# NUTLET MORPHOLOGY AND ITS TAXONOMIC SIGNIFICANCE IN THE GENUS MENTHA L. (LAMIACEAE) FROM TURKEY

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## **Abstract**

The nutlet morphology of 11 taxa of *Mentha* L. (*M. pulegium*, *M. aquatica*,  $M. \times piperita$ ,  $M. \times dumetorum$ , M. spicata subsp. spicata, M. spicata subsp. tomentosa,  $M. \times villoso-nervata$ , M. longifolia subsp. tomentosa, tomentosa, total subsp. total subsp. tomentosa, total subsp. total subs

## Introduction

Mentha L., one of the most important genera of the family Lamiaceae, has worldwide distribution and it consists of perennial aromatic herbs. Some Mentha species, such as M. pulegium L., M. longifolia (L.) Huds., M. spicata L., M. × piperita L. and M. × villoso-nervata Opiz, are traditionally used in folk medicine (Baytop, 1999). Mint oil and their constituents obtained from different species of Mentha are also used in perfumery, cosmetics and food industries (Kokkini, 1994).

Mentha is a taxonomically difficult genus because of extensive hybridization, vegetative propagation, polyploidisation and cultivation (Harley, 1972; Harley and Brighton, 1977; Tucker et al., 1980). The genus comprises 18 species and 11 hybrids placed into four sections, namely Pulegium, Tubulosae, Eriodontes and Mentha according to the latest taxonomic treatment (Tucker and Naczi, 2007). Harley (1982) recognized 11 Mentha taxa belonging to two sections (Pulegium and Mentha) from Turkey and then two hybrids have been added to Flora of Turkey (Tarimcilar and Kaynak, 1997a, b). In this study, the treatment of Harley (1982) has been followed for the nomenlature of Mentha.

There are some studies about monophyly of *Mentha* and phylogenetic relationships within the genus (Gobert *et al.*, 2002; Bunsawat *et al.*, 2004; Shasany *et al.*, 2005). Saric-Kundelic *et al.* (2009) investigated the utility of morphological, anatomical and phytochemical characters for the identification of *Mentha* species, hybrids, varieties and cultivars in Bosnia-Herzegovina and Slovakia. In various genera of family Lamiaceae, the nutlet morphology, anatomy, pericarp structure and their taxonomic significance have been reported by some studies (Husain *et al.*, 1990; Marin *et al.*, 1994; Ryding, 2010). However, accounts on the mericarp morphology of some

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taxa of *Mentha* examined in this study are rather limited (Duletic-Lausevic and Marin, 1999; Moon *et al.*, 2009). We aim in this study, with the aid of scanning electron microscope (SEM), to provide detailed data on nutlet morphology of 11 *Mentha* taxa found in Turkey and to determine which characteristics of their nutlets may be used for taxonomic purposes.

## **Materials and Methods**

#### Plant materials:

Nutlets of 11 taxa of *Mentha* collected from different parts of Turkey were investigated. The materials used in this study were composed mainly of herbarium specimens, which were deposited in the herbarium of Uludag University (BULU). The specimens used for SEM micrographs were presented in Table 1.

# Nutlet size and SEM analyses:

For nutlet length and width, 50 nutlets were measured per taxon. However, at least 10 nutlets were measured for hybrids. In order to ensure that the nutlets were of normal size and maturity, they were examined using a stereomicroscope. For SEM, nutlets of taxa were transferred directly to a double-sided tape-affixed stub and were coated with gold-palladium, using a BAL—TEC SCD 005 sputter. The micrographs were obtained from a CARL ZEISS Evo 40 SEM using a voltage of 20 kV at the Microscopy Laboratory of Science and Art Faculty of Uludag University. The micrographs were used to describe surface sculpturing type of nutlets. The terminology for nutlet shape and surface sculpturing mainly follows that of Barthlott (1981) and Stearn (1983).

Table 1. List of taxa used for SEM micrograph (GT- Gül Tarımcılar).

No.	Taxon	Collection data	Vouchers
1	M. aquatica L.	A2 Bursa: Fadilli village, 9 m, 3.9.2004	GT 30514
2	M. × dumetorum Schult.	A1Kirklareli: Babaeski, 60 m, 23.8.2003	GT 30448
3	M. pulegium L.	A2 Istanbul: Cavusbasi, 16.8.2005	GT 30533
4	<i>M. longifolia</i> (L.) Huds. subsp. <i>longifolia</i>	A2 Bursa: Gemlik, Hayriye village, 10 m, 8.9.2006	GT 30592
5	M. longifolia (L.) Huds. subsp. typhoides (Briq.) Harley	A2 Istanbul: Sile, 15.8.2005	GT30530
6	$M. \times piperita$ L.	A2 Istanbul: Cavusbasi, Kavaklık, 16.8.2005	GT 30535
7	M. × rotundifolia (L.) Huds.	B1 Balikesir: Bandırma to Erdek, 130 m, 27.8.2004	GT 30508
8	M. spicata L. subsp. spicata	A1 Tekirdag: 1 km to Hayrabolu, 70 m, 23.8.2003	GT 30452
9	M. spicata L. subsp. tomentosa (Briq.) Harley	A2 Bilecik: Pazaryeri, Bahcesultan, 1050 m, 6.9.2006	GT 30562
10	M. suaveolens Ehrth.	A2 Yalova: Sultaniye, 25 m, 7.6.2006	GT 30570
11	M. × villoso-nervata Opiz.	B1Canakkale: Saros, Kocacesme village, 35 m, 25.8.2004	GT 30470

# **Results and Discussion**

The characteristics of nutlet (i.e. size, colour, presence or absence of trichomes and surface sculpturing) are summarized in Table 2. Micrographs of nutlets belonging to all studied taxa are presented in Figures 1-4. We found that the shape of all studied nutlets was broadly oblong or ovoid and that nutlet colour varied from pale to dark brown. The nutlets of *M. pulegium, M. aquatica* and *M. dumetorum* were pale brown, while those of *M. × piperita*, *M. × villoso-nervata* 

and  $M. \times rotundifolia$  were dark brown. However, the colour of the nutlets in M. spicata subsp. spicata, M. spicata subsp. tomentosa, M. longifolia subsp. longifolia, M. longifolia subsp. typhoides and M. suaveolens varied from chestnut brown to dark brown. Moreover, short or long trichomes were observed on the surface of nutlets of M. aquatica,  $M. \times dumetorum$ , M. spicata subsp. tomentosa and M. longifolia subsp. longifolia. Nutlet size ranged from 0.54 to 0.97 mm in length and from 0.37 to 0.66 mm in width. The smallest nutlet was found in  $M. \times villoso-nervata$  and the biggest nutlet was found in M. aquatica (Table 2).

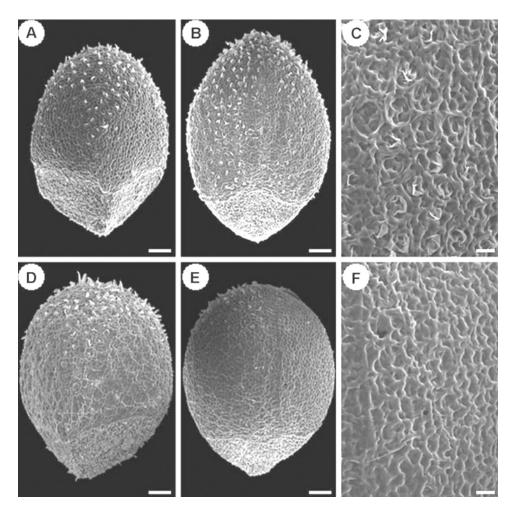


Fig. 1. SEM micrographs of nutlets of *Mentha aquatica* (A-C); *M*. × *dumetorum* (D-F); Ventral view (A, D); dorsal view (B, E); surface sculpturing (C, F). Scale bars: A, B, D, E = 100 μm; C, F = 20 μm.

Under SEM, three types were observed in the *Mentha* taxa based on surface sculpturing pattern:

**Type I.** Distinctly bireticulate: a surface with penta- or hexagonal-shaped small cells, and the walls of these cells are high, irregular and having depressions. This sculpturing pattern was seen in M. aquatica and M.  $\times$  dumetorum (Fig. 1C, F).

Table 2. Nutlet characteristics of the studied taxa of Mentha L.

Taxon	Length (mm)	Width (mm)	Sculpture	Presence/	Colour	Figures
	Mean± SD Mean± SD		absence of trichomes			
M. aquatica	0.9± 0.07	0.6± 0.05	TYPE I	short hair	pale brown	Fig. 1A-C
$M. \times dumetorum$	$0.8 \pm 0.15$	$0.6 \pm 0.06$	TYPE I	short hair	pale brown	Fig. 1D-F
M. pulegium	$0.7 \pm 0.01$	$0.5 \pm 0.04$	TYPE II	absent	pale brown	Fig. 2A-C
M. × piperita	$0.7 \pm 0.04$	$0.5 \pm 0.04$	TYPE II	absent	dark brown	Fig. 2D-F
M. spicata subsp. spicata	$0.8 \pm 0.02$	$0.6 \pm 0.01$	TYPE II	absent	chestnut to dark brown	Fig. 2G-I
M. spicata subsp. tomentosa	$0.7 \pm 0.01$	$0.5 \pm 0.01$	TYPE III	scarcely hair	chestnut to dark brown	Fig. 3A-C
M. longifolia subsp. longifolia	$0.6 \pm 0.06$	$0.5 \pm 0.07$	TYPE III	long hair	chestnut to dark brown	Fig. 3D-F
M. longifolia subsp. typhoides	$0.7 \pm 0.01$	$0.5 \pm 0.03$	TYPE III	absent	chestnut to dark brown	Fig. 3G-I
M. × villoso-nervata	$0.6 \pm 0.02$	$0.4 \pm 0.03$	TYPE III	absent	dark brown	Fig. 4A-C
M. suaveolens	$0.6 \pm 0.02$	$0.5 \pm 0.02$	TYPE III	absent	chestnut to dark brown	Fig. 4D-F
$M. \times rotundifolia$	$0.6 \pm 0.02$	$0.4 \pm 0.01$	TYPE III	absent	dark brown	Fig. 4G-I

**Type II.** Inconspicuously bireticulate: a surface covers inconspicuously penta- or hexagonal-shaped small cells, and these cells having various walls. *M. pulegium*, *M. × piperita* and *M. spicata* subsp. *spicata* exhibited this type of sculpturing. Only in *M. pulegium*, the nutlets with cells having rigid cell boundary and having star-shaped extensions at their centres (Fig. 2C). The nutlets of *M. × piperita* and *M. spicata* subsp. *spicata* with cells having wrinkled or often unclear walls (Fig. 2F, I).

**Type III.** Reticulate: a surface with penta- or hexagonal-shaped cells having large lumen and smooth, regular walls and forming a net-like appearance on their surface. The nutlets of *M. spicata* subsp. *tomentosa*, *M. longifolia* subsp. *longifolia*, *M. longifolia* subsp. *typhoides*, *M.* × *villosonervata*, *M. suaveolens* and *M.* × *rotundifolia* exhibited this type (Figs 3C, F, I; 4C, F, I).

When the nutlet characteristics of the investigated *Mentha* taxa were compared with previous literature (Ball, 1972; Borisova, 1977; Tarimcilar and Kaynak, 2002), our results are more or less similar to their findings. The shape of nutlets examined in this study was broadly oblong or ovoid. Borisova (1977), Harley (1982) and Tarimcilar and Kaynak (2002) have reported that the nutlet shape of the genus *Mentha* varies from globose to ovoid or obovoid.

Duletic-Lausevic and Marin (1999) found nutlet dimensions  $0.7 \times 0.5$  mm in M. pulegium and M. longifolia,  $0.8 \times 0.6$  mm in M. aquatica,  $0.6 \times 0.4$  mm in M. spicata and M.  $\times$  rotundifolia, and  $0.6 \times 0.5$  mm in M. suaveolens. Moon et al. (2009) examined nutlet characteristics (i.e. size, colour, shape and surface sculpturing) of Mentha aquatica, M. longifolia, and M. suaveolens and reported the length and width measurements (mm) as  $1\pm0.05 \times 0.7\pm0.02$ ,  $0.6\pm0.03 \times 0.5\pm0.02$  and  $0.6\pm0.03 \times 0.4\pm0.02$ , respectively. Nutlet shape of these taxa is widely elliptic, surface sculpturing type is reticulate, and colour varies from yellowish brown to reddish dark brown (Moon et al., 2009).

According to our results, the nutlets of *M. aquatica*, *M. × dumetorum*, *M. spicata* subsp. *tomentosa* and *M. longifolia* subsp. *longifolia* have trichomes. The presence or absence of trichomes on nutlet is an important character to discriminate *M. longifolia* subsp. *longifolia* and subsp. *typhoides* which have the similar nutlet size, sculpturing and colour (Table 2). On the other

hand, Duletic-Lausevic and Marin (1999) stated that the nutlets of *M. spicata*, *M. rotundifolia* and *M. suaveolens* lack trichomes and that *M. aquatica* and *M. longifolia* exhibit nutlets with or without trichomes.

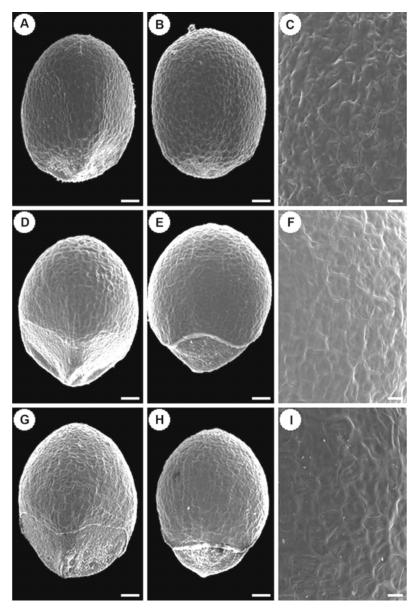


Fig. 2. SEM micrographs of nutlets of *M. pulegium* (A-C);  $M. \times piperita$  (D-F); M. spicata subsp. spicata (G-I). Ventral view (A, D, G); dorsal view (B, E, H); surface sculpturing (C, F, I). Scale bars: A, B, D, E, G, H = 100  $\mu$ m; C, F, I = 20  $\mu$ m.

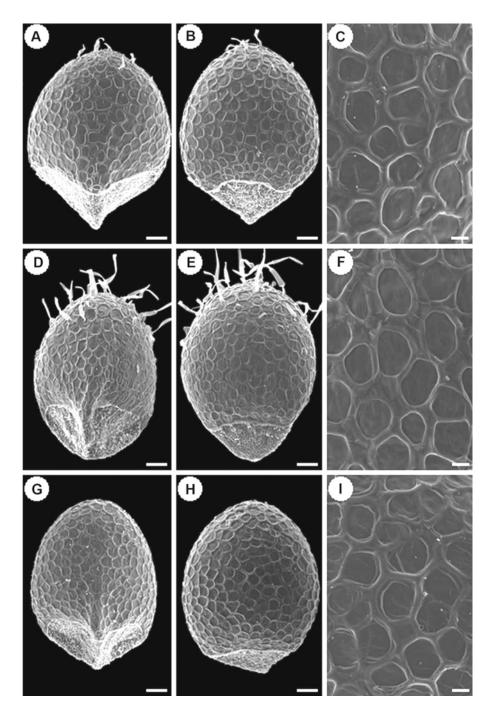


Fig. 3. SEM micrographs of nutlets of *M. spicata* subsp. *tomentosa* (A-C); *M. longifolia* subsp. *longifolia* (D-F); *M. longifolia* subsp. *typhoides* (G-I). Ventral view (A, D, G); dorsal view (B, E, H); surface sculpturing (C, F, I). Scale bars: A, B, D, E, G, H =  $100 \mu m$ ; C, F, I =  $20 \mu m$ .

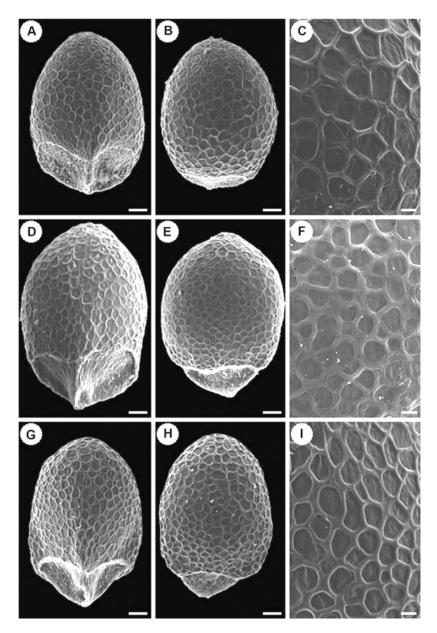


Fig. 4. SEM micrographs of nutlets of *M.* × *villoso-nervata* (A-C); *M. suaveolens* (D-F); *M.* × *rotundifolia* (G-I). Ventral view (A, D, G); dorsal view (B, E, H); surface sculpturing (C, F, I). Scale bars: A, B, D, E, G, H = 100 μm; C, F, I = 20 μm.

Mentha taxa employed in this study can be divided into three informal groups, with regard to nutlet characteristics basically sculpturing patterns. Group I includes M. aquatica, M.  $\times$  dumetorum (M.  $aquatica \times M$ . longifolia) and they are similar to each other both in terms of the morphological features and the nutlet characteristics. However, M.  $\times$  dumetorum differs from M. aquatica in its more oblong spikes and narrower leaves (Tarimcilar and Kaynak, 1997a, 2002).

Group II consists of M. pulegium, M. spicata subsp. spicata and M.  $\times$  piperita (M.  $aquatica \times M$ . spicata). Of the studied M entha taxa, only M. pulegium is located in sect. Pulegium, whereas the others are included in sect. M entha. Sect. Pulegium is distinguished from sect. M entha by its bracts similar to leaves, tubular calyx, weakly 2-lipped, with distinctly unequal calyx teeth, hairy within calyx throat, gibbous corolla tube. Sect. M entha have variable bracts, calyx tubular or campanulate, with more or less equal calyx teeth, glabrous calyx throat and straight corolla tube (Harley, 1982). Moreover, the inflorescence of M.  $\times$  piperita is morphologically similar to M. spicata in that it forms a terminal spike, but it differs from M. aquatica in its more lanceolate leaves that have shorter petioles (3-9 mm or rarely more).

Group III includes *M. spicata* subsp. *tomentosa*, *M. longifolia* subsp. *longifolia*, *M. longifolia* subsp. *typhoides*, *M.* × *villoso-nervata*, *M. suaveolens* and *M.* × *rotundifolia*. The nutlet surfaces of this group are covered with penta- or hexagonal-shaped cells that form a particularly net-like appearance. *M.* × *villoso-nervata* (*M. spicata* × *M. longifolia*) is morphologically different from the parents in its narrower spikes and smaller leaves and calyx (Tarimcilar and Kaynak, 1997b, 2002). *M.* × *rotundifolia* (*M. suaveolens* × *M. longifolia*) resembles *M. suaveolens* in its pale green and strongly rugose leaves, but it differs in that its leaves are more oblong and have an acute apex (Harley, 1982; Tarimcilar and Kaynak, 2002).

Hybrids can be distinguished from their parental species in terms of some nutlet features. As seen in Table 2, M. × dumetorum mainly differs from M. aquatica and M. longifolia with its smaller and distinctly bireticulate sculpturing nutlet. The nutlets of M. × piperita are smaller than those of M. spicata subsp. spicata, but they are more similar to M. spicata than M. aquatica in terms of nutlet characteristics. They are easily distinguishable from M. aquatica due to its inconspicously bireticulate, glabrous and dark brown nutlet. M. × villoso-nervata differs from M. longifolia subsp. longifolia and M. spicata subsp. spicata by its glabrous, dark brown and reticulate nutlet, respectively. The nutlet characteristics of M. × rotundifolia and M. suaveolens display a great similarity with each other.

A key can be established based on nutlet chacteristics for Turkish *Mentha* taxa:

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1	Nutlet sculpturing bireticulate Nutlet sculpturing reticulate	2 6
2	Nutlet sculpturing distinctly bireticulate Nutlet sculpturing inconspicously bireticulate	3 4
3	Nutlets at least 0.83 mm long Nutlets at least 0.65 mm long	M. aquatica M. × dumetorum
4	Nutlets 0.78-0.82 mm long Nutlets shorter than 0.78 mm	M. spicata subsp. spicata 5
5	Nutlet cells with star-shaped extensions at their centres Nutlet cells without star-shaped extensions at their centres	M. pulegium M. × piperita
6	Nutlets without hair Nutlets with hair	7 10
7 -	Nutlets 0.47-0.53 mm wide Nutlets 0.37-0.43 mm wide	8 9
8	Nutlets 0.69-0.71 mm long Nutlets 0.58-0.62 mm long	M. longifolia subsp. typhoides M. suaveolens

- 9 Nutlets at least 0.39 mm wide
- Nutlets at least 0.37 mm wide
- 10 Nutlets 0.69-0.71 mm long
- Nutlets 0.54-0.66 mm long

M. × rotundifolia M. × villoso-nervata

M. spicata subsp. tomentosa M. longifolia subsp. longifolia

The utility of nutlet characters, i.e. shape, size, presence or absence of hairs, nature of indumentum, surface sculpturing, exocarp cellular morphology and anatomy of the nutlet has been shown at various taxonomic levels in different genera of Lamiaceae (Husain *et al.*, 1990; Marin *et al.*, 1994; Duletic-Lausevic and Marin, 1999; Moon and Hong, 2006). Our findings also showed that the nutlet size, presence/absence of trichomes, surface sculpturing pattern are valuable diagnostic characteristics for separating closely related taxa of *Mentha*. In conclusion, we can say that nutlet morphological characteristics combined with other morphological characters can be used for delimination of taxa at the species and infraspecific levels in the genus *Mentha*. Furthermore, this study provides the detailed data on the nutlet features of Turkish *Mentha* taxa.

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