

FRUIT MORPHOLOGY, ANATOMY AND MIXOCARPY IN *NEPETA CATARIA* L. 'CITRIODORA' AND *NEPETA GRANDIFLORA* BIEB. (LAMIACEAE)

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The results of a complete study of nutlet morphology, anatomy and mixocarpy in two cultivated species of *Nepeta* genus (*Lamiaceae*, *Nepetoideae*) are investigated. The cultivated species are represented by *Nepeta cataria* L. 'Citriodora' and *Nepeta grandiflora* Bieb. (Sect. *Nepeta*). Two main types of pericarp pattern, smooth and sculptured, are described. A detailed description of the anatomy, macromorphology and micromorphology of *Nepeta*'s nutlets is presented. The mucilage production (mixocarpy) has also been tested. Original photographs and SEM images for mentioned taxa are provided. A polytomous morpho-anatomical identification key based on the nutlets's characters was made.

Key words: *Nepeta*, *Lamiaceae*, nutlets, micro- and macromorphology, anatomy, mixocarpy.

INTRODUCTION

The genus *Nepeta* L. contains some 250 species that are distributed only in the Old World, from Pacific Ocean to the Atlantic (9). *N. cataria* is a perennial herb, stem erect to 1m, branched, grey-pubescent to tomentose. Leaves ovate, cordate at the base, serrate; 'Citriodora': lemon-scented. Formerly cultivated as a medicinal herb and widely naturalized in N. & W.C. Europe (15). *N. grandiflora* is a perennial, stem erect to 40-80cm, branched, glabrous to minutely pubescent. Leaves ovate, cordate at the base, crenulate (6); a frequent casual in E. & E.C. Europe and locally naturalized (15). *N. grandiflora* was cited for several authors in the Flora of Romania; it was probably cultivated in gardens for ornament and occasionally naturalized (4).

Morphology, shape, color and size of the nutlets were used as diagnostic characters in classification. Many authors (1, 5, 7, 11, 13, 15) have demonstrated the usefulness of pericarp structures in *Lamiaceae* classification. Nutlet morphology in the *Lamiaceae* has proved useful to varying degrees at different levels of the taxonomic hierarchy. The importance of the morphology of nutlet surface has already been demonstrated for *Nepeta* (7, 10). In recent times the importance of the SEM in the study of nutlet surfaces has been demonstrated for various species of *Nepeta* (1, 2, 3, 8).

The objective of this paper is to provide a detailed description of the pericarp anatomy and morphology in two cultivated species of *Nepeta*: *N. cataria* L. 'Citriodora' and *N. grandiflora* Bieb. The SEM images and anatomical photographs of investigated species are presented for comparison.

MATERIAL AND METHODS

The morpho-anatomical study is based on the nutlets taken from herbarium material of different Botanical Gardens or collected from fieldwork. The investigated taxa and their origin are given in Table 1. Study of shape and surface structure and measurements of the nutlets (macromorphology) were made using a Stereomicroscope. The images for micromorphology were taken with a Scanning Electron Microscope (SEM) Hitachi S-570 at 10 kV. Samples were coated with 30nm gold using an Emitech K 550 Sputter Coater.

Table 1

Nepeta L. taxa and the source of investigated materials

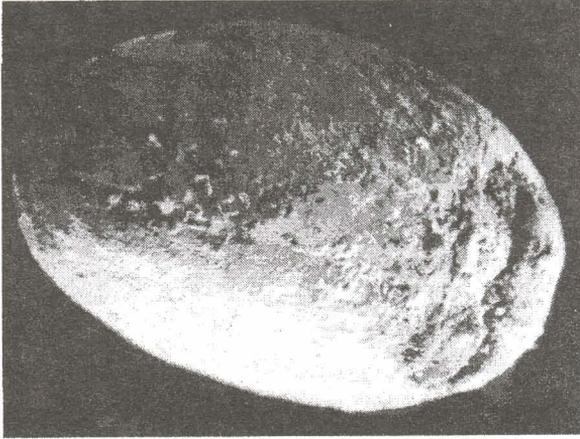
Taxa	Provenience and year of harvesting	Investigations
<i>Nepeta cataria</i> L. ' <i>Citriodora</i> '	Iași, 1999 (Romania); Toplița, 2001 (Romania)	Macromorphology and mixocarpy
	Iași, 1999 (Romania); Mainz, 1998 (Germany)	Micromorphology (SEM)
	Iași, 1999 (Romania)	Anatomy
<i>Nepeta grandiflora</i> Bieb.	Zagreb, 2001 (Croatia); Poznan, 2000 (Poland); Ulm, 2000 (Germany); Innsbruck, 1999 (Austria); Giessen, 1999 (Germany)	Macromorphology and mixocarpy
	Meisse, 2001 (Germany); Kew, 1969 (England)	Micromorphology (SEM)
	Poznan, 2000 (Poland)	Anatomy

For the investigations of mixocarpy, at least five nutlets were treated with distilled water, and the thickness of the mucous layer was measured under light microscope. The measurements were taken every 15 minutes during the first hour, and then after each hour during 8 hours. No mucilage production after 8 hours was taken to indicate the absence of mucilage reaction (12).

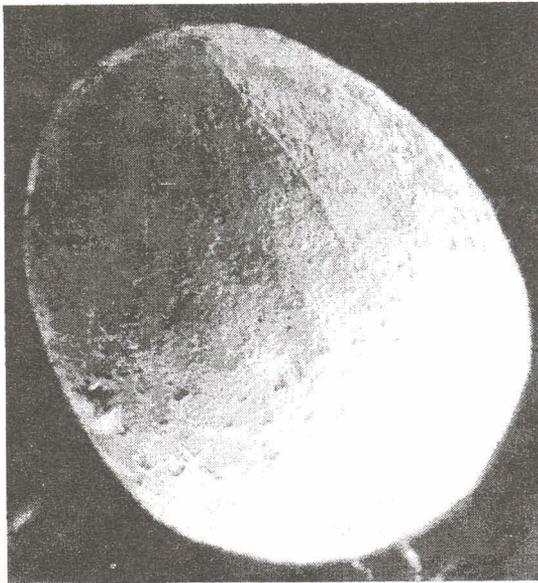
For the anatomical study, the nutlets were sectioned by microtome after fixing in ethyl alcohol fixative, and embedded in paraffin according to the standard method. After cutting (10 μ), removing the paraffin and staining with Blue methylene, the sections were finally embedded in Canada balsam. The cross sections of the nutlets were studied for the pericarp characteristics and the measurements of pericarp and schlerenchyma region thickness were done; afterwards, the sections were photographed from permanent slides by MC-7 microscope, using Exacta camera and Kodak Gold 200 Dx film. The SEM images and anatomical photographs of nutlets are presented and discussed in Figures 1, 3 for *N. cataria* '*Citriodora*' and in Figures 2, 4 for *N. grandiflora*.

RESULTS AND DISCUSSION

The nutlets size in *N. cataria* 'Citriodora' is 1.6–1.8 × 1.0–1.1 mm; they are brownish, elliptic, apically rounded or truncate, rounded-trigonous in transverse section with a whitish lateral straight (180°) or bilobed areole, slightly hidden (Figure 1A).



A.



B.

Fig. 1 – Nutlets of *Nepeta cataria* L. 'Citriodora' (SEM images):
A – Bilobed areole on the ventral side (113×);
B – Smooth pericarp on the dorsal side (119×).

The nutlets in *N. grandiflora* are brown, oblong or elliptic, with a hidden U-shaped or arcuate areole (Figure 2B).

The nutlet size is $1.8\text{--}2.1 \times 0.9\text{--}1.0$ mm, apically rounded, trigonous. Two main types can be recognized based on surface ornamentation: smooth (reticulate) at *N. cataria* '*Citriodora*' (Figure 1B) and sculptured (tuberculate) at *N. grandiflora* (Figure 2B).

Table 2 presents the anatomical data on the thickness of the pericarp and sclerenchyma region, the presence of crystals in the sclerenchymatous region or in the innermost layer of the mesocarp and mucilage reaction.

Table 2

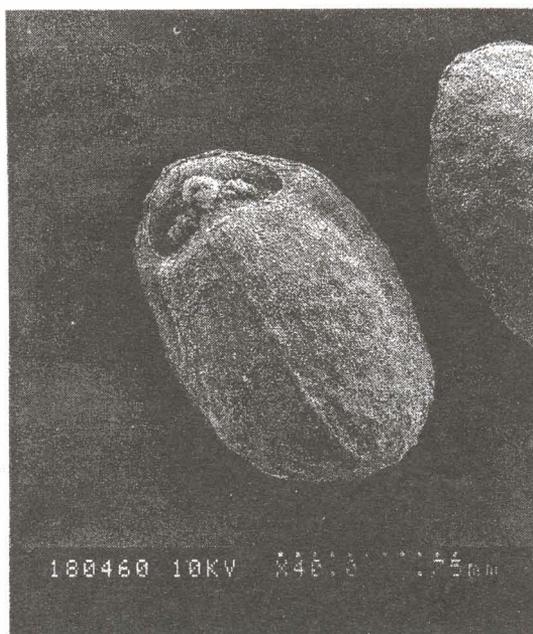
Anatomical characters of pericarp nutlets in *Nepeta* species

Anatomical characters of the pericarp	<i>N. cataria</i> ' <i>Citriodora</i> '	<i>N. grandiflora</i>
1. Number of cell layers in the mesocarp	2-3	4-5
2. Number of cell layers in the sclerenchyma zone	1	1
3. Presence or absence of prismatic crystals in the innermost layer of the mesocarp	c-	c-
4. Shape of sclerenchyma cells (two letters in combination indicate intermediates): bone cells with the lumen enlarged at the center (c), bone cells with the lumen enlarged below the center (b)	c/b	c
5. Presence or absence of crystals in the sclerenchyma region	c+	c+
6. Exocarp thickness (μm)	3.1-6.2	3.1-6.2
7. Mesocarp thickness (μm)	6.2-9.4	9.4-12.5
8. Sclerenchyma region thickness (μm)	59.2-68	46.8-53
9. Endocarp thickness (μm)	6.2-9.4	3.1-6.2
10. Pericarp thickness (μm)	74.7-93	62.4-77.9
11. Ratio of pericarp to sclerenchyma region thickness (%)	73	68
12. Mucilage production [#] on the nutlets upon wetting	+/-	+/-

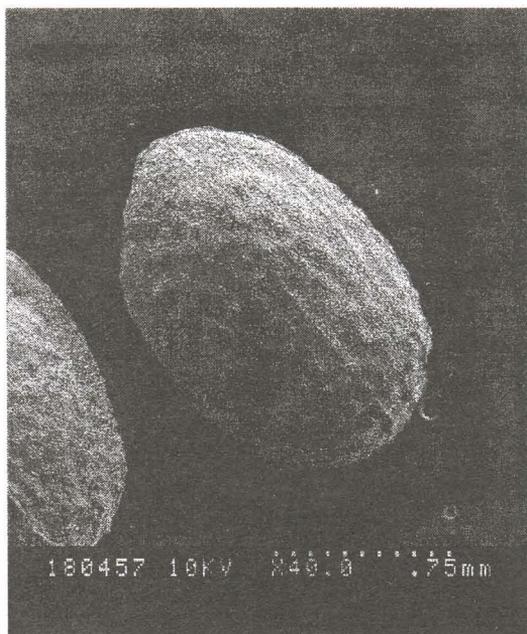
* All measurements were made at oc. $12.5\times$; $20\times$

+/-: very weak reaction or swelling (< 0.05 mm)

The nutlet pericarp of *N. cataria* '*Citriodora*' was $78\text{--}93$ μm . The exocarp is one-layered ($3.1\text{--}6.3$ μm) and the mesocarp ($6.3\text{--}9.3$ μm) is dark-coloured due to the pigments. The sclerenchyma region was one-layered with vertically arranged bone cells ($59.2\text{--}68$ μm) with a small and rounded lumen in the center. Numerous crystals were observed in the sclerenchyma region. The endocarp ($6.3\text{--}9.4$ μm) consisted of one thin layer of tabular parenchymatous cells with thickenings in the cell walls. The ratio of pericarp to sclerenchyma region thickness is about 73%. The pericarp structure of *N. cataria* '*Citriodora*' is shown in Figure 3.



A.



B.

Fig. 2 – Nutlets of *Nepeta grandiflora* Bieb. (SEM images): **A** – Arcuate areole on the ventral side (40×); **B** – Sculptured pericarp (tuberculate) on the dorsal side (40×).

The pericarp of *N. grandiflora* was 62.4–78 μm . Exocarp was one-layered (3.1–6.3 μm) and mesocarp (9.3–12.5 μm with 2-4-layers) was thin and dark-coloured. The bone cells of sclerenchymatous region (46.8–53 μm) were vertically arranged, with large crystals in the luminal cavity at the center. The endocarp is one-layered, very flat (3.1–6.3 μm). The ratio of pericarp to sclerenchyma region thickness is about 68%. Transverse section of the pericarp of *N. grandiflora* is shown in Figure 4.

Based on the characters of surface ornamentation and anatomical features, an original polytomous identification key is presented below:

A The color of the fruits: light brownish (**A_B** brown)

B The shape of the nutlets: elliptic (**B₀** \pm oblong to elliptic)

C The color of the areole: whitish

D Lack of multicellular simple hairs apically (**D_H** thin hairs occur in very young nutlets but later are absent)

E The fruit surface: unshiny

F The shape of the hilum: obviously bilobate areole, U-shaped or arcuate (**F_S** straight areole in 180°)

G The position of the hilum: hidden (**G_S** slightly hidden, **G_O** obviously hidden)

H The surface of nutlets (**H_S** smooth, **H_T** tuberculate)

I Mixocarp: present

J Number of cell layers in the mesocarp (**J₂₋₃** 2-3-layered, **J₄₋₅** 4-5-layered)

K The shape of the luminal cavity of the bone cells (**K_R** rounded, **K_O** oval)

L The lumen position in bone cells (**L_C** with a small lumen at the center, **L_B** bone cells with an elliptical lumen enlarged below the center)

M Presence of crystals in the sclerenchyma region in the mesocarp

Nepeta cataria L. 'Citriodora' – **A B C D_H E F_S G_S H_S I J₂₋₃ K_R L_C M**
Nepeta grandiflora Bieb. – **A_B B₀ C D E F G_O H_T I J₄₋₅ K_O L_B M**

CONCLUSION

The morphology of fruits was investigated in two cultivated species of the genus *Nepeta*. Two main types can be recognized based on surface ornamentation of fruits: smooth (reticulate) and sculptured (tuberculate). The nutlet size varies from 1.6–1.8 \times 1.0–1.1 mm in *N. cataria* 'Citriodora' and 1.8–2.1 \times 0.9–1.0 mm in *N. grandiflora*. Their shape is elliptic or oblong, trigonous or rounded trigonous in

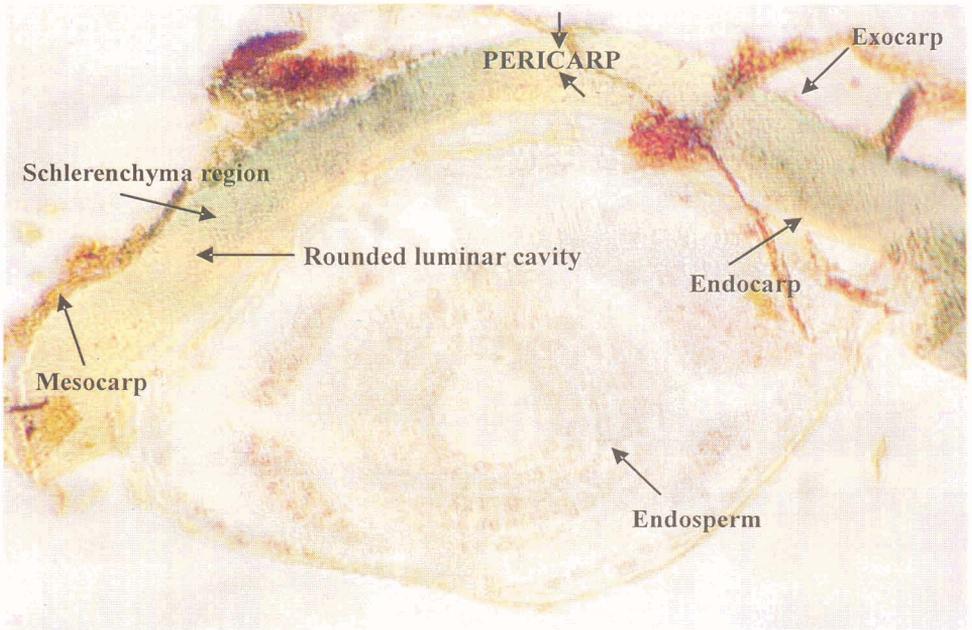


Fig. 3 – Fruit transverse section in *Nepeta cataria* L. 'Citriodora'
(orig., magnification oc. 12.5 × ob. 6).

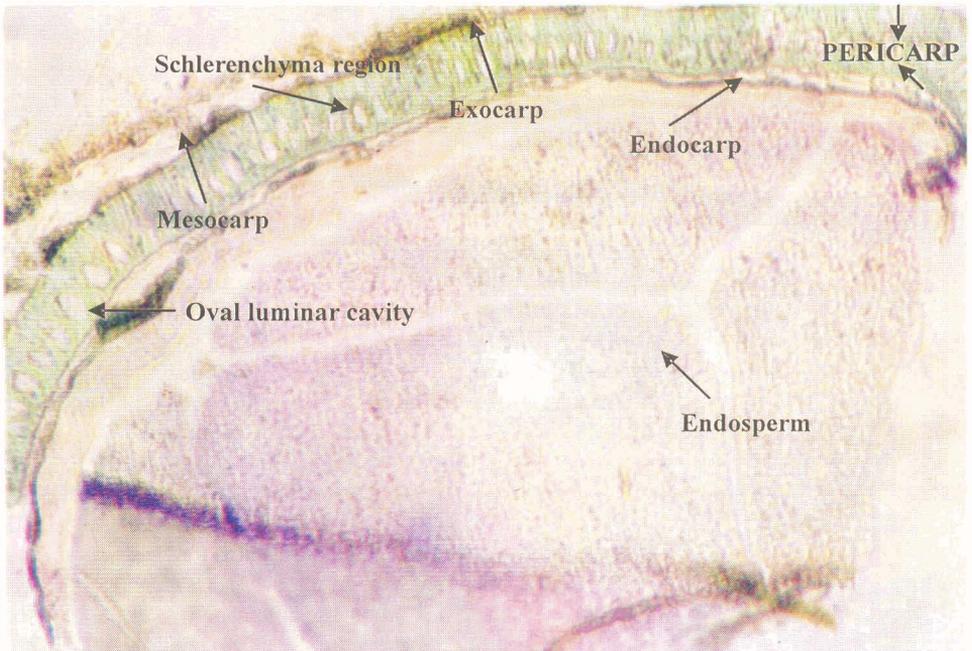


Fig. 4 – Fruit transverse section in *Nepeta grandiflora* Bieb.
(orig., magnification oc. 12.5 × ob. 6).

transverse section, and apically rounded, with a whitish lateral straight or bilobed and hidden areole, differently shaped. The exocarp and mesocarp are thin and dark-coloured. The bone cells of sclerenchyma region contain crystals. The shape of the luminal cavity in bone cells and their position are important in taxonomy on *Nepeta* L. genus.

From the original results obtained in this study it can be concluded that morphological and anatomical characteristics of the pericarp are useful as taxonomic characters at the species level in *Nepeta* genus.

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