Journal of Applied Biological Sciences 4 (2): 19-23, 2010
ISSN: 1307-1130, E-ISSN: 2146-0108, www.nobel.gen.tr

# Embryological study of *Gentiana cruciata L.* (Gentianaceae) from Bulgaria

Elina YANKOVA PetkaYURUKOVA
Bulgarian Academy of Sciences, Institute of Botany, Department of Plant Embryology, Sofia, BULGARIA

*Corresponding Author
**e-mail:** e_jankova@abv.bg

**Received :** May 15, 2010
**Accepted :** July 24, 2010

**Abstract**

Embryological study on the Bulgarian native populations of Gentiana cruciata L. has been carried out. The anther is tetrasporangiate. Its wall develops according to Dicotyledonous-type and consists of epidermis, fibrous endothecium, two middle layers and glandular tapetum. The simultaneous microsporogenesis leads to formation of predominantly tetrahedral microspore tetrads. The mature pollen grains are 2-celled. The ovule is anatropous, tenuinucellate and unitegmic with unicellular archesporium. The development of the embryo sac follows the Polygonum-type and rarely - the Allium-type. The legitimate embryo and endosperm develop after double fertilization. The embryogenesis runs according to Asterad-type. Initially, the endosperm is nuclear. In some ovules somatic apospory was established.

**Key words:** Gentiana, embryology, male and female gametophyte, embryo, endosperm

## INTRODUCTION

Gentiana cruciata belongs to the section Cruciata of the genus Gentiana [1]. In the Bulgarian flora it is found in rocky, grassy shrub-covered areas, in neglected arable lands and pastures of the foothills and mountains at the altitudes above 200 m, frequently between 1000 and 2000 m in Northwestern Bulgaria, Balkan Range (Northern and Central), districts of Sofia and Vitosha mountain, Western border mountains, Slaviyanka, Pirin, Rila, Sredna Gora, Rodopi Mts and part of the Tracian lowland. The national reserves of this species are abundant, but its populations are scattered and with a low density [2]. Up to now, embryological studies are carried out on the female generative sphere of G. cruciata [3,4,5,6]. The data on the anthers histology and development of the male gametophyte are incomplete, often contradictory [7,8] and on the embryogenesis – are missing. Bulgarian populations of G. cruciata are studied for the first time.

The aim of the present work is to receive some additional information that enriches the embryological characteristic not only of G. cruciata but also of the genus Gentiana.

## MATERIALS AND METHODS

The material of the study (flower buds and flowers at different stage of development) was collected from five native populations of G. cruciata (Sofia region, village Baikal, near the town of Pernik; Balkan Range (Central), the way to Yablanitsa; Rhodopi Mt (Eastern) - Beglika; Balkan Range (Western), Kozya stena summit); Pirin Mt (Banderitsa mountain hostel) and fixed in FAA mixture (formalin : glacial acetic acid : 70 % etanol in correlation 5:5:90 parts). The serial paraffin cuts with thickness 12-25 um made with rotary microtome are stained with Heidenhain's haematoxylin [9]. The permanent slides are embedded in Canada balsam. The observations and photography were carried out using an Olympus CX 21 microscope and "Infinity lite" Camera.

## RESULTS AND DISCUSSION

During the study, we observed no essential embryological differences between any of the studied populations of G. cruciata. The embryological features reported in the following descriptions, therefore, are common to all populations examined, unless particular comments are given.

**Anther and development of the male gametophyte.**

The anthers of G. cruciata are tetrasporangiate. The anther wall develops according to the Dicottyledonous-type [10] and prior to maturation usually comprises five layers: an epidermis, an endothecium, two middle layers and tapetum that are clearly distinguishable still at the time of prophase I of the meiosis in the microspore mother cells (MMCs) (Fig. 1 A).

The epidermal cells are large in size, almost rectangular and one-nucleate. During the anther ontogenesis they tangentially lengthened and rounded up. The cells of the endothecium are smaller in size but wider. They develop the typical for the family Gentianaceae fibrous thickenings after the microspore tetrads formation in the anthers.

The two middle layers are ephemeral that begin to degenerate to the end of the meiosis in MMCs. Then, their cells become flattened pressed from the enlarging cells of the endothecium and the tapetum and only a few darkly stained parts of this layers present at the uninucleate pollen stage.

The tapetum is one-rowed, glandular, consisting of large, rectangular or almost quadratic cells. During the anther ontogenesis, as a result of mitotic divisions (still at the stage of prophase I in MMCs) they become two-, four-nucleate. A peculiarity of the tapetum in G. cruciata, announced also for other species of the family Gentianaceae (G. lutea [11], Megacodon stylophorus and Veratrilla baillonii [12]; species of the genus Swertia [13]) is that the cells of tapetum on the connective side show radial elongation and intrude deeply into the anther locules to form "placentoids" [7] (Fig. 1B). At about the time of one-nucleate pollen grains formation, the tapetum degenerates forming together unusual mass in which the individual protoplasts are clearly distinguishable (Fig. 1C). The tapetal cells degenerate completely when mature pollen grains form. This manner of the tapetum development is described also in G. lutea [11], G. acaulis [8], G. angustifolia [14], species of the genera Leiphaimas and Voyria [15]. It no coincides with the interpretation of the tapetum development in G. cruciata by Steffen and Landmann [7]. These authors consider that the formation of one or more tapetum layers and one- or two "placentoids" is a result of sterilization of the sporogenous tissue. At the stage of maturity, the anther wall in G. cruciata comprises epidermis and fibrous endothecium (Fig. 1E).

The sporogenous tissue in the anthers is 3-4 layered and consists of small one-nucleate polygonal, with dense cytoplasm cells that initially are tightly packed one another with one. Later on, the sporogenous cells grow, round up, separate from each other and differentiate into MMCs. Meiosis runs normally with some insignificant deviations, most frequently expressed in the presence of lagging chromosomes behind the division spindle; chromosome bridges (predominantly during the heterotypic division); asymmetrical disposition of the spindles during the homeotypic division of the meiosis. After simultaneous microsporogenesis, predominantly tetrahedral and rarely isobilateral tetrads form (Fig. 1D). The mature pollen grains are 2-celled. During the development of the male gametophyte in G. cruciata, usually sterilization and degeneration processes take place. As a result of that, in the studied populations degenerating mature pollen grains in different amount are observed (Fig. 1F).

**Ovule and development of the female gametophyte.**

In the flowers of G. cruciata, as in all Gentianaceae species [4,8], the pistil is paracarpous and consists of two carpels. The ovary is upper, unilocular, with a great number of ovules in it. The well-developed ovule is anatropous, tenuinucellate (typical variation, according to Shamrov [6]), unitegmic with poor nucellus, that consists of a single archesporial cell surrounded by one-layered nucellar epidermis. The nucellar epidermis begins to degenerate when the one-nucleate embryo sac (ES) forms and at the stage of four-nucleate ES it completely disappears. The integument consists of five, seven layers of cells and forms deep and narrow micropyle that occupies 1/3 of the ovule length. The cells of it outermost layer (the integumental epidermis) are bigger than the other ones, rectangular, lengthened tangentially, with thickened outer walls. A similar characteristic of these cells is given in G. cruciata [3,6], G. lutea (own unpublished data) and G. punctata [16].

After degeneration of the nucellar epidermis, the cells of the two innermost integumental layers differentiate in integumentary tapetum (endothelium) (Fig.2 D) that is announced up to now only for G. cruciata [5,6] and Exacum pumilum [10,18]. In the studied species the cells of endothelium are elongated tangentially (not radially) that is not shown as typical for this structure [17]. No vascular bundles are differentiated in the ovule of G. cruciata that is typical for the Gentianaceae family [3,8]. In the chalaza a specific structure known as a hypostase was observed (Fig.2 B). Shamrov [5,6] reports this structure in G. cruciata too. In the ovule, an unicellular archesporium forms hypodermally that differentiates directly into megaspore mother cell (megasporocyte) without formation of parietal cells (Fig. 2 A). It is typical for Gentrianaceae family too [8,18]. The megasporocyte undergoes meiosis and a linear tetrad of megaspores forms (Fig. 2 B).

In the populations from Balkan Range and Rhodopes Mt the formation of a megaspore dyad in some ovules instead of a tetrad was observed (Fig.2 C) that is announced also in other Gentianaceae species, as: Centaurium ramosissimum [19]; Swertia minor [20]; G. punctata [16], G. lutea (own unpublished data).

The formation of a dyad in G. cruciata, clearly shows that besides the Polygonum (monosporic)-type, the embryo sac (ES) develops according to Allium (bisporic)-type. Usually the chalazal megaspore becomes functional and develops after consecutive mitotic divisions respectively into a two-, four (Fig.2 D) and eight-nucleate ES.

The mature ES is spindle-shaped with the typical differentiation of its elements (Fig.2 E). Multiplication of antipodal cells passes and their number becomes from three to six (Fig.2 F) that is also observed in G. cruciata [4,6] and Swertia carolinensis [18].

The legitimate embryo and endosperm form after double porogamous fertilization. The first division of the

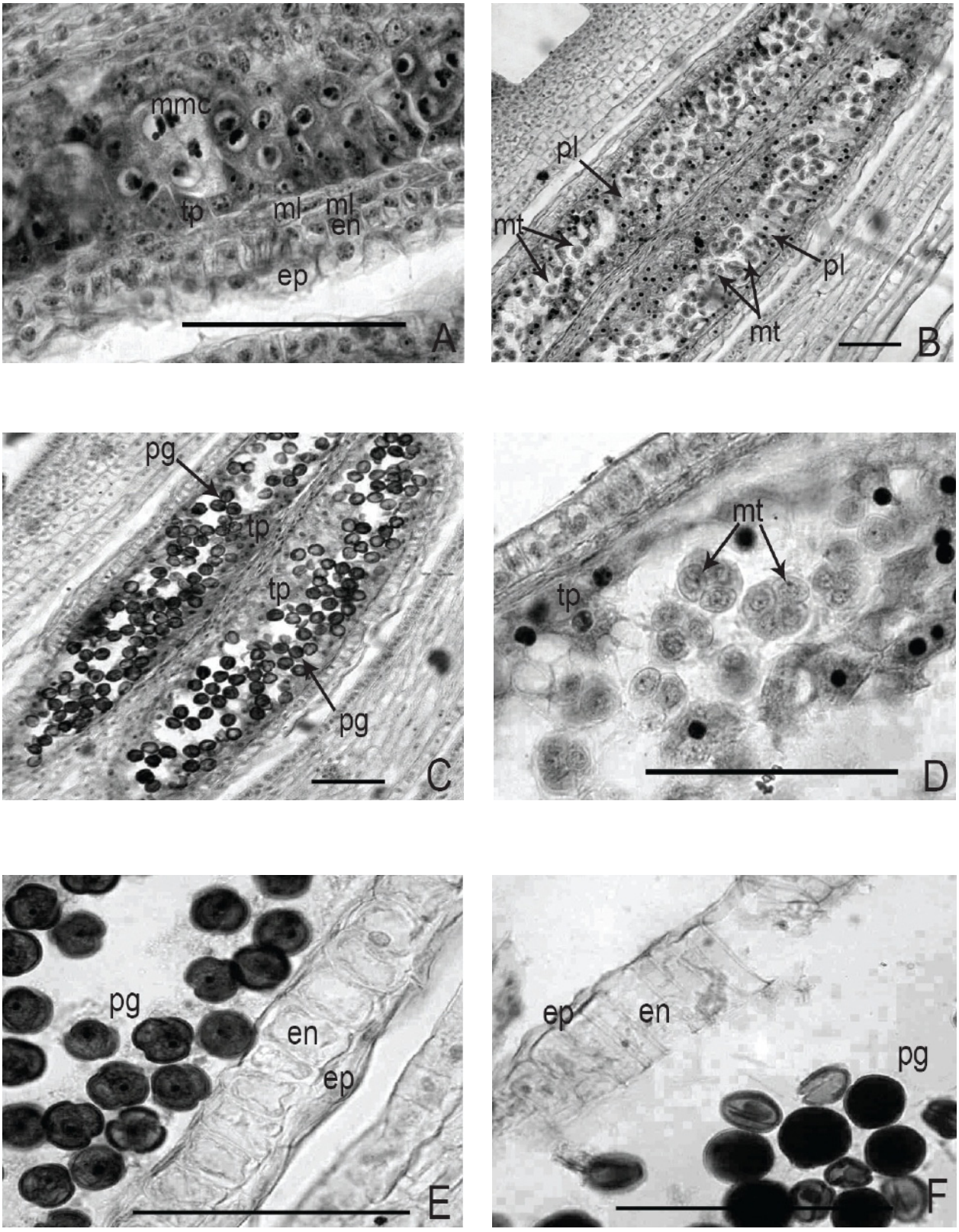


**Fig. 1.** Anther and development of the male gametophyte: A). Anther wall and microspore mother cells (MMCs), ep – epidermis, en – endothecium, ml – middle layer, tp – tapetum, mmc – microspore mother cell; B). Anther locules with placentoids, pl – placentoids, mt – microspore tetrads; C). One-nucleate pollen grains and degenerating tapetum, pg - pollen grain; D). Different types of microspore tetrads; E). Mature pollen and anther wall with epidermis and endothecium; F). Degenerating mature pollen grains. Scale bars = 100 μm

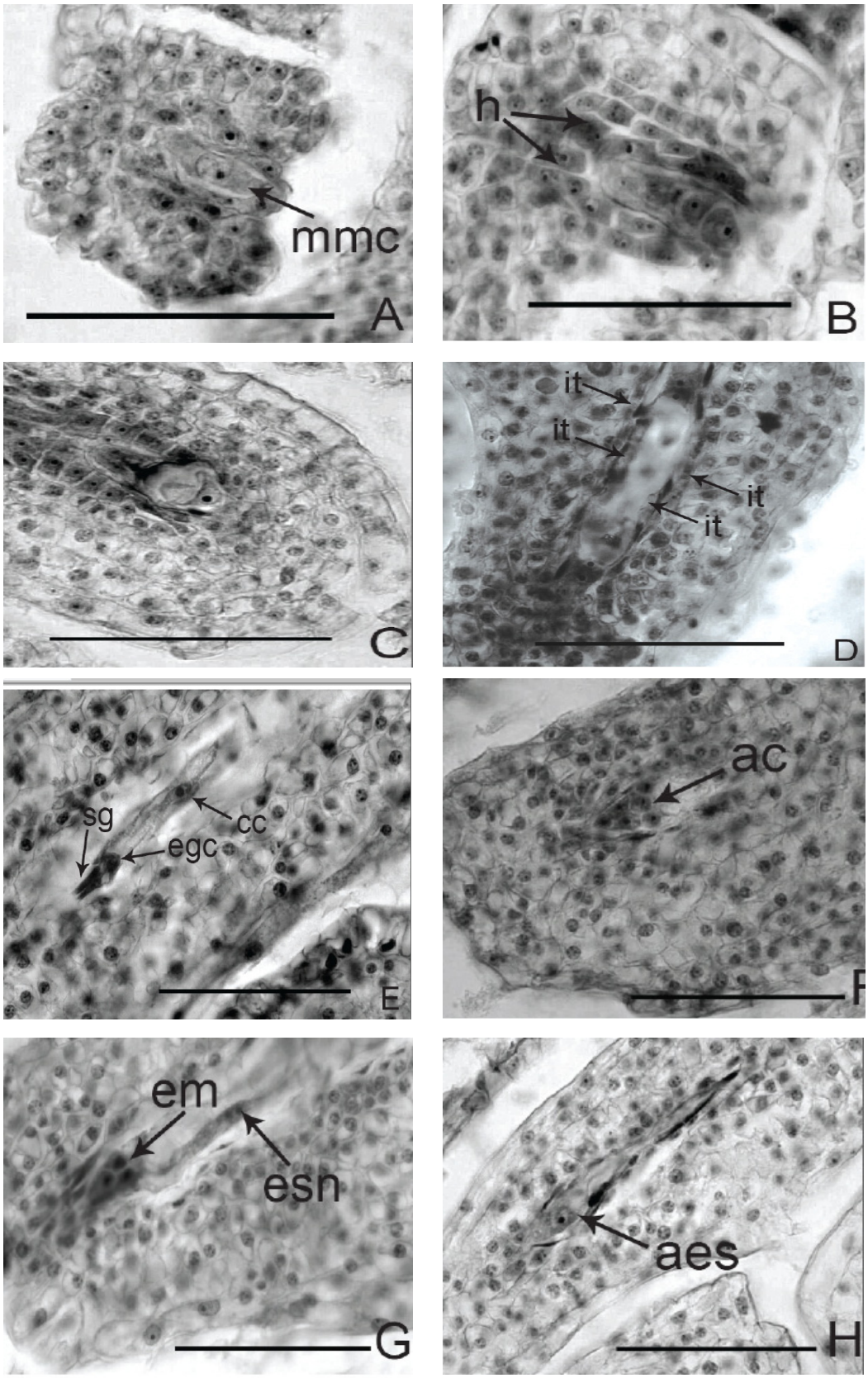


**Fig. 2.** Ovule and development of the female gametophyte: A). Megaspore mother cell (megasporocyte) in the ovule, mmc – megaspore mother cell; B). Linear megaspore tetrad in the ovule, h - hypostase; C). Megaspore dyad in the ovule; D). Four-nucleate embryo sac (ES) with endothelium, it – integumentary tapetum; E). Mature embryo sac, sg – sinergid, cc – central cell, egc – egg cell; F). Antipodal cells in the mature ES, ac – antipodal cells; G). Asterad-type young embryo and nuclear endosperm, em – embryo, esn – endospermal nuclei; H). Aposporous ES in the ovule, aes – aposporous embryo sac. Scale bars = 100 μm.

primary endosperm nucleus precedes that of the zygote. The proof of this, are the free endosperm nuclei observed in the ES cavity at the stage of zygote. The direction of the cell wall setting, during the first divisions of the zygote, show that the embryogenesis in G. cruciata follows the Asterad-type (Fig. 2 G). This type embryogenesis is marked as a characteristic one for the family Gentianaceae together with the Solanad-type[18]. The endospermogenesis passes a free nuclear stage and differentiates into completely cellular at the globular embryo stage.

In single ovules of the population from Balkan Range, an one-nucleate aposporous ES initiates from a somatic cell located near by the four-nucleate legitimate ES (Fig. 2 H). The somatic apospory is noticed for the first time in G. cruciata.

## CONCLUSION

The results of the study carried out demonstrate that the most of observed embryological characteristics of G. cruciata are already described as typical for Gentianaceae family: tetrasporangiate anthers; Dicotyledonous-type anther wall; simultaneous type of microsporogenesis; two-celled mature pollen grains; unicellular archespore in the ovule; development of the ES after Polygonum (monosporic)-type; the embryogenesis runs according to the Asterad- type and nuclear endosperm forms.

During the study were confirmed some features considered as specific for G. cruciata: the presence of integumentary tapetum and hypostase in the ovule. New embryological data that characterize excepting G. cruciata the whole family Gentianaceae were established, namely: development of the female gametophyte besides to the Polygonum-type according to the Allium-type too as well as an initiation of the aposporous ES in some ovules.

## REFERENCES

[1] Tutin T. G. 1972. Gentiana L. In: Flora Europaea, 3, Cambridge Univ. Press, Cambridge, 59-63.

[2] Bondev I. 1995. Chorological atlas of the medicinal plants in Bulgaria, Sofia. Acad. Izd. Prof. M. Drinov.

[3] Bouman FS, Schier S. 1979. Ovule ontogeny in seed coat development in Gentiana with a discussion on the evolutionary origin of the single integument. Acta bot. Neerl. 28(6): 468-478.

[4] Shamrov I. 1988. Ovule and structural characteristics of embryo sac in some members of the Gentianaceae family. Bot. Zhurn. 73 (2): 213-222.

[5] Shamrov I. 1990. The ovule of Gentiana cruciata (Gentianaceae): structural-functional aspects of dvelopment. Bot. Zhurn. 75 (10): 1363-1379.

[6] Shamrov I. 2000. Translocation pathways for metabolites in developing ovules of Gentiana cruciata L., Gymnadenia conopsea (L.) R.Br., Gagea stipitata Merklin and Luzula pedemontana Boiss. Et Rent. Acta Biol. Cracov. 42 (1): 61-77.

[7] Steffen K, Landmann W. 1958. Entwicklungsgeschichte und cytologische Untersuchungen am Balkentapetum von Gentiana cruciata und Impatiens glandulifera. Planta. 50 (4): 423-460.

[8] Shamrov I. 1987. Family Gentianaceae. In: Batygina TB, Jakovlev MS, (eds.), Comparative embryology of flowering plants. Vol. 4, Nauka, Leningrad, 137-145.

[9] Sundara R. S. 2000. Practical manual of plant anatomy and embryology. New Delhi. Anmol Publ. PVT LTD.

[10] Davis G. 1966. Systematic Embryology of the Angiosperms. New York, London, Sydney. John Wiley & Sons, Ltd.

[11] Shamrov I. 1986. Anther development of Gentiana lutea (Gentianaceae). Bot. Zhurn. 71 (6): 733-739.

[12] Xue CY, Li DZ. 2005. Embryology of Megacodon stylophorus and Veratrilla baillonii (Gentianaceae): descriptions and systematic implications. Bot. J. Lin. Soc.147 (3): 317-331.

[13] Xue CY, Ho TN, Li. DZ. 2007. Embryology of Swertia tetraptera Maxim. (Gentianaceae) relative to taxonomy. Bot. J. Lin. Soc. 155 (3): 383-400.

[14] Guèrin P. 1926. Le developpement de l'anthere chez les Gentianacees. Bul. Soc. Bot. France. 73 (1): 5-18.

[15] Öhler E. 1927. Entwicklungsgeschichtlich-zytologische Untersuchungen an einigfen saprophytischen Gentianaceen. Planta. 3 (4): 641-733.

[16] Bicheva R, Robeva-Davidova P, Yurukova-Grancharova. P. 2004. Macrosporogenesis and development of the female gametophyte in Gentiana punctata (Gentianaceae). Phytol. Balcan. 10 (2-3): 233-240.

[17] Poddubnaja-Arnoldi VA. 1976. Cytoembryology of Angiosperms. Moskva, Nauka.

[18] Poddubnaja-Arnoldi VA. 1982. Characteristics of the Angiosperms families on cytoembryological features. Moskva, Nauka.

[19] Vijayaraghavan MR, Padmanaban U. 1969. Morphology and embryology of Centaurium ramosissimum Druce and affinities of the family Gentianacae. Beitr. Biol. Pflanzen. 46 (1): 15-37.

[20] Sankara Rao K, Nagaraj M. 1982. Studies in Gentianaceae: embryology of Swertia minor (Gentianaceae). Can. J. Bot. 60 (2): 141-151.