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The exploitation of micromorphological parameters for identification in the section *Mentha*
Využitie mikromorfologických parametrov pri identifikácii v sekcii *Mentha*

Original research article

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Abstract The identification of species in the genus *Mentha* is especially difficult because of the ease of hybridization, favoured by gynodioecy, which is further complicated by polymorphism, cultivation, polyploidy and vegetative propagation. This all explains the genicpopulation and microevolutionary background for variability of mints. In this work we studied the usage of micromorphological and phytochemical parameters in identification and characterization of selected *Mentha* species. On abaxial surface of leaves of 20 *Mentha* populations we determined the size and the number of peltate glandular trichomes. The essential oil was analysed by GC MS. We identified and characterized populations of *M.* × *piperita, M. spicata, M. spicata* subsp. *condensata, M. spicata var. crispa, M. spicata var. citrata, M.* × *gentilis, M. aquatic, M. arvensis* amd *M. longifolia*. The size and the number of peltate glandular trichomes contribute to characterisation of some *Mentha* species (especially in *M. longifolia* and *M.* × *piperita*).

Slovak abstract Identifikácia druhov v rode *Mentha* L. je obzvlášť náročná. Je to spôsobené ľahkým krížením podporeným gynodioéciou, ktorá je ďalej skomplikovaná polymorfizmom, pestovaním, polyploiditou a vegetatívnym rozmnožovaním, čo vysvetľuje genetickopopulačné a mikroevolučné pozadie premenlivosti v rode *Mentha*. V tejto práci sme študovali využitie mikromorfologických a fytochemických parametrov pri identifikácii a charakterizácii vybraných druhov rodu *Mentha*. Na spodnej pokožke listov 20 populácii *Mentha* sme stanovili veľkosť a počet „žliazok typu Lamiaceae". Silicu sme analyzovali pomocou GC MS. Identifikovali a charakterizovali sme populácie druhov *M.* × *piperita, M. spicata, M. spicata* subsp. *condensata, M. spicata var. crispa, M. spicata var. citrata, M.* × *gentilis, M. aquatic, M. arvensis* a *M. longifolia*. Sledovanie veľkosti a počtu „žliazok typu Lamiaceae prispievajú k charakterizácii niektorých druhov *Mentha* (najmä *M. longifolia* a *M.* × *piperita*).

Keywords *Mentha – peltate glandular trichomes – quantitative microscopy – essential oil – GC MS*

Kľúčové slová: *Mentha – žliazky typu Lamiaceae – kvantitatívna mikroskopia – silica – GC MS*

1. INTRODUCTION

The genus *Mentha* L. belongs to the family Lamiaceae, subfamily Nepetoidae. The taxonomy of the genus *Mentha* is still not definite but on the basis of a phylogenetic analysis of morphology, chromosome numbers, and major constituents of essential oil, the genus *Mentha* is now redefined to include 18 species and 11 hybrids placed in four sections: *Pulegium, Tubulosae, Eriodentes* and *Mentha* (Tucker & Naczi, 2007). The pharmacological activities of mints are chiefly bound to the presence of two main groups of secondary metabolites: essential oil and phenolic compounds. Essential oil of mints is composed of monoterpenes and sesquiterpenes, which content vary from species to species (Lawrence, 2007). Essential oil is produced by specialized secreting tissues – glandular trichomes (Gershenzon et al., 1989; McCaskill et al., 1992). Two types of glandular trichomes occur on mint´s leaf surface: small, capitate trichomes, with a single secretory head cell (**Figure 1**) and peltate glandular trichomes (**PGT**), with an eight-celled head and huge subcuticular oil storage cavity (**Figure 2**). Recently, we published works aimed in identification by usage of different parameters: anatomical, morphological, phytochemical (Fialová et al., 2011; Šarić-Kundalić et al., 2009). The aim of this study was further

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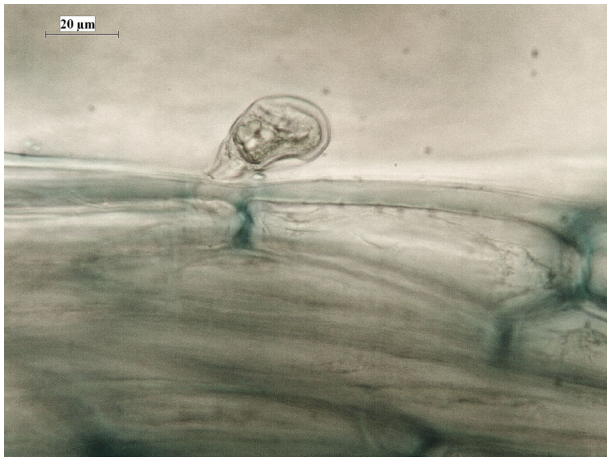

Figure 1. Capitate trichome, with a single secretory head cell

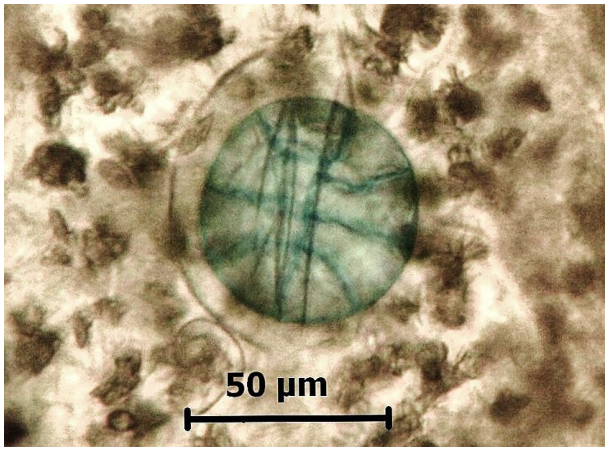

Figure 2. Peltate glandular trichome, with an eight-celled head

examination of usage of micromorphological parameters, specifically the size and number of peltate glandular trichoms, for the identification and characterization of different mints. We investigated the frequency and size of peltate glandular trichomes and validated this method as useful for the differentiation of *Mentha* species. For disquisitional identification it was necessary to determine the main components of essential oils.

2. MATERIAL AND METHODS

2.1. Plant material

The plants have been collected in different areas of south, south-west and middle Slovakia (**Table 1**). All plants were harvested in the stage of flowering, in sunny days. Plants was dried in an oven at 30-35 °C, afterwards were kept in paper bags at room temperature. The voucher specimens are deposited at the Department of Pharmacognosy and Botany, Faculty of Pharmacy, Comenius University in Bratislava, Slovakia.

Table 1. Investigated mints and their origin

Sample	Collection area
Mentha spicata (V10)	old genetic resources SUA Nitra
Mentha × *piperita* (V11)	old genetic resources SUA Nitra
Mentha longifolia (V12)	old genetic resources SUA Nitra
Mentha × *gentilis* (V13)	Komárno
Mentha longifolia (V14)	Garden of Medicinal Plants, Bratislava
Mentha × *piperita* (V15)	old genetic resources SUA Nitra
Mentha × *piperita* (V16)	old genetic resources SUA Nitra
Mentha spicata (V17)	Garden of Medicinal Plants, Bratislava
Mentha × *gentilis* (V18)	Komárno
Mentha spicata (V19)	Podkylava (Myjava*)
Mentha spicata var. *crispa* (V22)	old genetic resources SUA Nitra
Mentha × *gentilis* (V24)	Garden of Medicinal Plants, Bratislava
Mentha longifolia (V43)	Zochova chata (Pezinok*)
Mentha spicata subsp. *condensata* (V44)	Modra (Pezinok*)
Mentha spicata (V46)	Modra (Pezinok*)
Mentha arvensis (V50)	Revište (Žarnovica*)
Mentha aquatica (V55)	Pezinok
Mentha spicata (V57)	Modra (Pezinok*)
Mentha spicata var. *crispa* (V59)	Komárno
Mentha spicata var. *citrata* (V60)	old genetic resources SUA Nitra

*SUA - Slovak University of Agriculture, * region*

2.2. The quantitative microscopic analysis

Anatomical analyses were carried out using Leica DME microscope (Leica, Germany) equipped with a Leica EC3 Digital Camera (Leica, Germany) and its equipment of software (Leica Application Suite 2.4.0 R1 LAS EZ ver. 1.3.0, Germany). Leaves were placed on a glass slide, embedded in few drops of 60% chloralhydrate and shortly gently boiled in order to clear the samples. For the observation we have selected only the leaf on stem placed as the fourth from the end of inflorescence (from the top).

2.3. The analysis of essential oil by GC-MS

For GC MS (Shimadzu GCMS-QP2010) a Column Zebron capillary ZB-WAX (0.25 µm film thickness, 60 m length x 0.25 mm) was used, 50 mg dry leaf sample were extracted in 500 µL dichloromethane and treated by ultrasonication for 10 minutes at room temperature. 1 µL aliquots were analysed. The composition was reported as a relative percentage of the total peak area.

3. RESULTS AND DISCUSSION

We observed the abaxial surface of leaf due to the higher density of PGT in comparison to adaxial surface (Maffei et al., 1989; Turner et al., 2000). We measured the size of PGT and their number per 1 mm^2. PGT appear from the top as formations of rounded shape; their size was expressed as the diameter of the circle in microns (µm). For this analysis we used an optical microscope. The measurements were performed by the digital camera with measuring software. The camera was calibrated manually using a lens micrometre (1 mm/0.01). For the analysis of each population we used at least 10 samples (10 leaves). The formation of peltate glandular trichomes is not synchronized; different developmental stage of PGT could be found on the same leaf. The PGT are developing while the meristematic activity of protoderm lasts. The size of PGT is therefore dependent on the leaf ripening. In our work, the size of PGT was measured on the fourth leaf from the end of inflorescences, which is considered to be sufficiently developed, meristematic activity is stopped and the constant size of the PGT is supposed. Nevertheless, some minor changes in the size of PGT are generally connected with the aging of leaves. The size of PGT is an endogenously controlled sign (Felklová et al., 1986). The differences in number and size of PGT in mints were recorded in the past. Anyway, the majority of published works advert to peppermint and menthol type of mints (Maffei et al., 1989; Nátherová & Lindauerová, 1986; Felklová et al., 1986).

Number of PGT on leaves

Maffei et al. (1989) investigated the correlation between PGT number and leaf length in peppermint. They found out a positive correlation between trichome number and leaf length, whereas the trichome density decreased with increasing leaf length. Our investigations comprised different mint species, where we observed the correlation between average leaf length and average density of PGT on surface area 1 mm^2. Our results confirm the previous investigation. Anyway there are also some exceptions (**Table 2**). The largest PGT number on area 1 mm^2 we found on *M. spicata* var. *citrata* (14.7 PGT/mm^2). Similarly, large amount of PGT was found in all populations of *M.* × *piperita* (8-14 PGT/mm^2) and *M. longifolia* (9 - 14.7 PGT/mm^2). The less number of PGT occurred in *M. arvensis* (3.6 PGT/mm^2) and *M.* × *gentilis* (3.6 - 5.6 PGT/ mm^2).

The size of PGT

As we already published; the size of PGT could be helpful in mints identification (Fialová et al., 2011; Šarić-Kundalić et al., 2009). If the size of PGT on developed leaves is constant; our results showed that the largest PGT are presented in peppermint and spearmint (**Figure 3**).

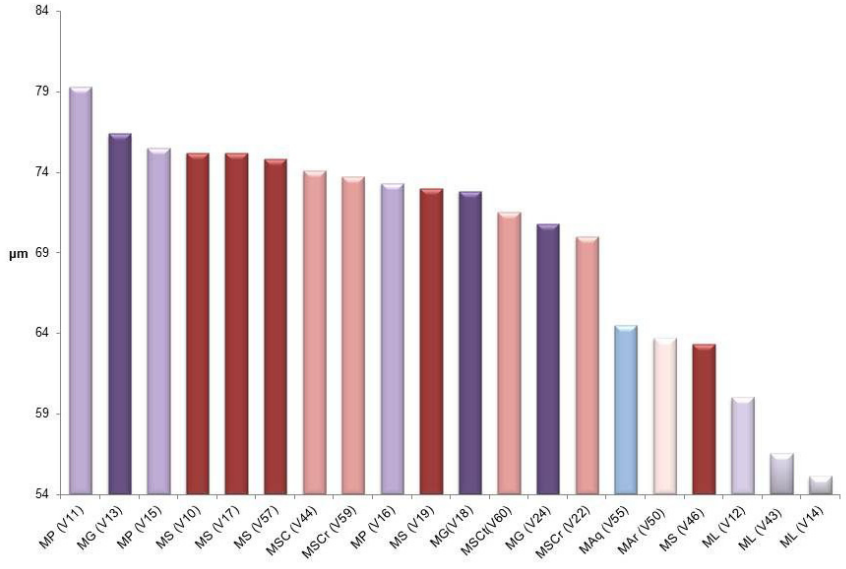

Figure 3. The size of PGT in µm on abaxial surface of different mints.
MP - M. × *piperita, MS - M. spicata, MSC - M. spicata subsp. condensata, MSCr - M. spicata var. crispa, MSCt - M. spicata var. citrata, MG - M.* × *gentilis, MAq - M. aquatic, MAr - M. arvensis, ML - M. longifolia*

Table 2. The results of measurements of leaf length and the number of peltate glandular trichomes.

Sample	Average leaf length [cm]* ± SD	PGT average density on 1 mm^2 ± SD
M. × *piperita* (V11)	**4.0** ± 0.36	**8.1**± 2.14
M. × *piperita* (V15)	**2.5** ± 0.25	**12** ± 1.79
M. × *piperita* (V16)	**3.9** ± 0.25	**8.3** ± 0.89
M. spicata (V10)	**1.9** ± 0.15	**9.1** ± 1.42
M. spicata (V17)	**4.1** ± 0.40	**8.1**± 0.42
M. spicata (V19)	**4.3** ± 0.45	**3.7** ± 0.21
M. spicata (V46)	**2.7** ± 0.33	**4.4** ± 0.81
M. spicata (V57)	**6.3** ± 0.51	**4.3** ± 0.4
M. spicata var. *crispa* (V22)	**3.6** ± 0.35	**4.8** ± 0.64
M. spicata var. *crispa* (V59)	**3.0** ± 0.35	**10.91** ± 1.95
M. spicata var. *citrata* (V60)	**3.5** ± 0.39	**14.73** ± 2.16
M. spicata subsp. *condensata* (V44)	**4.7** ± 0.51	**10.9** ± 0.93
M. × *gentilis* (V13)	**2.8** ± 0.25	**3.6** ± 0.31
M. × *gentilis* (V18)	**3.8** ± 0.42	**5.6** ± 1.7
M. × *gentilis* (V24)	**4.9** ± 0.45	**4.1** ± 0.2
M. longifolia (V12)	**2.3** ± 0.24	**13.1** ± 2.27
M. longifolia (V14)	**2.2** ± 0.17	**14.7** ± 3.07
M. longifolia (V43)	**4.8** ± 0.46	**9** ± 0.21
M. arvensis (V50)	**3.6** ± 0.36	**3.6** ± 0.76
M. aquatica (V55)	**4.1** ± 0.39	**1.2** ± 0.31

**Average is count from 10 leaves observations; SD – standard deviation*

It is difficult to distinguish species with similar sizes of PGT, but this parameter could be important for the characterization and description of the species. The significantly smallest PGT were found in three populations of *M. longifolia* (55-60 µm). We suggest that a large number of PGT in *M. longifolia* populations could compensate a relatively small size of PGT, while maintaining the relatively high content of essential oil (2.5% [V/m], unpublished results). Our observations confirmed previous results of published papers (Fialová et al., 2011; Šarić-Kundalić et al., 2009) and thus it appears to be one of the most important evaluation criteria for this species.

Phytochemical parameter

Third step of our research was GC MS analysis of essential oil of investigated mints. For a clear distinction of *Mentha* species it is important to establish phytochemical parameters. In this work we identified and quantified determined the main components of essential oil, which significantly vary from species to species. The main compounds important for identification are presented in **Table 3**. As it is markedly the investigated mints could be divided in 2 groups according their essential oil: menthol type and carvone type. In interpretation of results, it is necessary to consider that compounds in essential oil of one species could vary depending on variety, cultivar or chemotype. To know the main compound in essential oil is very helpful in identification, but also this phytochemical parameter as a sole identification parameter would be not sufficient.

4. CONCLUSION

Exploitation of micromorphological observation in identification seems not to be sufficient for all mints. The measurement of the size of PGT, supported by counting of PGT number on

Table 3. The main components (%) of essential oil of investigated mints

	V11	V15	V16	V12	V17	V10	V57	V19	V59	V60	V22	V46	V18	V13	V24	V44	V43	V14	V50	V55
1.8-cineol			5.3							11.6			7			6.2				15
3-octanol													11.5						**16.6**	
α-cedrol																		5.1		
alloaromadendrene				1.7																
β-pinene											4.5			3.3	4.8					
β-terpinylacetate					6.2		6			3.6										
borneol																			3.1	
dihydrocarveol					7.9			7.9	13.8			4								
dihydrocarvone					11.8	4.4	9.3	11.8	12.3			12.5				5.3				
germacrene D				8.3										**6.5**		5	6.9		2.4	
isomentone	10.8	11.6												6.5						
carvone					**39.4**	**66.2**	**22.5**	**47.8**	**37.4**	**45.1**	**39.6**	**19.6**	**21.3**		**45.5**	**42.8**				
caryophyllene													8.1	3.7			4			12.5
L-limonene						3.2				6.9	6.4				9.2					
menthofuran																				**22.8**
menthol	**44.4**	**41.2**	**43.4**																	
menthone																	**63.3**			
menthylacetate	10.7	9.6																		
myrcen				3.1		3.7													1.9	
neodihydrocarveol							8	4.9				6.9								
neomenthol	6.9	7.4	7.5																	
piperitone																		3.9		
piperitone-oxide				**54.6**													4.3	**57.1**		
p-menthan-1.2.3-ol																		8		
trans-sabinene hydrate			8.8						5.4		5.4				5.9					
virdiflorol																				5.8

selected area, allows us to distinguish some individual taxa. Anyway, micromorphology along with phytochemical analysis is appropriate tool in *Mentha* identification and characterization; nevertheless it still needs further research.

References

[1] Tucker AO, Naczi RFC. *Mentha*: An Overview of Its Classification and Relationships. In: Lawrence BM. *Mint. The genus Mentha.* Medical and Aromatic Plants – Industrial Profiles. Boca Raton, London, New York: CRC Press, Taylor & Francis Group; 2007.

[2] Lawrence BM. The Composition of Commercially Important Mints. In: Lawrence BM. *Mint. The genus Mentha.* Medical and Aromatic Plants – Industrial Profiles. Boca Raton, London, New York: CRC Press, Taylor & Francis Group; 2007.

[3] Gershenzon J, Maffei M, Croteau R. Biochemical and histochemical localization of monoterpene biosynthesis in the glandular trichomes of Spearmint (*Mentha spicata*). Plant Physiol. 1989; 89:1351–7.

[4] McCaskill D, Gershenzon J. Croteau R. Morphology and monoterpene biosynthetic capabilities of secretory cell clusters isolated from glandular trichomes of peppermint (*Mentha piperita* L.). Planta. 1992; 187:445–54.

[5] Fialová S, Halušová L, Leitmanová G, Tekeľová D, Grančai D. Hodnotenie vybraných druhov rodu *Mentha* L. na základe kvantitatívnej analýzy silicových orgánov. Farm Obzor. 2011; 80:165-8.

[6] Šarić-Kundalić B, Fialová S, Dobeš Ch, Ölzant S, Tekeľová D, Grančai D, Reznicek G , Saukel J. Multivariate numerical taxonomy of *Mentha* species, hybrids, varieties and cultivars. Sci Pharm. 2009; 77:851-76.

[7] Felklová M, Nátherová L, Lindauerová T. Siličné orgány na listech různých taxonů rodu *Mentha* L. Českoslov. Farm. 1986; 35(1):1 - 5.

[8] Nátherová Ľ, Lindauerová T. Silicové orgány na listoch súkvetia niektorých taxónov rodu *Mentha* L. Farm.Obz., 1986; 55(6):241-7.

[9] Maffei M, Chialva F, Sacco T. Glandular trichomes and essential oils in developing peppermint leaves. New Phytol. 1989; 111:707-16.