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A study of 34 cultivars of basil (*Ocimum* L.) and their morphological, economic and biochemical characteristics, using standardized descriptors

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Basil is an important medicinal and aromatic plant that Abstract. is widely used in many fields. The aim of this study was to evaluate the oil content and ornamental value of a wide range of species and cultivars of Ocimum L. from various different sources, using standardized descriptors. 34 cultivars of basil (Ocimum L.) were grown in two different years at The Faculty of Horticulture in Lednice, Czech Republic. The cultivars were divided into groups according to leaf colour and leaf size. The cultivars displayed a wide diversity of morphological, biological and economic characteristics. The groups of green small-leafed and purple-leafed varieties were judged to be the most decorative. To improve the ornamental value of the Ocimum species the uniformity, colour stability and earliness of flowering should be examined. The essential oil was extracted using hydro-distillation methods. Most of the samples had high essential oil levels $(1.8 - 14.3 \text{ ml.kg}^{-1})$, which were influenced by cultivar, environmental conditions and storage length. The major

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volatile oil constituents were determined by gas chromatography. Levels of linalool, 1,8-cineol and eugenol were determined in all samples. The small-leaved cultivars, *O. basilicum* (pravá trpasličí) and *O. b. minimum* 'Spicy Bush', were richest in essential oil content, followed by cv. 'Citriodora' and the traditional Czech cultivars 'Ohře' and 'Litra'. The results suggest that further research to improve the quality of the essential oil content is desirable for essential oil production.

1 Introduction

Basil has been used as a medicinal and aromatic plant for centuries. Nowadays it is used in many fields of human activity, especially in the food industry and in gastronomy. Basil is also important for the pharmaceutical industry and is still used in traditional medicines in many parts of the world. It is appreciated for its essential oil, which gives it a unique flavour.

The genus *Ocimum* is characterized by great variability among its constituent species, including morphology, growth habit, the colour of flowers, leaves and stems, and chemical composition. The ease of cross-pollination has given rise to a large number of species, subspecies, varieties and forms [28, 30]. However, [7] have found that their chemical constituents are not necessarily correlated with their taxonomy. Different authors have divided basil into groups based on various points of view, ranging from their chemical composition to morphology [1, 5, 7, 10, 11, 20, 27, 33]. Some systems of descriptors combining morphological, economical and biochemical characteristics of *Ocimum* species have also been proposed [8, 18].

The levels of essential oil and other compounds vary between the different basil species and cultivars, and are also influenced by growing conditions [7, 18, 34, 35]. According to [12], the variation in essential oil content and composition of *Ocimum basilicum* coming from different countries might be attributed to different environmental conditions, as well as genetic factors, different chemotypes and differences in the nutritional status of the plants. Most of the essential oil is concentrated in the leaves, and the amounts obtained from branches and stems is not economically significant [3, 17, 23].

In the last few decades the importance of basil as an ornamental plant has grown and the number of cultivars on sale has increased significantly, it is now available in a wide range of habits, colours and flavors. Apart from their ornamental value, these cultivars may also be rich in essential oils and could offer a new source of oil for industrial exploitation [34]. However, according to [30], for example, only a few of the North-American lemon-scented cultivars could compete successfully as industrial sources of citral. On the other hand, they suggest that the cultivar 'Camphor' could be a potential industrial source of camphor and the cultivars 'East Indian' and 'Tree' could be a useful source of eugenol.

This is the first study of a large number of basil cultivars using a large number of standardized descriptors and with an emphasis on detailed morphological characteristics. The aim was to evaluate basil cultivars according to their essential oil content and composition as well as their ornamental value. Domestic and foreign cultivars of basil were collected to form a field trial. The morphological development of cultivars was observed to assess the best time of harvest for aromatic and medicinal purposes. The essential oil content was examined qualitatively and quantitatively to assess the potential of cultivars for industrial use.

2 Material and methods

Plant material

34 basil cultivars were purchased from commercial growers in different countries (Tab. 1).

Field trial

The plants were cultivated in a sandy-loam soil in the experimental grounds of Mendel University of Agriculture and Forestry (Brno), at the Faculty of Horticulture in Lednice, Czech Republic. Average temperature from April to August was 18.5°C with 199 mm of rainfall in 2003, and 16.8°C with 364 mm of rainfall in 2005. A drip-irrigation system was installed to provide water when necessary.

34 basil cultivars were sown in containers in a greenhouse in the middle of April. Newly germinated plants were pricked out into pots. The plants were planted out in the field from 40 to 45 days after germination. Each cultivar was represented by 20 plants in 2003 and by 15 plants in 2005. The distance between the plants was 0.4 m in the rows and 0.6 m between the rows.

Plants were harvested at the beginning of flowering from the middle of June to the end of July in 2003 and from the end of June to the middle of August in 2005. The fresh biomass was weighed immediately after harvest, then dried at room temperature and weighed again.

Table 1: Origin of the plant material

Semo Smržice, Czech Republic:
Ocimum basilicum L. 'Purple Opaal'
Ocimum basilicum L. 'Cinamonette'
Ocimum basilicum L. 'Lime'
Ocimum basilicum L. 'Compakt'
Ocimum basilicum L. 'Blue Spice'
Ocimum basilicum L. 'Dark Green'
Ocimum basilicum L. 'Lettuce Leaf'
SEVA FLORA, Valtice, Czech Republic:
Ocimum basilicum L. 'Ohře'
Ocimum basilicum L. 'Litra'
NOHEL GARDEN, Dobříš, Czech Republic:
Ocimum basilicum L. (pravá trpasličí)
Ocimum basilicum L. (pravá zelená)
Ocimum basilicum L. (pravá červená)
Botanical garden of medicinal plants,
Hortus Plantarum Medicinarum Academii Mediceae, Wroclav, Polland:
Ocimum basilicum L. 'Kardinal'
Ocimum basilicum L. 'Cinnamon'
Ocimum tenuiflorum L.
IHP ŽALEC, Slovenia:
Ocimum basilicum L. 'Genovese'
Ocimum basilicum L. 'Citriodora'
Ocimum basilicum L. 'Grant Vert'
Ocimum basilicum L. 'Sweet Dani'
JELITTO, Schwarmstedt, Germany:
Ocimum basilicum L.
Ocimum basilicum L. 'Großblättrig'
Ocimum basilicum L. 'Purple Ruffles'
Johny's Selected Seeds, Maine, USA:
Ocimum basilicum L. 'Italian Large Leaf'
Ocimum basilicum L. 'Nufar'
Ocimum basilicum L. 'Magical Michael'
Ocimum basilicum L. 'Genovese Compact Improved'
Ocimum basilicum L. 'Red Rubin'
Ocimum basilicum L. 'Osmin Purple'
Ocimum basilicum L. 'Cinnamon'
Ocimum basilicum L. 'Fino Verde'
Ocimum basilicum citriodora 'Mrs. Burns' Lemon'
Ocimum basilicum minimum 'Spicy Bush'
Ocimum americanum 'Lime'
Tsukuba Medicinal Plant Research Station,
National Institute of Health Sciences, Japan:
Ocimum basilicum L.

Descriptors for the genus *Ocimum* L. [8] was used to evaluate morphological, biological and economic characters. An evaluation of plant width, stem branching, inflorescence length and density of flowers were added to the list of morphological characters to be assessed. The number of days of flowering was added to the list of biological characters. Fresh and dry yields of biomass per plant and vigour rating were added to the list of economic characters.

Analysis of essential oil content

The analysis were made in the laboratories of Mendel University of Agriculture and Forestry (Brno), at the Faculty of Horticulture in Lednice, Czech Republic. The essential oil content was measured by hydrodistillation. 20 g of the dry biomass with 400 ml of distilled water was boiled in the distillation apparatus. After three hours volume of distilled essential oils was noted.

Gas chromatography (GC) analysis of essential oils

The analysis were made by the Prague-Ruzyne Crop Research Institute at their Gene Bank Department in Olomouc, Czech Republic. The essential oil was analyzed on a Hewlett-Packard gas chromatograph model 5890A SERIES II equipped with flame ionization detector (FID) and HP-INNOVAX capillary column (60 m – 0.53 mm). Injector and detector temperatures were set at 220°C. The column temperature programme was: 100°C (3 min, 3°C/min), 150°C (2°C/min) and 200°C (15 min). Nitrogen was used as carrier gas at 39 kPa and at a flow rate of 50 ml/min. A sample of 1.0 µl was injected, using split mode (split ratio, 1:20). The relative retention times of the compounds were compared with those of standards. The content of 1,8-cineole, linalool and eugenol was determined for each sample. Other essential oil compounds were identified if having a significant percentual content in comparison with the other compounds.

Statistical analysis

Statistical analysis of the data was performed by analysis of variance (ANOVA) using Unistat 4.53 software.

3 Results

Morphological characteristics

The plant heights varied from 143 to 570 mm. The smallest was *Ocimum* basilicum minimum 'Spicy Bush', the tallest were *Ocimum* basilicum from Germany (Jelitto) and *Ocimum* americanum 'Sweet Dani'. The plant spread was from 213 mm in *O. basilicum* minimum 'Spicy Bush' to 610 mm in *O. basilicum* 'Ohře'.

Most plants had a semi-erect habit, semi-dense branching and sparse stem pubescence. The prevalent leaf-shape was lanceolate. In green-leafed basils with large leaves, and purple-leafed basils, a serrate leaf margin was prevalent. In the green small-leafed basils the leaf margin was entire.

Most cultivars had glabrous leaves apart from the cultivars 'Blue Spice', 'Piperitum' and *O. tenuiflorum*, whose leaves are sparsely pubescent. Plants had prevalently plain leaves in the medium and small green-leafed and purpleleafed cultivars. In the large green-leaved cultivars most leaves were undulate with small or sparse blisters. The prevalent leaf colour varied from green to dark green according to the RHS colour chart.

Flower size was intermediate. The green-leafed cultivars predominantly had a green calyx and a white corolla. In the purple-leafed cultivars the calyx and corolla were various shades of purple, and in most cultivars the calyx was pubescent. The length of the flowering spike varied from 29 to 178 mm. Sparse and semi-sparse inflorescence density was prevalent.

Biological characteristics

Most cultivars do not show stand uniformity, although the following cultivars do: *O. basilicum* 'Litra', 'Blue Spice', 'Cinnamon', 'Cinamonette', 'Citriodora', 'Kardinal', 'Lime', 'Piperitum', *O. americanum* 'Lime', *O. tenuiflorum* and *O. basilicum minimum* 'Spicy Bush'.

The beginning of flowering varied from 19 to 64 days after planting. The cultivars which were the latest to initiate flowering were *O. basilicum* (Tsukuba, Japan), 'Fino Verde', 'Magical Michael' and 'Purple Ruffles'. The earliest flowering cultivars were 'Blue Spice', 'Sweet Dani' and *O. tenuiflorum*.

Economic characteristics

Fresh weight yields varied from 14 to 713 g per plant. The lowest fresh weight yield was given by *O. americanum* 'Lime', the highest by *O. basililcum* 'Fino Verde'. Dry weight yields varied from 25 to 499 g per plant. The lowest dry weight yield was given by the cultivar 'Spicy Bush', the highest by 'Fino Verde'. Vigour ratings varied from 4:1 to 9:1.

Biochemical characteristics

Essential oil contents varied from 1.8 to 14.3 ml.kg⁻¹ (0.18 - 1.43%) in the dry herbage. According to the descriptors [8] the oil content was high in most cultivars (over 4 ml.kg⁻¹ of dry biomass). In 94.1% of the cultivars the

essential oil content was more than 0.3% of dry weight. The lowest oil content was found in the cultivar 'Piperitum', the highest oil content in dwarf basil *O. basilicum* (pravá trpasličí) and *O. basilicum* 'Lime'.

Linalool content varied from 3.9 to 19.9%. The highest linalool content was found in the cultivar 'Red Rubin' and the lowest in 'Blue Spice'. The content of 1,8-cineole varied from 0.9 to 18.5%. The highest levels were found in the cultivar 'Piperitum', the lowest in *O. americanum* 'Lime'. The eugenol content varied from 0.3 to 13.3%, with the highest levels in the cultivar 'Magical Michael' and the lowest in *O. americanum* 'Lime'. Some cultivars also contained significant levels of one or more of the following compounds: methylchavicol, camphor, citral, limonene, methylcinnamate, caryophyllen- β , anethol, terpinen-4-ol, myrcene, thymol, ocimene and cinnamaldehyde. (Tab. 2 - 5)

Statistical results

Statistically highly significant differences were observed between the two years (due to environmental factors) regarding plant height, essential oil content and eugenol content, and also in the fresh and dry weight yields per plant and in the vigour rating. Statistically highly significant differences were observed between cultivars regarding plant height, leaf-length, flower-size, flowering time, essential oil content, anethol and terpinen-4-ol content, and also in the fresh weight yield per plant and levels of linalool, 1,8-cineole, eugenol, methylchavicol, citral and β -caryophyllene. There were differences seen between the first and the second harvest in yield, content of 1,8-cineole and eugenol, but not in essential oil content and linalool content.

4 Discussion

Only a few articles about basil have focussed on essential oil levels and its composition, and also on morphological characteristics as well e.g. [7, 16, 18, 19, 22, 30]. The present study gives a detailed overview of the morphological, biological, economic and biochemical characteristics of 34 basil cultivars evaluated using Dušková's minimal set of descriptors [8].

According to [2] plant height varies with the cultivar and the number of harvests in the season, as is confirmed in this research too. [16] found that harvesting only the secondary branches of *O. tenuiflorum* led to maximum plant height and number of secondary branches per plant. On the other hand, secondary branches give the least biomass yield.

Ta	bel 2:	Biot	chem	iical	char	actei	istic	S - 19	reen	leafé	sd cu	ıltiva	rs with large	Tabel 2: Biochemical characteristics - green leafed cultivars with large leaves (> 60 mm)	u)
Green leafed	Essential oil content Linalool content 1,8-cineol content Eugenol content [ml.kg ⁻¹] [%] [%]	ial oil co [ml.kg ⁻¹]	ontent 	Linal	lool coi [%]	ntent	1,8-cii	neol co [%]	ontent	Euge	nol cor [%]	ntent	Content	Content of other essential oil compounds	spunodu
cultivars with	2003	20	2005	2003		2005	2003		2005	2003	2005	05	2003	2005	
large leaves (> 60 mm)	1 st Harves t		2 nd Harv.	1 st 2 nd 1 st 1 st 2 nd 1 st 2 nd 1 st 2 nd 1 st 2 nd <th< th=""><th>1st Harv.</th><th>2nd Harv.</th><th>1st Harv.</th><th>1st Harv.</th><th>2nd Harv.</th><th>1st Harv.</th><th>1st Harv.</th><th>2nd Harv.</th><th>1st Harvest</th><th>1st Harvest</th><th>2nd Harvest</th></th<>	1 st Harv.	2 nd Harv.	1 st Harv.	1 st Harv.	2 nd Harv.	1 st Harv.	1 st Harv.	2 nd Harv.	1 st Harvest	1 st Harvest	2 nd Harvest
Ocimum basilicum L. ¥ Dark Green¥	3.25	3.88	4.63	18.00	14.25	15.60	14.40	14.40 13.50 14.90	14.90	9.65	9.20	11.10			methylcinnamate (11,3 %)
Ocimum basilicum L. ¥ Genovese¥	1.88	6.00	•	14.40	16.50		11.75 13.50	13.50	-	11.85	8.50		methylchavicol (14,35 %)	-	
Ocimum basilicum L. ¥ Genovese Compact Improved¥	4.25	4.00		14.40	14.40 18.10		14.40 15.80	15.80		10.95	9.90		ı		·
Ocimum basilicum L. ¥ Grant Vert¥	3.75	4.75	5.50		14.65	16.25	13.15 14.65 16.25 13.15 12.75 16.25 10.85	12.75	16.25	10.85	8.75	7.75			
Ocimum basilicum L. ¥ Grofi bl%åtrig¥	3.88	4.25	4.88	14.35	17.35	15.10	14.35 17.35 15.10 11.90 6.60 15.05 11.15 9.40	6.60	15.05	11.15		8.20	,	myrcene (12,75 %), limonene (5,35 %)	
Ocimum basilicum L.¥ Italian Large Leaf¥	- *	4.50		'		,									
Ocimum basilicum L. (Jelitto)	1.90	2.65	3.88	14.80	11.25	19.65 13.80	13.80	8.80	14.95	12.45	•	9.50		thymol (9,1 %)	
Ocimum basilicum L. ¥ Lettuce Leaf¥	4.00	4.50	4.25		13.70 14.20	17.10	4.00	12.00 13.45	13.45	7.00	9.10	6.70	camphor (10 %)	-	
Ocimum basilicum L. ¥ Litra¥	6.00	7.05	8.00	15.90	16.85	16.50	8.80	9.15	16.05	9.90	7.50	6.40	anethol (15,8 %)	anethol (15,7 %), camphor (5,25 %)	anethol (16,5 %), camphor (1,4 %)
Ocimum basilicum L. ¥ Nufar¥	ı	6.00	·	14.90	15.80		ı	ı	,			1	·		·
Ocimum basilicum L. (prav-zelen-)	3.75	4.00	,	12.30 14.40	14.40	ī	14.95 14.40	14.40	ı.	11.90 10.20	10.20		ı	ı	ı
Ocimum basilicum L. (Tsukuba)	8.00	5.00	•	'	'		12.30 14.40	14.40	'	9.65	8.10	•			

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