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Intraspecific variability of sweet basil (*Ocimum basilicum* L.) in special respect to essential oil content and composition

INTRODUCTION

The genus *Ocimum* (*Lamiaceae* family) includes annual and perennial herbs and shrubs native to the tropical and subtropical regions of Asia, Africa and South America [1,2]. Sweet basil (*Ocimum basilicum* L.) is one of the most popular species of this genus, used predominantly as a spice in a fresh form. The herb of this annual plant is rich in essential oil, that is utilized in the food and cosmetic industry as a flavoring agent. Sweet basil (both herb and essential oil) reveals various pharmacological activities, e.g. spasmolytic, antimicrobial, antioxidant and diuretic. Thus, it can be applied in the gastrointestinal disorders and loss of appetite treatment [3].

Sweet basil is characterized by a high intraspecific variability, considering both morphological and chemical traits. The species is known to occur as several chemotypes or cultivars, different in respect of essential oil composition. Here, the dominant compounds are: linalool, methyl chavicol, eugenol, methyl eugenol and methyl cinnamate [4,5]. When regards morphological features, great variation in pigmentation, leaf shape and size and pubescence were noticed. Such diversity could be caused by intraspecific hybridization leading to the polymorphism within *Ocimum basilicum* species [6].

The aim of the present study was to determine the variability of selected sweet basil populations introduced into *ex situ* conditions, in terms of developmental and morphological traits as well as total content and composition of essential oil.

MATERIALS AND METHODS

Plant material

Objects of the study was 6 sweet basil accesions, analysed in ex situ conditions. The field experiment was carried out in 2016 at the Experimental Station of Department of Vegetable and Medicinal Plants, WULS-SGGW. The morphological observations and harvest of raw material was carried out at the beginning of plants blooming (July). Collected herb was dried at 35 °C and subjected to chemical analysis. The voucher specimens of the populations' seeds are kept in the National Centre for Plant Genetic Resources (Polish GeneBank).

Developmental characteristics

Directly before harvest of raw material (July, 2016) the following morphological observations were carried out: type of growth habit, plant height (cm), number of shoots, length of vegetative stem, foliage density, length of leaf (mm), width of leaf (mm), leaf shape, surface, margin and colour, the length and shape of inflorescence (mm), color of corolla and anthocyanin coloration of bracts. Fresh and dry mass of herb (g × plant^{-1}) was determined, as well. At each population, the observations were conducted on 10 plants.

Chemical analysis

The essential oil was isolated according to European Pharmacopeia 8th edition. 50 g of air-dried raw material was submitted to hydrodistillation for 3 h using Clevenger-type apparatus. Obtained essential oils were stored in dark vials, at 4 °C. The analysis of essential oil was carried out by GC/MS and GC/FID. All measurements were performed in triplicate.

The qualitative GC/MS analysis was carried out using Shimadzu GC-MS QP210S gas chromatograph equipped with Phenomenex Zebron ZBFFAP polar column (30 m × 0.25 µm × 0.25 µm film thickness). The operating conditions were as follows: oven temperature 2 min. isothermal at 60 °C, then rising at 4 °C per min to 210 °C and held isothermal for 5 min. Injector temperature: 210 °C. The carrier gas (He) flow was 1.1 ml × min-1. The split ratio was 1:20. Diluted samples were injected at 210 °C by auto sampler. Ion source temperature -220 °C, ionization voltage 70 eV. Mass spectra were scanned in the range 40-500 amu. Essential oil compounds identification was based on comparison of mass spectra from the Mass Spectral Databases and on comparison of retention indices (RI) relative to retention times of a series of n-hydrocarbons (C7-C30) with those reported in literature.

The quantitative GC/FID analysis was performed using a Hewlett Packard 6890 gas chromatograph equipped with a flame ionization detector (FID) and capillary, polar column HP 20M (25 m × 0.32 mm × 0.3 µm film thickness). The analysis was carried out using the same temperature programme as described earlier. The carrier gas (He) flow was 1.1 ml × min^{-1}. The split ratio was 1:70. Manually injection of 0.5 µl essential oil was applied. The percentage composition of the essential oils was computed by the normalization method from the GC peak areas, without the use of correction factors.

RESULTS

Obtained results indicate that investigated populations differed significantly both in respect of developmental and chemical traits. It was observed that they were the most differentiated when regards the length and width of leaves (CV 0.26; from 73.9 to 155.8 mm and from 27.1 to 58.10 mm, respectively), as well as the length of inflorescences (CV 0.26; 73.6-170.50 mm). The height of plants ranged from 51.0 to 71.6 cm, the number of shoots was a level of 3-5, while the length of vegetative shoots varied from 52.4 to 76.2 cm. The fresh mass of herb ranged from 420.74 to 635.11 g per plant, while dry mass 82.11-98.22 g per plant (Tab. 1). Investigated sweet basil populations differed as to the other morphological traits, described in Tab. 2 and shown of Photo 1 and 2.

The total content of essential oil ranged from 1.25 % in herb of populations no. 2 and 5 to 2.00% in populations no. 3 (Tab. 3). 25 compounds were identified in the essential oil, comprising 91.64 – 99.28% of the sample. The oxygenated monoterpenes fraction was a fundamental part analyzed essential oils, since it formed from 78.31 to 91.99%. Here, the dominant were: linalool (42.89 - 72.74%), methyl chavicol (0.41-25.11%) and eugenol (0.53-15.95%). Based on this major compounds, investigated populations can be recognized as following chemotypes: populations no 1, 3, 4 – linalool + methyl chavicol, populations no. 5 and 6 – linalool + eugenol, while population no 2 can be regarded as pure linalool chemotype (Tab. 3).

Such variable plant material may be used in future investigations to provide interesting, cultivated forms of sweet basil.

Photo 1. Shape of leaves of sweet basil investigated populations

Population no. 1 | Population no. 2 | Population no. 3 | Population no. 4 | Population no. 5 | Population no. 6

Photo 2. Types of growth habits of sweet basil investigated populations

Population no. 1 | Population no. 2 | Population no. 3 | Population no. 4 | Population no. 5 | Population no. 6

Table 1. Developmental and morphological traits of investigated sweet basil populations

Traits/population no	1	2	3	4	5	6	Mean	CV
Plant height (cm)	51.0	59.4	71.6	56.8	70.0	56.0	60.80	0.14
Number of shoots	4	5	3	4	4	3	3.83	0.20
Length of vegetative stem	52.4	64.8	76.2	59.2	72.4	58.4	63.90	0.14
Length of leaf (mm)	73.9	97.9	92.4	106.1	105.8	155.8	105.32	0.26
Width of leaf (mm)	27.1	39.7	34.3	39.3	42.4	58.10	40.15	0.26
Length of inflorescence (mm)	73.60	156.50	170.50	116.40	124.90	134.50	129.40	0.26
Fresh mass of herb (g × plant^{-1})	525.32	510.45	420.74	595.12	525.10	635.11	535.31	0.14
Dry mass of herb (g × plant^{-1})	90.15	86.47	82.11	94.13	86.47	98.22	89.59	0.07

Table 2. Morphological traits of investigated sweet basil populations

Traits/population no.	1	2	3	4	5	6
Growth habit	upright	upright	upright	upright	semi upright	upright
Foliage density	medium dense	dense	sparce	dense	dense	sparce
Leaf shape	narrow elliptic	rhomboid	elliptic	ovate	elliptic	broad ovate
Leaf surface	smooth	smooth	medium corr.	medium corr.	smooth	corrugated
Leaf margin	strong serrated	medium serrated	weak serrated	weak serrated	weak serrated	weak serrated
Leaf color	dark green	light green	dark green	green	green	light green
Shape of inflorescence	elongate	elongate	elongate	elongate	elongate	elongate
Anthocyanin coloration of bracts	absent	present	present	absent	present	absent
Corolla color	creamy	creamy	white	white	white	creamy

Table 3. The total content (g × 100 g^{-1}) and gas chromatographic composition (% peak area) of investigated populations essential oils

No.	Compound	RI	1	2	3	4	5	6
1	α-pinene	1027	0.16	0.00	0.12	0.11	0.21	0.26
2	β-pinene	1110	0.15	0.00	0.00	0.00	0.11	0.00
3	myrcene	1162	0.12	0.35	0.15	0.14	0.59	0.82
4	limonene	1199	0.25	0.00	0.00	0.11	0.06	0.22
5	1.8-cineole	1214	0.60	0.90	1.88	2.36	6.88	7.01
6	γ-terpinen	1245	1.38	0.99	0.79	0.27	3.39	0.35
7	cis ocimen	1251	0.38	0.24	0.15	0.00	0.05	0.00
8	p cymen	1270	0.28	0.00	0.00	0.00	0.24	0.00
9	α-copaene	1493	0.44	1.13	0.10	1.54	1.54	0.17
10	camphor	1518	1.30	0.54	0.29	0.20	1.78	0.27
11	linalool	1547	53.86	72.74	71.29	72.48	42.89	64.99
12	terpinen-4-ol	1584	0.94	2.61	1.08	1.36	0.61	3.55
13	β-caryophyllene	1593	0.39	0.13	0.47	0.45	2.66	0.14
14	isoborneol	1659	0.34	0.35	0.28	0.37	1.08	0.78
15	methyl chavicole	1672	25.11	1.34	7.46	9.74	1.04	0.41
16	α-terpineol	1697	1.69	2.64	1.71	2.44	3.78	2.13
17	germacren D	1711	0.71	2.40	0.91	1.18	3.78	1.03
18	nerol	1794	0.77	1.25	0.72	1.27	2.82	2.08
19	geraniol	1843	2.88	0.45	2.65	0.29	1.05	0.18
20	methyl eugenol	2013	0.29	0.37	0.27	0.38	0.43	0.50
21	spathulenol	2134	2.31	2.51	1.87	2.89	0.36	0.35
22	eugenol	2162	2.01	4.26	4.14	0.53	15.95	9.43
23	thymol	2176	0.46	0.31	0.22	0.00	0.00	0.00
24	α-bisabolol	2214	1.46	1.13	0.41	0.14	0.23	0.38
25	α-cadinol	2227	0.23	0.00	0.86	1.03	0.11	0.00
	Total		98.51	96.64	97.82	99.28	91.64	95.05
	Monoterpene hydrocarbons		2.72	1.58	1.21	0.63	4.65	1.65
	Oxygenated monoterpenes		90.25	87.76	91.99	91.42	78.31	91.33
	Sesquiterpene hydrocarbons		1.54	3.66	1.48	3.17	7.98	1.34
	Oxygenated sesquiterpenes		4.00	3.64	3.14	4.06	0.70	0.73
	Essential oil total content		1.75	1.25	2.00	1.50	1.25	1.50

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REFERENCES

1. Darrah H.H., 1974. Investigations of the cultivars of basils (*Ocimum*). Econ. Bot. 28, 63-67.
2. Darrah H.H., 1988. The Cultivated Basil. Buckeye Printing, Independence, MO.
3. Hiltunen R., Holm Y., 1999. Basil. The genus Ocimum. Medicinal and Aromatic Plants – Industrial Profiles. Hardwood Academic Publishers.
4. Grayer R.J., Kite G.C., Goldstone F.J., 1986. Intraspecific taxonomy and essentials oil chemotypes in sweet basil (*Ocimum basilicum*). Phytochem 43, 1033-1039.
5. Telci I., Bayram E., Yılmaz G., Avcı B., 2006. Variability in essential oil composition of Turkish basils (*Ocimum basilicum* L.). Biochem Syst Ecol 34, 489-497.
6. Hasegawa Y., Tajima K. Toi N., Sugimura J. 1977. Characteristic components found in the essential oil of *Ocimum basilicum* L. Flav Fragr J. 12, 195-200.

CONCLUSIONS

1. Investigated populations of sweet basil differed both in terms of morphological and chemical traits.
2. Plants from population no 3 were the highest and characterized by the longest inflorescences, while population no 6 was distinguished by the largest leaves and the highest mass of herb.
3. Three chemotypes were recognized within investigated populations: linalool + methyl chavicol, linalool + eugenol and pure linalool chemotype.