10.8%) and S42 (6.5%), while in the rest of the accessions it was absent or in small quantities (average of 0.6%). Additionally, 35 compounds were identified in concentrations higher than 1% but lower than 10%, and a further 35 compounds were identified in concentrations lower than 1%. In each sample, the identified compounds represented more than 90% of the total oil.

There were weak to moderate correlations among the seven major compounds (Table 2), with the exception of the strong negative correlation between linalool and methyl chavicol ($r = -0.78$).

Seven major compounds were included in the Principal Components Analysis (PCA). The first three principal components explained 72.8% of the total variation and had an eigenvalue higher than 1 (2.16, 1.66 and 1.28, respectively) (Table 3).

The relative position of accessions and major compounds in the three-dimensional space defined by the first three principal components is shown in Fig. 2. The first component explained 30.9% of the

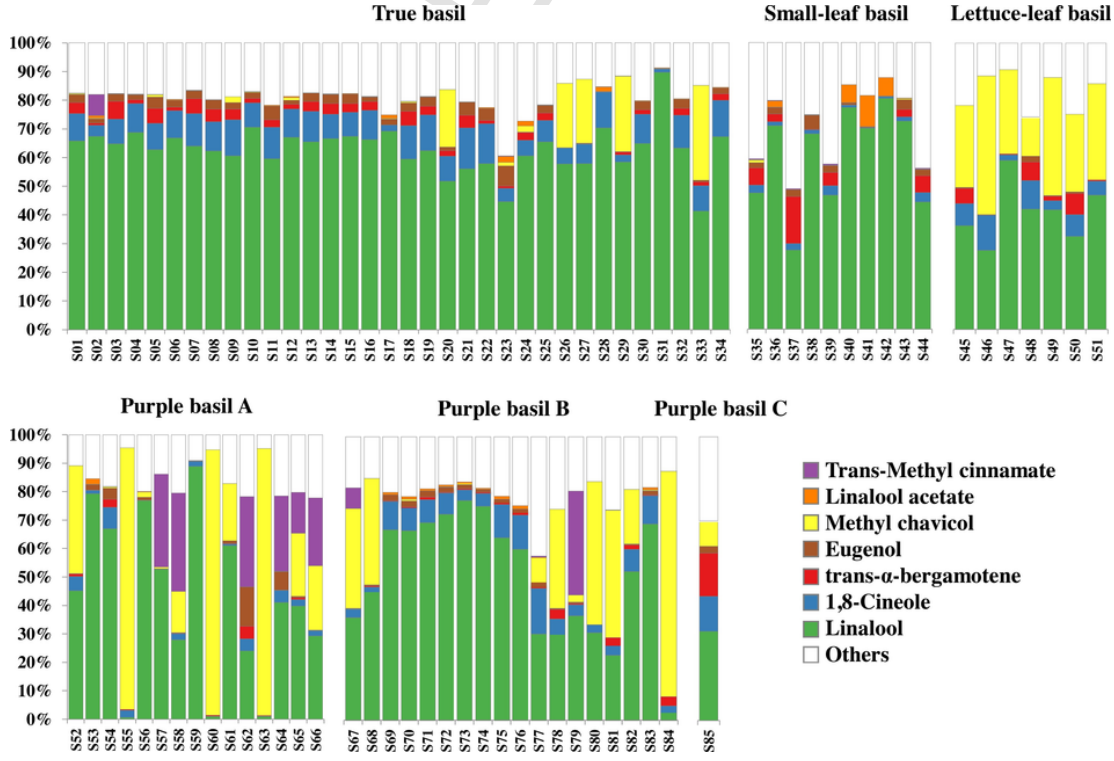

Fig. 1. Concentrations of major compounds (expressed as percentage) in *O. basilicum* accessions, grouped according to morphotype.

Table 2
Pearson correlation coefficients for the most abundant compounds of *O. basilicum* essential oil.

Major compound	1,8-Cineole	Linalool	Methyl chavicol	Linalool acetate	Eugenol	*Trans*-methyl cinnamate	*Trans*-α-bergamotene
1,8-Cineole	1.00						
Linalool	0.16^{ns}	1.00					
Methyl chavicol	−0.32**	−0.78***	1.00				
Linalool acetate	−0.23*	0.30**	-0.21^{ns}	1.00			
Eugenol	0.25*	0.09^{ns}	−0.44***	−0.10^{ns}	1.00		
Trans-methyl cinnamate	−0.26*	−0.28**	−0.07^{ns}	−0.11^{ns}	0.20^{ns}	1.00	
Trans-α-bergamotene	0.25*	−0.20^{ns}	−0.16^{ns}	−0.19^{ns}	0.26*	−0.14^{ns}	1.00

ns – non-significant.
* Significant at $P < 0.05$.
** Significant at $P < 0.01$.
*** Significant at $P < 0.001$.

Table 3
Pearson correlation coefficients between seven major compounds of *O. basilicum,* essential oils and scores of the first three principal components.

Major compounds	Principal components					
	PC1	*r*	PC2	*r*	PC3	*r*
1,8-Cineole	0.52	***	0.48	***	−0.38	***
Linalool	0.80	***	−0.48	***	−0.07	ns
Methyl chavicol	−0.93	***	0.07	ns	−0.25	*
Linalool acetate	0.21	*	−0.70	***	0.05	ns
Eugenol	0.51	***	0.48	***	0.47	***
Trans-methyl cinnamate	−0.21	*	0.12	ns	0.90	***
Trans-α-bergamotene	0.20	ns	0.67	***	−0.19	ns
Eigenvalue	2.16		1.66		1.28	
% of variance	30.92		23.69		18.23	
Cumulative%	30.92		54.61		72.84	

ns – non-significant.

* Significant at $P < 0.05$.
*** Significant at $P < 0.001$.

variation and was bipolar with linalool ($r = 0.8$), 1,8-cineole ($r = 0.52$) and eugenol ($r = 0.51$), contrasted with methyl chavicol ($r = -0.93$). PC2 accounted for 23.7% of the variation with *trans*-α-bergamotene ($r = 0.67$), 1,8-cineole ($r = 0.48$) and eugenol ($r = 0.48$), contrasted with linalool acetate ($r = -0.70$) and linalool ($r = -0.48$), as major compounds. PC3 accounted for 18.2% of the variation, and consisted of *trans*-methyl cinnamate ($r = 0.9$) and eugenol ($r = 0.47$), contrasted with 1,8-cineole ($r = -0.38$). A strong negative correlation between linalool and methyl chavicol was observed ($r = -0.78$).

Cluster analysis grouped accessions into five clusters (Fig. 3). Forty-seven accessions belonged to Chemotype A, characterized by a high content of linalool, which varied from 56% to 89.7% with a mean value of 68.3%. And, 1,8-cineole (0.1–14.3%) was the other important component of this chemotype, comprised largely of True basil morphotype accessions and a number of Small-leaf basil morphotype accessions. Several authors consider this chemotype, as a typical European sweet basil chemotype (da Costa et al., 2015; Vieira and Simon, 2006; Zheljazkov et al., 2008).

The main component of Chemotype B (S23, S35, S37, S39, S44, S48, S77 and S85) was linalool, ranging from 27.6% to 47.5% with a mean value of 39.4%. Furthermore, this chemotype was characterized by high *trans*-α-bergamotene content, ranging from 0.6% to 16.4% and methyl chavicol content, ranging from absent to 13.4%. *Trans*-α-bergamotene was previously reported in some papers at lower concentrations (Bernhardt et al., 2014; Grayer et al., 1996; Liber et al., 2011; Marotti et al., 1996) and was considered as a main constituent in a chemotype, in only a few cases (da Costa et al., 2015; Zheljazkov et al., 2008). Half of the accessions in this chemotype (S35, S37, S39 and S44) were Small-leaf basil morphotype accessions. 'Purple ruffles', a single accession of the Purple basil C morphotype belongs to this chemotype.

Linalool was also the main component of Chemotype C (S20, S26, S27, S29, S33, S45, S46, S47, S49, S50, S51, S52, S61, S67, S68, S78, S80, S81 and S82). This chemotype is characterized by both higher methyl chavicol content, ranging from 19.3 to 50.4% and lower *trans*-α-bergamotene content (0–7.4%) than Chemotype B. Eugenol content in this chemotype was lower (0–1.3%) than in Chemotype B (1.7–7.1%). Chemotype C matches the European chemotype (Vernin and Metzger, 1984), and this is well known in the literature (da Costa et al., 2015; Liber et al., 2011; Marotti et al., 1996). All but one accession (S48) of Lettuce-leaf basil morphotype belonged to this chemotype.

The two main compounds in Chemotype D (S57, S58, S62, S64, S65, S66 and S79) were linalool (24.1%–52.7%) and *trans*-methyl cinnamate (14.4%–36.7%). This chemotype was also characterized by the notable presence of eugenol in accessions S62 and S64 (13.9% and 6.6%), and methyl chavicol in accessions S58, S65 and S66 (14.5%, 22.2% and 22.6%). In addition to an accession of Purple basil B morphotype, this chemotype consisted exclusively of accessions belonging to Purple basil A morphotype. This chemotype, predominantly consisting of *trans*-methyl cinnamate, was described as Tropical chemotype (Vernin and Metzger, 1984) Our chemotype corresponds to the Tropical chemotype and is observed in the research of many authors (Grayer et al., 1996; Liber et al., 2011; Telci et al., 2006; Zheljazkov et al., 2008).

Chemotype E (S55, S60, S63 and S84) was dominated by methyl chavicol, ranging from 79.4% to 93.8%. One accession of Purple basil B morphotype and three accessions of Purple basil (A) morphotype were included in this chemotype. It was previously reported by several authors (Grayer et al., 1996; Liber et al., 2011; Telci et al., 2006;

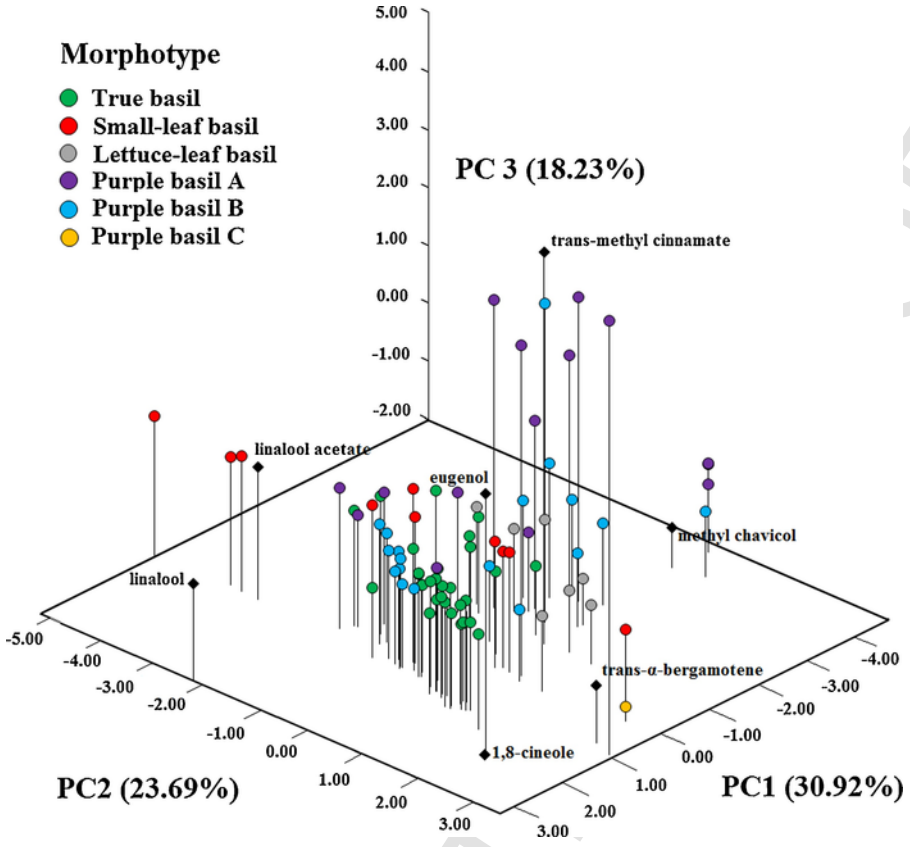

Fig. 2. Principal components analysis based on seven major essential oil compounds in 85 *O. basilicum* accessions.

Zheljazkov et al., 2008) and corresponds to the Reunion chemotype (Vernin and Metzger, 1984).

Bernhardt et al. (2014) found two chemotypes in this species; (1) Linalool-rich and (2) Linalool/methyl chavicol chemotype. Marotti et al. (1996) described three chemotypes: (1) Linalool, (2) Linalool/methyl chavicol and (3) Linalool/eugenol chemotypes. De Masi et al. (2006) reported five chemotypes based on the relative abundance of three or four compounds: (1) Citral/linalool, (2) Linalool/methyl cinnamate/methyl chavicol/eugenol, and three chemotypes with different ratios of linalool, methyl chavicol and eugenol. Telci et al. (2006) reported seven chemotypes in this species: (1) Linalool, (2) Methyl-cinnamate, (3) Methyl cinnamate/linalool, (4) Methyl eugenol, (5) Citral, (6) Methyl chavicol and (7) Methyl chavicol/citral chemotype. In our study, citral (neral + geranial) was not a major component in any of the analysed samples. These compounds are typically found in *O. americanum* and *O. africanum* (*O.* x *citriodorum*) as well as in cultivars that resulted from hybridization between *O. basilicum* and the above-mentioned taxa, e.g., 'Sweet Dani' (Carovic-Stanko et al., 2010; Morales and Simon, 1997; Vieira et al., 2003).

By comparing the characterizations based on both morphological and biochemical traits, we find the majority of True basil morphotype accessions (28 out of 34) grouped within Chemotype A. The majority of Lettuce-leaf basil morphotype (6 out of 7) belonged to Chemotype C. Purple basil B morphotype was the most diverse (accessions scattered through all chemotypes). Chemotypes D and E are found exclusively in accessions belonging to Purple A and B morphotypes (Table 4).

The major compounds found in our samples are synthesized via one of three biosynthetic pathways. Monoterpenes (1,8-cineole, linalool, linalool acetate) derive from the non-mevalonate biosynthetic pathway. Sesquiterpene *trans*-α-bergamotene derives from the mevalonate biosynthetic pathway, while phenylpropane derivatives (methyl chavicol, eugenol, *trans*-methyl cinnamate) are synthesized via the phenylpropene biosynthetic pathway (Iijima et al., 2004). Based on the major compounds present in the chemotype, the dominant biosynthetic pathway utilized by each chemotype can be determined. Chemotype A predominantly utilizes non-mevalonate, while Chemotype E utilizes the phenylpropene biosynthetic pathway. Chemotype D utilizes two biosynthetic pathways (phenylpropene and non-mevalonate), and Chemotypes B and C utilize all three of the biosynthetic pathways. Combining this information with molecular data could assist in breeding distinct chemotypes with unique essential oil composition (Rastogi et al., 2014).

Great intraspecific biochemical diversity was also observed in other taxa of the Lamiaceae family, specifically in species belonging to genera *Mentha* and *Thymus* (Tétényi, 1970). The great biochemical diversity among accessions of *O. basilicum* can be attributed to a long tradition of breeding for different purposes (Vieira and Simon, 2000). Hybridization between different accessions has generated novel compounds in the essential oil of hybrids that are not present in the essential oils of either parents (da Costa et al., 2014). Due to a long breeding tradition (including interspecies crosses), the relationship between morphological diversity and chemical composition is complex. Because *O. basilicum* L. was and still is cultivated for different purposes, many hybrids exist as a result of combining different genotypes. In breeding programs aimed at obtaining ornamental cultivars, a selection of certain morphological features occurred. On the other hand, programs used for creating genotypes that exhibited specific aromas used as spices or in pharmacology, selected accessions with certain biochemical profiles. As a result, we have a great number of cultivars that exhibit different morphological traits but show the same biochemical profile, and vice versa. Thus, biochemical traits, coupled with morphological analysis should be used for intraspecific characterization and germplasm management and determining the origin of cultivars.

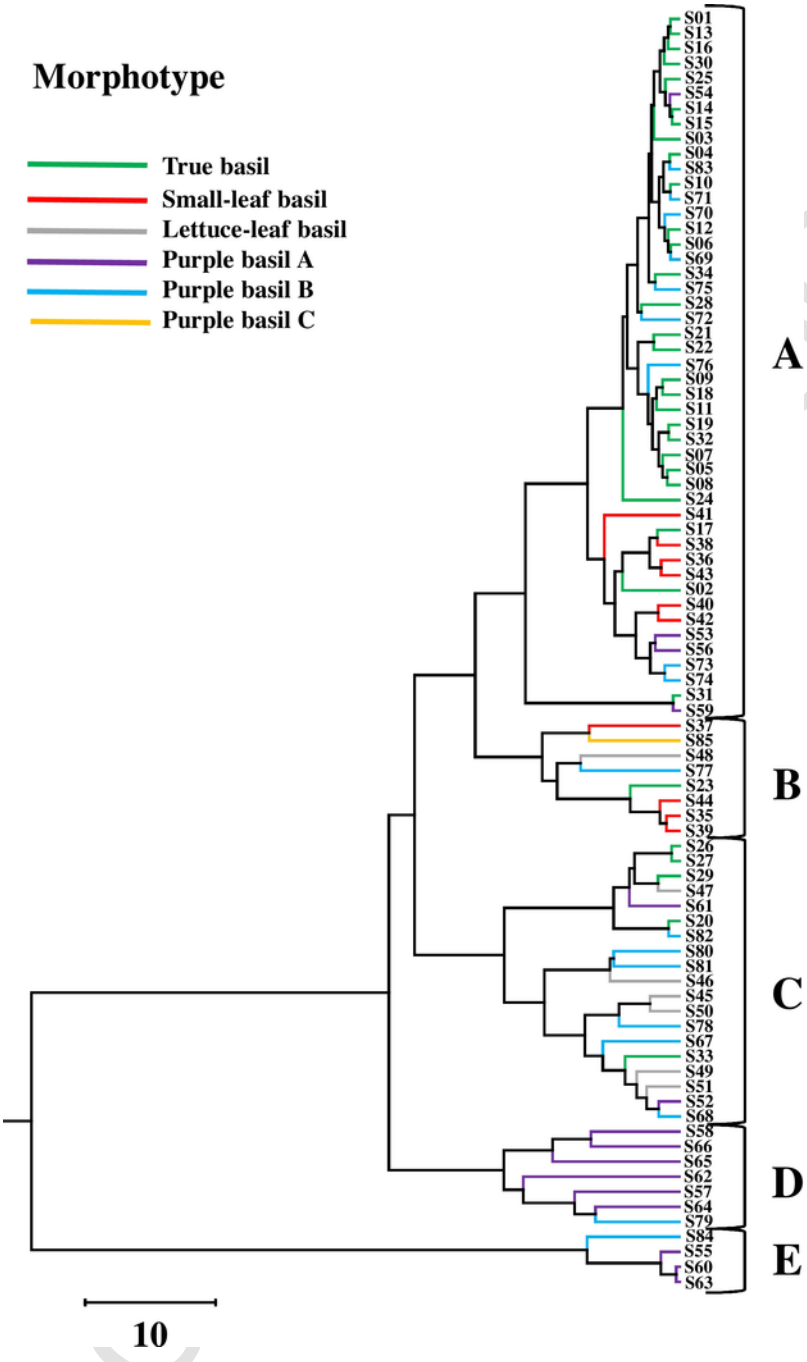

Fig. 3. UPGMA tree of 85 accessions of *O. basilicum* based on seven major essential oil compounds.

Previous investigations of *O. basilicum* chemotaxonomy differ in approach, by some degree, leading to different results. The type of material used for analysis plays an important role when conducting this type of research. Grayer et al. (1996) reported significant differences in the analysis of chemotypes when using fresh and freeze-dried material of the same plant. Environmental factors and plant growth stage should also be taken into consideration. In order to minimize the impact that pedo-climatic factors have on material, most researchers grow plants in a controlled environment, and collect material at the same growth stage(Vieira et al., 2001). The inclusion of different cultivars as well as the threshold and number of compounds included in PCA or CA affects the results.

4. Conclusions

We detected 77 volatiles in 85 accessions of *O. basilicum*, revealing great biochemical diversity within the species. Based on seven major compounds, we propose intraspecific characterization into five chemotypes: (A) Linalool, (B) Linalool/*trans*-α-bergamotene, (C) Linalool/methyl chavicol, (D) Linalool/*trans*-methyl cinnamate and (E) Methyl chavicol. Chemotypes A and C can be considered to be European chemotypes. Chemotype D is a Tropical chemotype, while Chemotype E is described as a Reunion chemotype. A total correspondence between morphotypes and chemotypes does not exist. Due to the wide variety of basil accessions included in this research, the information

Table 4
Relationship between morphotype and chemotype characterizations of *O. basilicum* accessions.

Morphotype	Chemotype[a]					
	A	B	C	D	E	Total
True basil	28	1	5	–	–	34
Small-leaf basil	6	4	–	–	–	10
Lettuce-leaf basil	–	1	6	–	–	7
Purple basil A	4	–	2	6	3	15
Purple basil B	9	1	6	1	1	18
Purple basil C	–	1	–	–	–	1
Total	47	8	19	7	4	85

[a] Chemotypes: (A) Linalool, (B) Linalool/*trans*-α-bergamotene, (C) Linalool/methyl chavicol, (D) Linalool/trans-methyl cinnamate, (E) Methyl chavicol.

obtained will enable an effective management of germplasm collections and facilitate their use in future breeding programs aimed specifically at obtaining genotypes with certain traits for usage in pharmacology, medicine, phytopathology, and especially horticulture, where morphological traits and essential oil composition are major factors for cultivation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.indcrop.2017.09.018.

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