Histological structure of the microsporangia, microsporogenesis and development of the male gametophyte in *Nepeta cataria (Lamiaceae)*

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Received: February 06, 2003 ▹ Accepted: April 04, 2003

Abstract. Histological structure of the anthers and embryological processes in the male generative sphere of the flower are studied for the first time in *Nepeta ca taria*. Within the genus formation of peltate glandular trichomes on the epidermis of the anthers has been established also for the first time. A strong trend towards formation of functionally female flowers has been observed.

Key words: *Lamiaceae*, male gametophyte, microsporogenesis, *Nepeta*, pollen, peltate glandular trichomes

Nepeta cataria L. is a perennial herbaceous plant, widely distributed in Bulgaria in weedy and stony places, at altitudes up to 1200 m. (Assenov 1989). It is a valuable aromatic (0.2–0.7 %), melliferous and medicinal plant used in perfumery and confectionery. In the official medicine the plant is applied as a vermicidal, coughing and spasmolytic herb, and in the folk medicine (Stojanov & Kitanov 1960) it is used to treat diarrhoea. The latest data mention its application in the treatment of cancer too (Hornok 1992).

N. cataria has not been studied embryologically. Data exist only on the pollen morphology from Erdtman (1945) who identified 6-colpate pollen and Waterman (1960) who found out 3-celled pollen.

The Bulgarian populations of *N. cataria* have been studied karyologically and $2n = 34$ chromosome numbers has been established (Markova & Nguen Thin Thu 1974).

The present work is aimed at a detailed study of the embryological processes in the male generative sphere in *N. cataria*.

Material and methods

The material for study (flower buds and flowers at different age) was collected in the period 2000–2001, in the vicinities of Balchik town (Northern Black Sea Coast). It was fixed with Navashin's mixture and treated according to the classical paraffin methods (Nikolov & Daskalov 1966). The staining was made with Heidenhain's hematoxylin. Sections 8–12 μm thick were cut with Minot rotation microtome and the observations were made with Amplival light microscope. The microphotos were made with MF-matik.

Results and discussion

In *N. cataria* four microsporangia differentiated in each anther, with walls formed according to the *Dicotyledonous* type. Apparently, this species is on a somewhat lower degree of evolutionary development of the anther loculi, because only two sporang-

iate anthers have been reported for the species of genus *Nepeta* (Turner 1972). Obviously, the number of loculi in the anthers within *Lamiaceae* is not the same for the different investigated species and varies from two (Yurukova-Grancharova & Daskalova 1992; Daskalova 1993) to four (Yurukova-Grancharova & Daskalova 1995, 2002).

A very characteristic element of the anthers is the formation of distinct placentoids typical of the representatives of *Lamiaceae* (Davis 1966; Dzevaltovsky 1979). They emerge at the earliest phases of anther development and degenerate a little prior to their splitting, predominantly at the stage of mature 3-celled pollen.

The anther walls in *N. cataria* are 4-layered, built of epidermis, endothecium, middle layer and tapetum of the secretory type (Plate I, Fig. 1), a specificitiy established so far in all investigated Bulgarian representatives of family *Lamiaceae* (Daskalova & Genova 1996a, b).

During the initial moments of anther development the three layers of the anthers (epidermis, endothecium and tapetum) are rather similar in their morphological features, and only the middle layer is built of narrower and tangentially elongated cells. Gradually, with the development of the anthers the layers undergo a number of significant morphological changes (Plate I, Fig. 1).

The most characteristic element of the epidermal cells is the formation of 1–4 glandular trichomes, often built of 6–8 cells. They are always arranged on the connective side of the anther and reach the maximum of their development in the period of mature flowers, when the embryo sacs are already ripe and probably help the successful pollination. Similar trichomes have been described in *Agastache foeniculum* (Yurukova-Grancharova & Daskalova 2002), *Hyssopus officinalis* (Yurukova-Grancharova & Daskalova 1995) and *Salvia tomentosa* (Daskalova & Genova 1996a). The establishment of glandular trichomes on the epidermis of the anthers is a valuable contribution made by this work, because so far it has been reported in the embryological literature (Wunderlich 1967) that the species of genus *Nepeta* do not contain trichomes. Already Schnarf (1917) had reached the conclusion that the absence or presence of glandular trichomes in the different species could be used as a valuable diagnostic characteristic within the *Lamiaceae*.

The epidermis in *N. cataria* preserves its vitality until the period of anther splitting.

In most anthers observed in *N. cataria* the endothecium develops fibrous thickenings, predominantly at the stage of uninucleate pollen, when the tapetum has already degenerated. Along with this, we have observed no fibrous thickenings in some loculi of the anthers in *N. cataria*. This phenomenon is typical chiefly for the male sterile plants (Tsikov & Tsikova 1981) and is an indication of the formation of functionally female flowers.

The middle layer in the anther can not be defined as ephemeral, contrary to the reports of Kamelina & Dzevaltovsky (1987) for *Lamiaceae*. Our observations have shown that it lingers for a comparatively long time and its cells continue to function occasionally up to the stage of microspore tetrads and in single anthers up to the stage of uninucleate pollen.

The tapetum in *N. catria* is of the secretory type and is built of polygonal or irregularly shaped cells, filled with thick, intensively stained cytoplasm (Plate I, Fig. 1). Already during the prophase, in most archesporial cells, the nucleus in the tapetum cells begins to divide. It is because at a later stage of the meiosis we have observed binucleate tapetum cells in the anthers, and in single cells the number of nuclei was reaching even four. Another typical specificity is the formation of 1–4 vacuoli in the tapetum cells at a later stage of the anther development, somewhere towards the end of heterotypic division in the macrospore mother cells. The tapetum in *N. cataria* degenerates mainly at the tetrad stage (Plate II, Fig. 2) in the anthers.

The sporogenous tissue in the anthers is prediominantly 1–3-layered, as it has been established in most investigatged species of *Lamiaceae* (Yurukova-Grancharova & Daskalova 1992, 1995, 2002).

At the initial stages of their development, the archesporic cells resemble much in their morphology the tapetum cells, but are larger in size (Plate I, Fig. 1). They gradually get rounded, grow in size, move apart from each other, and differentiate into microspore mother cells.

Meiosis in *N. cataria* runs normally (Plate I, Fig. 2; Plate II, Fig. 1), as it has been established for the species of *Lamiaceae* (Davis 1966; Dzevaltovsky 1979). We have observed some insignificant deviations in its running, mostly expressed in some lagging behind chromosomes and chromosome bridges. An indication of properly running meiosis is the presence of equal in size pollen grains at a later stage of development of most anthers.

Meiosis is simultaneous in type because the division of the four formed nuclei takes place only at the end of meiotic division (Plate II, Fig. 1).

Mention deserves the fact that after the simultaneous meiosis in the anthers of *N. cataria* only tetrahedral tetrads form (Plate II, фиг. 2), contrary to the reports of all researchers so far (Poddubnaya-Arnoldi 1982; Daskalova & Genova 1996a, b), who have found, although in a more insignificant degree, isobilateral tetrads in the investigated by them species of *Lamiaceae*. Single microspore tetrads in *N. cataria* have a very thick callose wall occurring mainly in the male sterile plants (Tsikov & Tsikova 1981; Daskalova 1993).

The mature pollen is 3-celled 6-colpate, as it was established by all researchers who have dealt in detail with the pollen morphology in the species of *Nepeta* (Leitner 1942; Waterman 1960) and occurs in the evolutionary most advanced taxa within *Lamiaceae*. The three-celled pollen consists of a sticke in shape vegetative nucleus and two spheroid sperms in close proximity to it. Often one of the two sperms cannot be seen because it is set behind the vegetative nucleus. The above-mentioned data about the pollen do not coincide with those reported by other authors (Kamelina & Dzevaltovsky 1987) who have found in the investigated species of *Nepeta* two fusiform sperms and a vegetative nucleus of irregular shape. Apparently, there are differences in this important embryological characteristic in the different species of this genus, which could be used for the more precise differentiation of the species on cytological level.

Mention also deserves the fact that in single anthers we have often observed germinated pollen grains, always through germanous colpi, i.e. these grains were of the monosyphonous type, contrary to the polysiphonous type established in *S. sclarea* (Daskalova 1995). In the germinated pollen grains usually first enters one sperms, followed by the vegetative nucleus and finally the other sperms. Germination of pollen in tetrasporangiate anthers contradicts the reports of Endress & Stumpf (1990), who have found it only in twosporangiate anthers in the species of *Lamiaceae*.

During the splitting of the anther we have often established in single loculi 1-2-3-celled uninucleate, pollen grains. Distribution of the pollen occasionally takes place by single pollen grains, and most frequently by polyades of 3 (Table II, Fig. 3), 5, 7 or more pollen grains combined in different configurations. In single anthers we have observed by way of exception conglomeration of all pollen grains in the still unsplit anthers, most of them germinated, which indicates cleistogamy in this species too, moreover that within the *Lamiaceae* cleistogamy was established in *Lamium amplexicaule* (Gorczynski 1929).

We have also established degeneration of pollen grains, usually most of them with a formed exine, but without cell content: a phenomenon typical of the male sterile plants (Tsikov & Tsikova 1981).

In *N. cataria* we have observed some strong degeneration processes, starting in single anthers already at the phrophase in some MMC. The tapetum in them was usually degenerated too. These facts have been also observed in the male sterile plants of the species of genus *Salvia* (Mohan & Kans 1987; Daskalova 1993). The degeneration processes continued at a later stage of development too, affecting single tetrads, pollen grains and even entire loculi of the anther. The degeneration processes observed by us, as well as formation of tetrads with a thick callose wall, the absence of starting off of fibrous thickenings in some anthers testified to the possibility that male sterile flowers may be present, which is not rare within the species of *Nepeta* (Turner 1972).

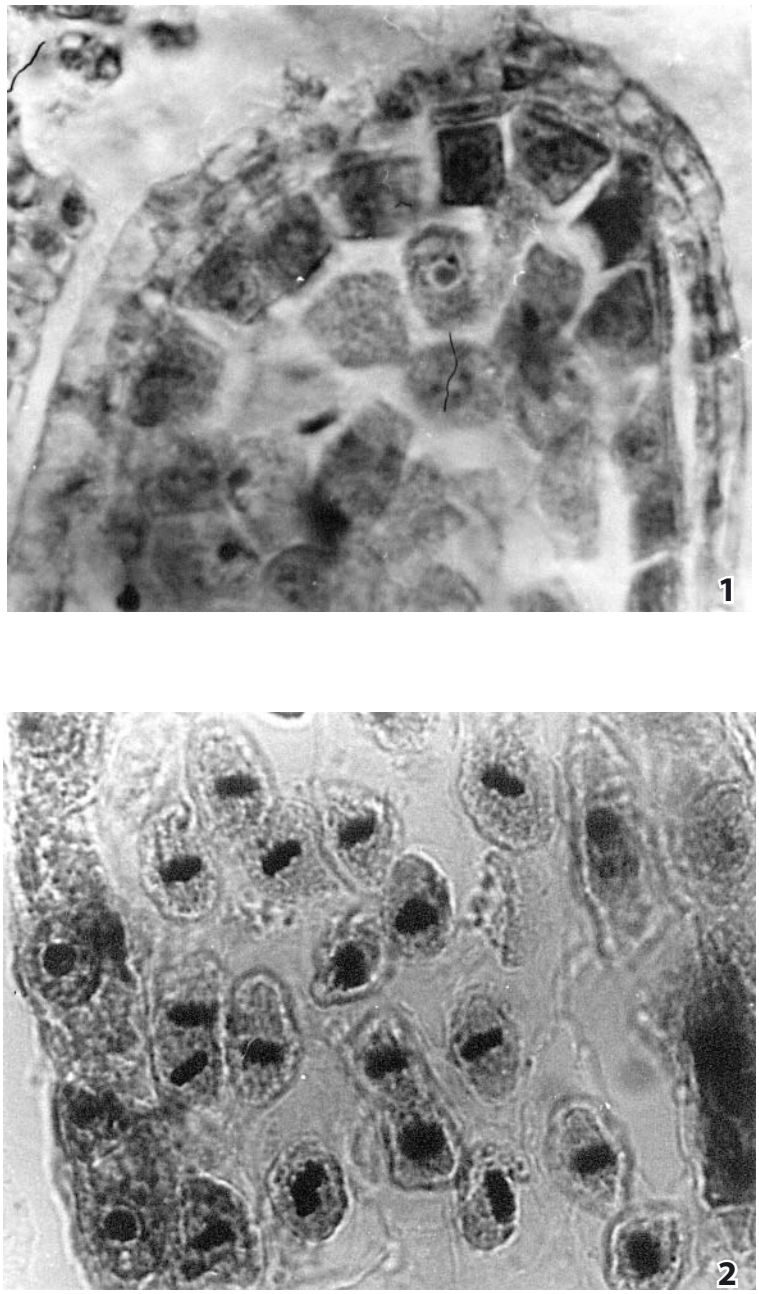
Conclusion

Most of the established embryological features – tetrasporangiate, 4-layered anthers, tapetum of the secretory type, normal meiosis of the simultaneous type, and 3-celled 6-colpate pollen – are typical of the so far studied species of *Lamiaceae*. They are another evidence of the invariable embryological characteristics in this family, as it was established by other researchers too (Wunderlich 1967). Along with this, the formation of tetrahedral tetrads alone testifies that not all embryological features established so far for *Lamiaceae* are characteristic on the species level.

The peltate glandular trichomes on the epidermis of the anthers, found for the first time within *Nepeta,* could be used for a more precise differentiation of the species.

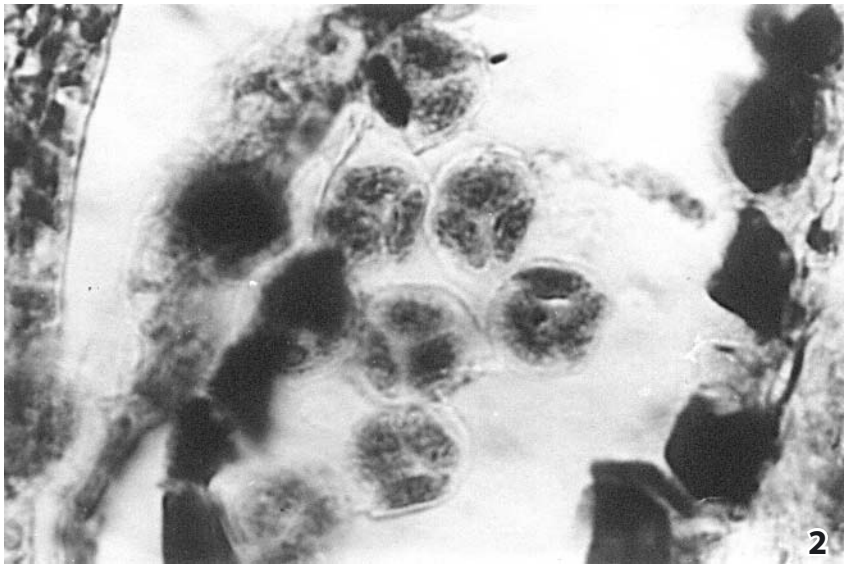
The strong degeneration processes observed during microsporogenesis and development of the male gametophyte, starting in some anthers already at the stage of prophase in MMC and affecting the tapetum, showed a distinct trend for transition to functionally female and even female flowers, widely distributed within the *Nepeta* (Turner 1972).

Plate I



Figs 1–2. *N. cataria*
1, a four-layered wall of the anther loculus with archesporial cells (×130); **2,** metaphse I in MMC (×130).

Plate II



Figs 1–3. *N. cataria*
1, metaphase I in MMC (×130); **2,** tetrahedral tetrads in the anther loculus with degenerated tapetum (×130); **3,** configuration of three interlinked pollen grains (×600).

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