Reproductive biology of *Atropa belladonna*: embryological features, pollen and seed viability

Petka Yurukova-Grancharova[1], Elina Yankova-Tsvetkova[1], Georgi Baldjiev[1] & Manuel Cantos Barragan[2]

[1] Department of Plant and Fungal Diversity and Resources, Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Acad. Georgi Bonchev St., bl. 23, 1113 Sofia, Bulgaria, e-mail: y_grancharova@abv.bg; y_grancharova@mail.bg (corresponding author)

[2] Department of Plant Biotechnology, Institute of Natural Resources and Agrobiology, CSIC, P.O. Box 1052, 41080 Sevilla, Spain, e-mail: cantos@irnase.csic.es

Received: December 30, 2010 ▷ Accepted: March 7, 2011

Abstract. An embryological study of the Bulgarian native populations of *Atropa belladonna* has been carried out. Some embryological features typical for the genus *Atropa* and the family *Solanaceae* were observed. A new feature, namely endosperm embryo, was also established. The recognised high plasticity of the female gametophyte, as well as the high pollen and embryo viability play an essential role for maintaining the size of populations of the studied species. The specific reproductive features observed are important for the use of *A. belladonna* as a source of raw material for the pharmaceutical industry and for its status as an endangered species in the Bulgarian flora.

Key words: Anther, *Atropa*, embryo, endosperm, male and female gametophyte, ovule

Introduction

Atropa belladonna L. is a perennial herbaceous plant of the family *Solanaceae* Juss., included under the threat category Rare in the *Red Data Book of the People's Republic of Bulgaria* (Genova 1984) and as Vulnerable in the *Red List of Bulgarian vascular plants* (Genova 2009). Since ancient times, it has been known as a medicinal plant with spasmolytic and mydriatic effect (Petkov 1982). The leaves and berries of *A. belladonna* are toxic and contain tropane alkaloids, including atropine, scopolamine and hyoscyamine. Even to this day, atropine is used by eye doctors to dilate the pupils during examination of patients' retina. Scopolamine and hyoscyamine, similarly to the compound atropine, have a sedative effect and bring about relaxation in the smooth muscles of the body. The curative effect of the drugs from roots and leaves of *A. belladonna* is due to the high alkaloid content that makes them a valuable material mainly for the production of spasmolytic medicines (Ganchev 1995).

Hitherto, the studies of this species have been mainly focused on overcoming the problems of poor seed germination in relation to its cultivation as a raw material for the pharmaceutical industry (Jankulov 1961; Genova & al. 1997). In this connection, the chemical composition and pharmacological effect of the active substance in *A. belladonna* drug have been already established (Petkov 1982). Data on embryology of this species are scanty and fragmentary (Tognini 1900; Zhukova & Poddubnaya-Arnoldi 1987). The Bulgarian populations of this very useful medicinal plant have not been studied so far.

The increased wide application of this valuable plant in pharmaceutical industry has significantly reduced in size and numbers its wild populations in Bul-

garia. Elucidation of the reproductive pathways of *Atropa belladonna* will provide important information for estimation of the reproductive success of the studied populations in connection with the potential possibilities for its *in vitro* cultivation and preservation.

The aims of the present study were: (1) To reveal the mode of reproduction of the native Bulgarian populations of *A. belladonna*; (2) To characterize the peculiarities of the reproductive structures and processes; (3) To analyze the pollen and embryo viability.

Material and methods

Two Bulgarian native populations of *A. belladonna* were studied:

1. Sofia Region, Mt Lyulin (in the vicinity of the city of Sofia), near St. Spas Monastery in a wood of *Fagus sylvatica*, at an altitude of about 1200 m (Fig. 1). The population was with low density, consisting of a comparatively small number of sparsely growing individuals, usually with low fertility.

2. Balkan Range (*Western*), Vrachanski Divide (near Parshevitsa chalet), in a wood of *Fagus sylvatica,* at an altitude of about 1280 m (Fig. 2). The population was dense, consisting of many individuals, with high viability and fruitfulness.

For the embryological study and pollen viability assessment, flower buds and flowers (at different stages of development) were collected and fixed in a mixture of FAA (formalin: glacial acetic acid: 70 % ethanol in correlation 5:5:90 parts), embedded in paraffin, cut up into 8–15 μm sections with a rotary microtome and treated according to the classical paraffin methods (Sundara 2000). The sections were stained with Heidenhain's and Delafield's haematoxylin and finally included in Enthelan.

For proving the content of starch in the pollen grains, anthers were detached from flowers, washed with water and then cut open to separate their content on a slide. A drop of iodine-potassium-iodine (IKI, known as Lugol`s iodine solution) was added to the pollen, in order to reveal the presence of starch. Stainability of pollen grains was estimated immediately by light microscope. The relative amount of starch accumulation within the pollen grains was established according to the coloration intensity (the greater the starch accumulation, the deeper the bluish-brown staining of pollen).

To estimate embryo viability, a quick viability test completed within 24 h was applied (known as tetrazolium test). For this purpose, mature seeds of two studied populations were preliminarly imbibed on filter paper overnight at 20–25 °C. Then the seeds were cut deeply on the leaving part of the seed coat and incubated in a diluted (1 %) solution of 2, 3, 5-triphenyltetrazolium chloride (TTC), according to Peters (2000). Initially, the tetrazolium solution is colourless, but changes to red, when it is in contact with the hydrogen (a reduction) derived from the enzymes in the respiration process of the embryos and endosperm. Embryos showing active respiration turn red and are considered viable (the darker the colour, the higher is the respiratory activity in the seed). The embryo viability is estimated depending on the intensity of staining: (1) Viable embryos display entire embryo and en-

Fig.1. *Atropa belladonna* in a wood of *Fagus sylvatica*, Sofia region (Mt Lyulin, near St. Spas Monastery).

Fig. 2. *Atropa belladonna* in a wood of *Fagus sylvatica*, Balkan Range (*Western*), Vrachanski Divide.

dosperm staining (normal staining); (2) Nonviable embryos display abnormal or no staining of any part of the embryo or endosperm.

Observations were carried out on permanent slides, using an Olympus CX21 light microscope. Microphotographs were made with Digital Infinity Lite Camera 1.4 Mpx.

Results

The features revealed during the present embryological study are generally the same for the two native Bulgarian populations of *Atropa belladonna*. Therefore, only special comments are given below.

Anther and development of the male gametophyte

The anthers are tetrasporangiate (Fig. 3). Placentoids (parenchymatous longitudinal bulges of the septa intruding into each pollen sac) are always present (Fig. 4).

The anther wall consists of an epidermis, endothecium, one-two middle layers and tapetum (Fig. 4). The epidermis comprises one row of almost rectangular one-nucleated cells. During the anthers ontogenesis, these cells enlarged tangentially and rounded up. The cells of initially one-rowed endothecium are one-nucleated and rectangular. Subsequently, they divide in a radial direction and the endothecium becomes 2–3-rowed outside, up to multi-rowed towards the connective tissue (Fig. 6). The endothecium cells develop fibrous thickenings after the formation of one-nucleated pollen (Fig. 6). The middle layers are ephemeral and degenerate to the end of meiosis in the microspore mother cells – MMCs (Fig. 7). Initially, the tapetum is glandular with one-nucleated cells. As a result of mitotic divisions, a multiplication of the nuclei occurs. The tapetum cells become 4-nucleated on the outer side of the anther wall, up to 8-nucleated towards the connective tissue. They also differ in their morphological characteristics: the former are rectangular and relatively uniform, while the latter are lengthened in a radial direction (Fig. 8). At the stage of mature pollen grains, only the epidermis and endothecium persist in the anther wall, although occasionally with destroyed continuity (Fig. 9).

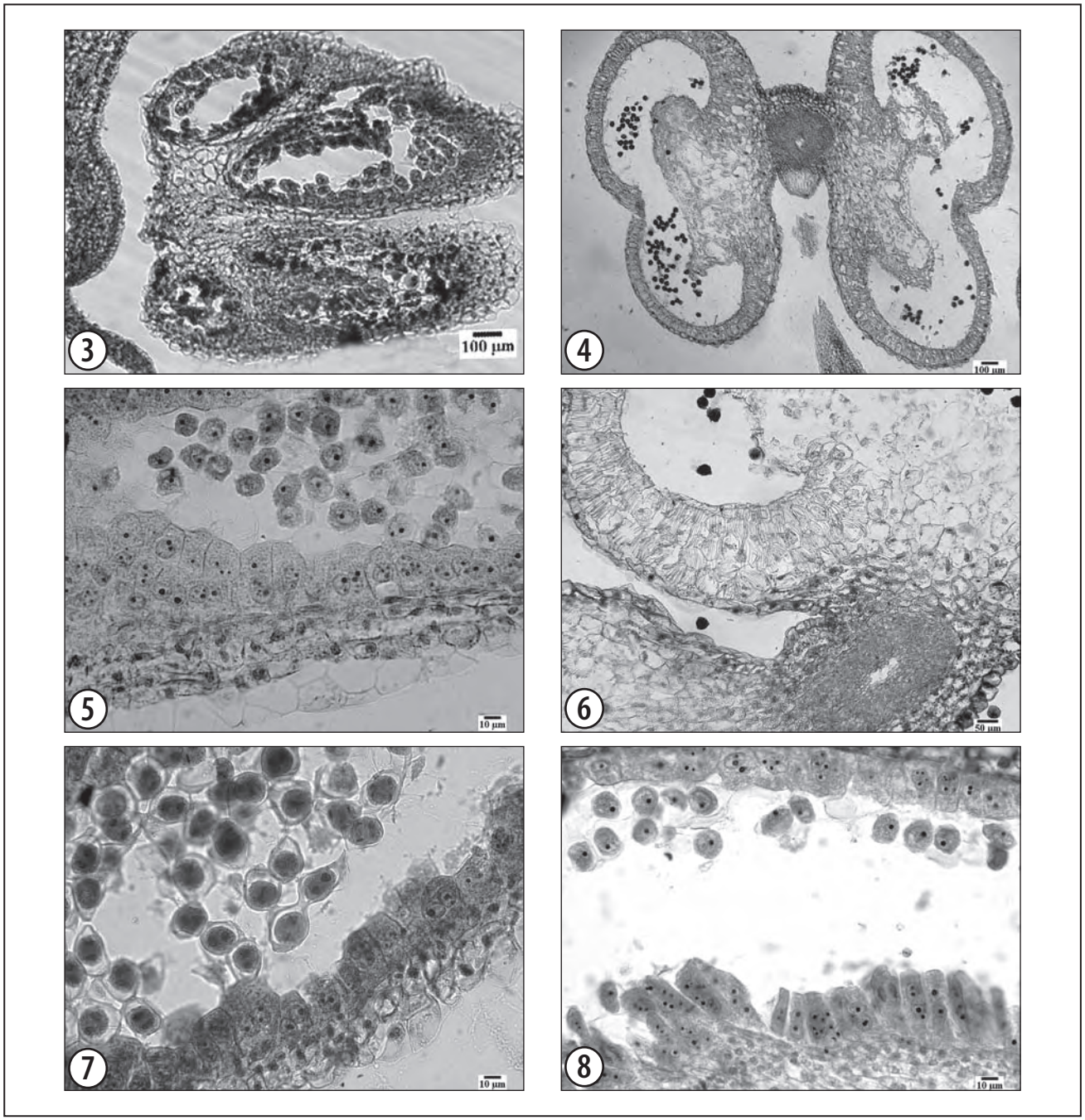
The sporogenous tissue is multilayered and consists of small polygonal cells with dense cytoplasm that initially fit in closely side by side. During the anther development, these cells round up, separate from each other and differentiate into MMCs (Fig. 5). Meiosis runs almost normally, although some deviations have been established, such as: individual lagging chromosomes behind the division spindle; chromosomes out of the spindle; asymmetrical disposition of the spindles especially during the homeotypic division of the meiosis (Fig. 7). After simultaneous microsporogenesis, tetrahedral, isobilateral and decussate microspore tetrads form, often connected with cytoplasmic ligaments up to their disintegration (Fig. 10). The mature pollen grains at the time of shedding are predominantly 2-celled, sporadically 3-celled, three-colporate (rarely 4-colporate), prolate-spheroidal, exine-striate, starch-contained (Figs. 11, 27). Some of the pollen grains begin to germinate through the pores inside the anthers (Fig. 12).

Ovule and development of the female gametophyte

In a flower, the pistil characterizes by a solid style and bilobed stigma with a papillate surface (Fig. 13). The gynoecium is bicarpellar, syncarpous, ovary superior, tetralocular due to false septa, with 5–8 up to 15–20 ovules within each of the locules (Fig. 14). The well-developed ovule is anatropous, tenuinucellate and unitegmic. Within the still young ovule of unicellular archesporium forms hypodermally (Fig. 15). It differentiates directly into a megaspore mother cell (megasporocyte) without the formation of any parietal cells (Fig. 16). As result of the meiosis in the megasporocyte, a linear megaspore tetrad or dyad formed. The embryo sac (ES) development initiated from the chalazal megaspore of the tetrad or the dyad runs according to *Polygonum* (monosporic)-type (Fig. 17) or *Allium* (bisporic)-type (Fig. 18) respectively. The sister megaspores, however, gradually degenerated. After three mitotic divisions of the functional megaspore (usually the chalazal one), consecutively two- (Fig. 19), four- (Fig. 20) and eight-nucleate ES formed. The mature *Polygonum*- or *Allium*-type ES consists of: a 3-celled egg apparatus with a pyriform egg cell and two synergids (Fig. 21), a central cell and three antipodals. The central cell formed before the fertilization, after the fusion of the two polar nuclei. The three antipodals, located deeply into the chalaze, are more often in linear arrangement, especially in the population

of the Balkan Range. In the studied populations of *A. belladonna* long-lived antipodal cells in the ES (up to the early stages of endospermogenesis) were observed. The integumentary tapetum (endothelium) differentiates from the innermost layer of the integument to the stage of two-nucleate ES. The cells of the endothelium were bigger than the other integument cells. Up to the mature ES, these cells lengthened radially and became vacuolated (Fig. 21).

The legitimate embryo and endosperm formed after a porogamous double fertlization. The first transversal division of the zygote and direction of the cell



Figs 3-8. Anther and development of the male gametophyte:
3, Tetrasporangiate anther. Scale bar, 100 µm; **4,** Anther sacs with placentoids (pd). Scale bar, 100 µm; **5,** Microspore mother cells (mmc) and five-cell-layered wall structure: epidermis (epi), endothelium (end), two middle layers (ml), and tapetum (tap). Scale bar, 10 µm; **6,** Four-cell-layered wall structure: epidermis (epi), and three endothecial layers (end) and mature pollen grains. Scale bar, 50 µm; **7,** Homeotypic division of the meiosis in MMC (mmc) and anther wall (AW). Scale bar, 10 µm; **8,** Secretory tapetum (tap) with different morphological characteristics and MMCs (mmc). Scale bar, 10 µm.

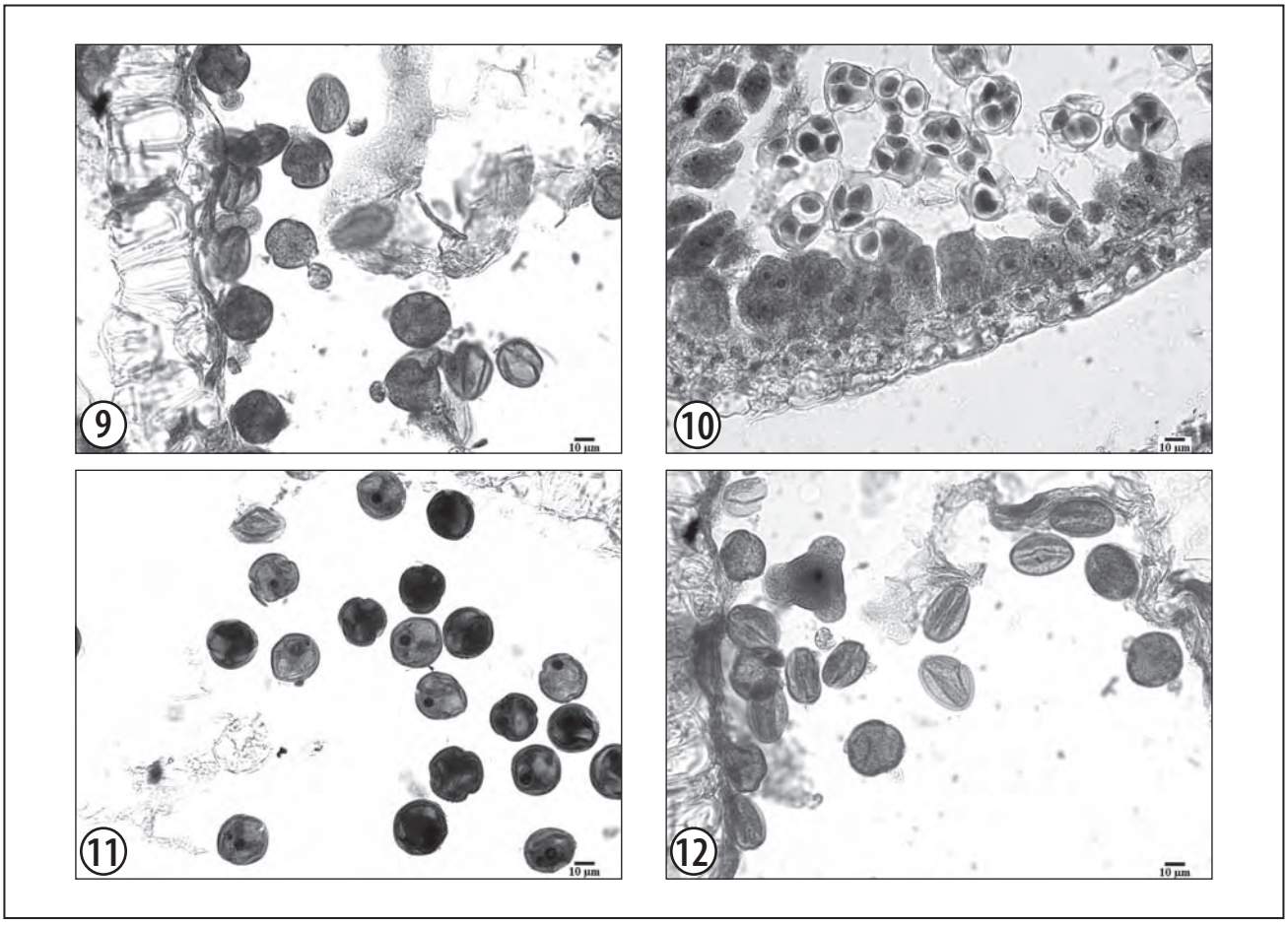
wall setting after the mitotic divisions in the young embryo indicate that the embryogenesis follows the Solanad-type. The young globular embryo characterizes with short and massive suspensor, consisting of three, four one-nucleated cells in a row (Figs. 22, 23). The mature embryo is small, curved (Fig. 24), non-chlorophyllous, located within abundant endosperm not exceeding ¼ part of the seed length.

The first division of the fertilized central cell (primary endosperm cell) precedes the division of the zygote. The endosperm formation passes a free nuclear stage. Its transformation from nuclear into cellular one begins from the micropylar end of the ES (Fig. 25). This process runs rapidly and initially nuclear endosperm becomes cellular usually during the first division of the zygote. As result of a degeneration of the endosperm cells, around and ahead of the young embryo, a large lysis area forms.

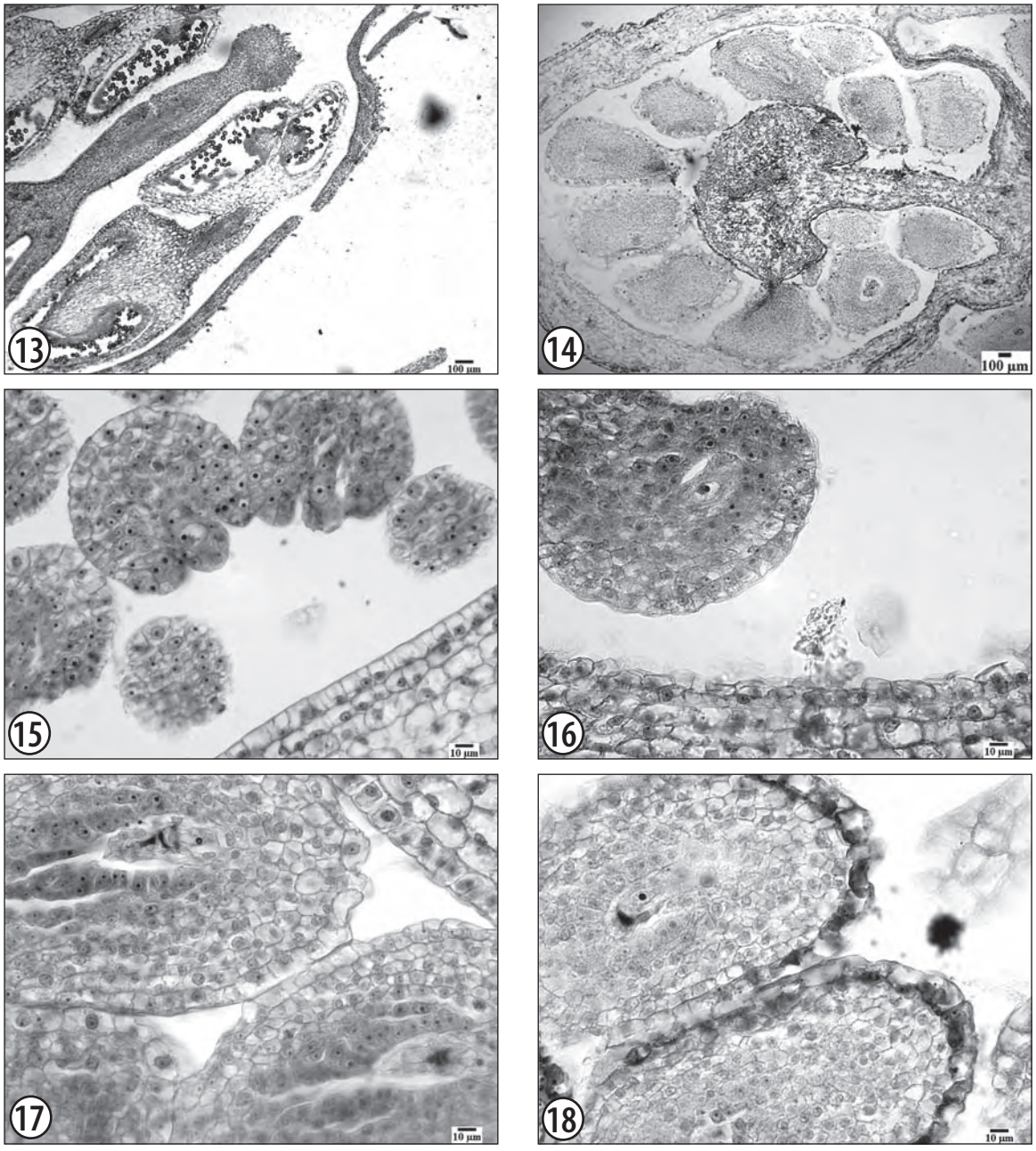
In some ovules of the population from Balkan Range, we observed the development of an embryo without suspensor and situated deeply in the endosperm. The topography and morphology of such embryo give us the reason to suppose that its initiation begins from the endosperm cells (Fig. 26).

Pollen and embryo viability

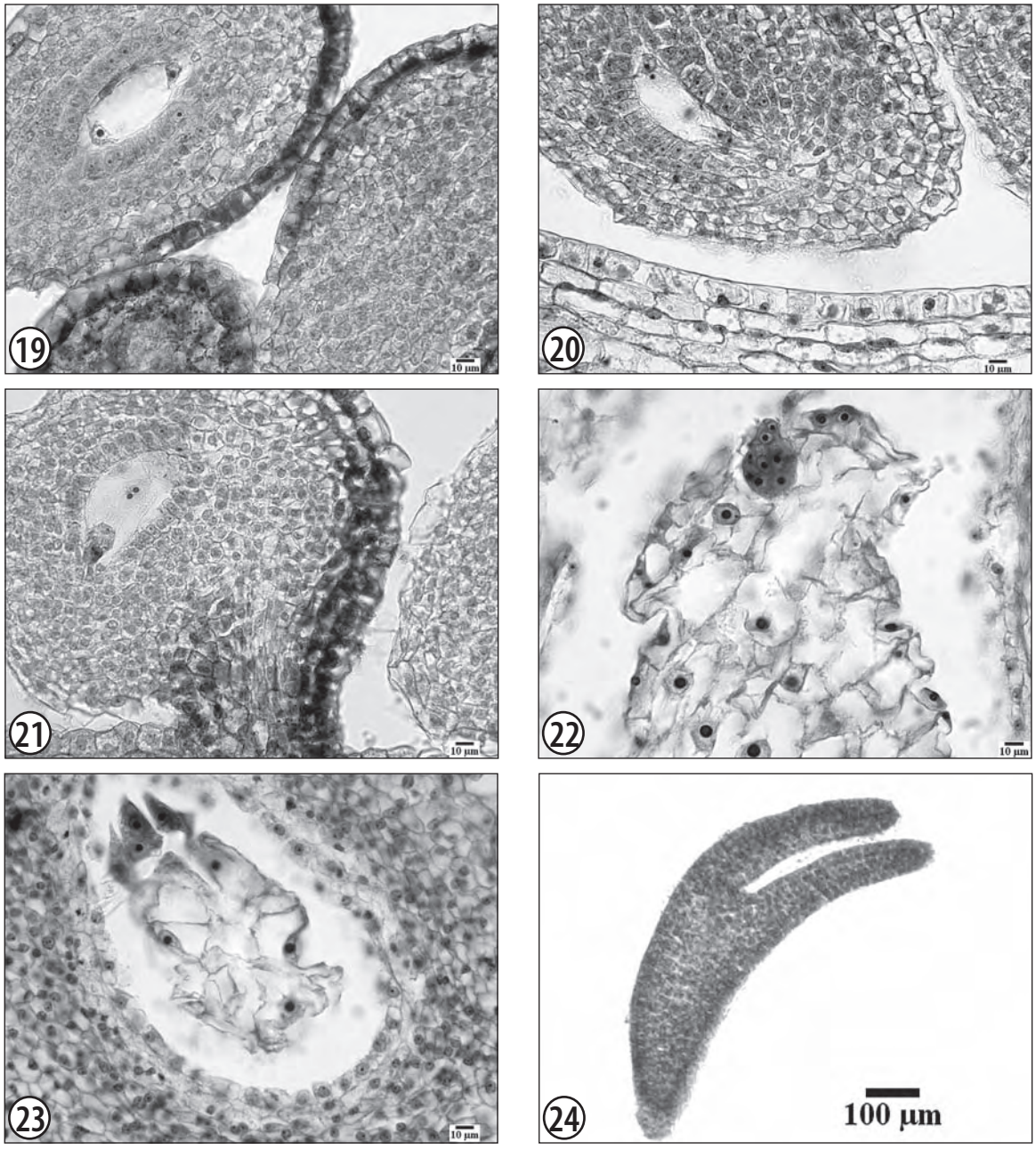
The estimated pollen viability of the specimens from the two studied sites was approximately 100 % (Fig. 27). The embryos viability was evaluated by the intensity of their staining after tetrazolium treatment (Figs 28-30). As a result, the embryos were divided in two classes: Class I, representing completely unstained and light pink-stained embryos, considered as nonviable (Fig. 29), and Class II with red-stained viable embryos (Figs 28, 30). In the two studied populations, the frequency of viable embryos formation was evaluated almost as similar but yet higher in the population from the Balkan Range (Table 1).



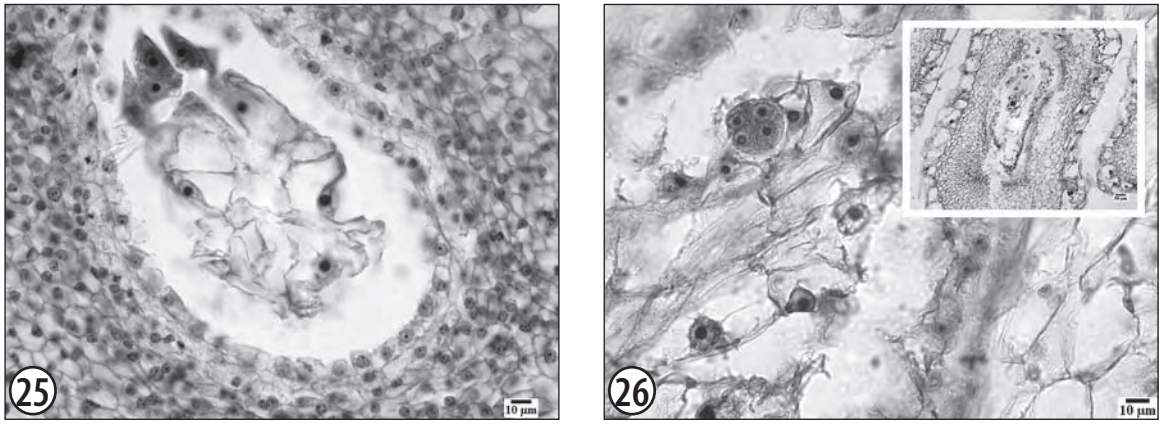
Figs 9-12. Microsporogenesis and development of the male gametophyte:
9, Anther wall with epidermis (epi) and endothecium (end) and mature pollen grains (pg). Scale bar, 10 µm; **10,** Anther wall (AW) and microspore tetrads (mtd). Scale bar, 10 µm; **11,** Mature pollen grains (pg). Scale bar, 10 µm; **12,** Pollen grains germinating inside the anther sac. Scale bar, 10 µm.



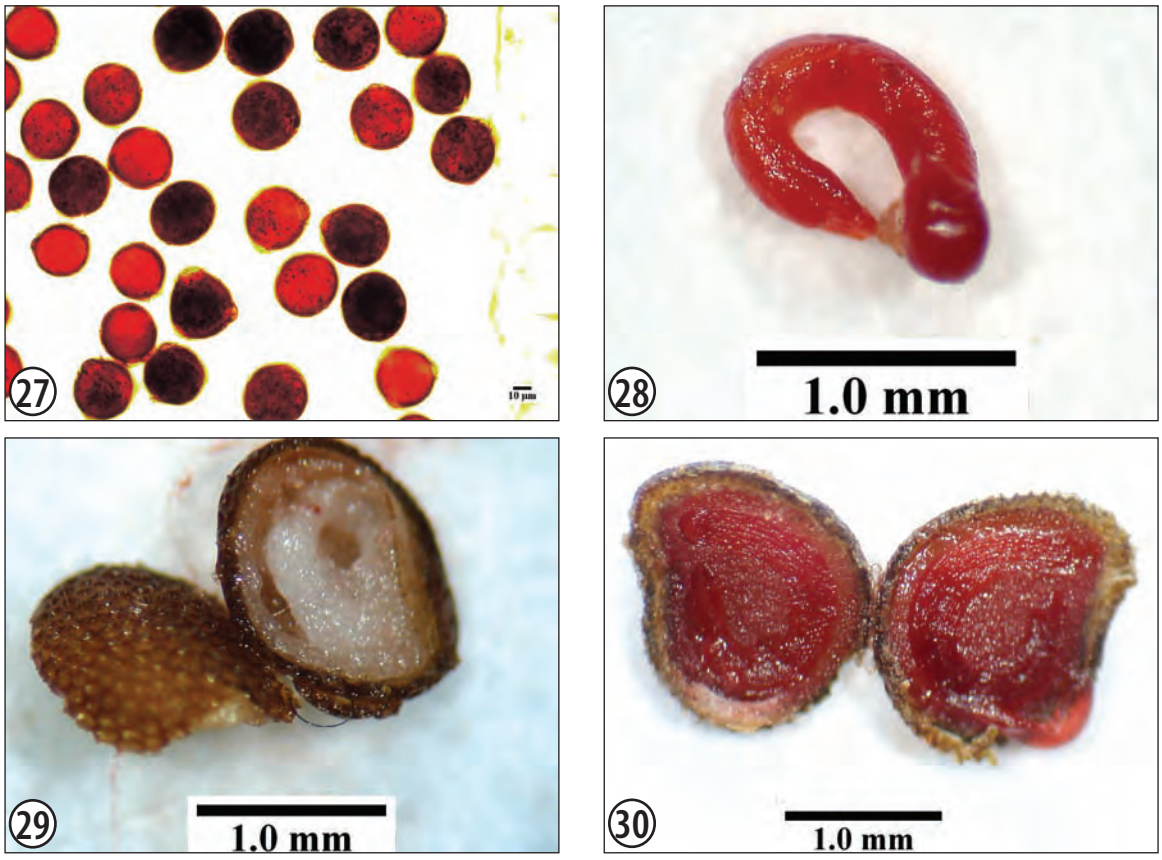
Figs 13-18. Ovule and development of the female gametophyte:
13, Pistil with style (st) and papillate stygma (sg). Scale bar, 100 µm; **14,** Tetralocular ovary with false septa (fs) and ovules. Scale bar, 100 µm; **15,** One-celled archesporium in the ovule. Scale bar, 10 µm; **16,** Megasporocyte. Scale bar, 10 µm; **17,** Initiation of the *Polygonum* (monosporic)-type ES from the chalazal megaspore of the tetrad. Scale bar, 10 µm; **18,** One-nuclear *Allium* (bisporic)-type ES from a chalazal megaspore of a dyad. Scale bar, 10 µm.



Figs 19-24. Development of the female gametophyte, embryo- and endospermogenesis:
19, Two-nuclear ES. Scale bar, 10 µm; **20,** Four-nucleate ES. Scale bar, 10 µm; **21,** Egg cell apparatus and two polar nuclei (pn) in the mature ES with endothelium. Scale bar, 10 µm; **22,** Young legitimate globular embryo (em) and nuclear endosperm (ends). Scale bar, 10 µm; **23,** Legitimate globular embryo (em) with suspensor (sp). Scale bar, 10 µm; **24,** Mature embryo with cotyledons. Scale bar, 100 µm.



Figs 25-26. Embryo- and endospermogenesis:
25, Differentiation of the nuclear endosperm (endn) into cellular one (endc) from the micropyle (my) to the chalaze (ch) of ES. Scale bar, 10 µm; **26,** Endospermal embryo (em) within the endosperm (ends) and in the small photo – the topography of this embryo. Scale bar, 10 µm.



Figs 27-30. Pollen and seed viability tests:
27, Mature starch-containing pollen grains after treatment with Lugol's iodine sollution. Scale bar, 10 µm; **28,** Viable mature embryo after tetrazolium testing. Scale bar, 1 mm; **29,** Nonviable seed after tetrazolium testing. Scale bar, 1 mm; **30,** Viable seed after tetrazolium testing. Scale bar, 1 mm.

Table 1. Estimation of embryo viability (according to AOSA rules for tetrazolium testing).

Population (locality)	Total number of examined seeds	Viable seeds (number)	Nonviable seeds (number)	Viability (%)	Notes
Sofia Region, Mt Lyulin 20.09.2008	236	168	68	71.19	Population with low density and fertility
Balkan Range (*Western*), Vrachanski Divide 20.09.2008	240	184	56	76.67	Dense population with high viability and fruit abundance

Discussion

In general, the present study of *A. belladonna* has revealed not only the typical embryological features of the family *Solanaceae* and the genus *Atropa*, but also some new specific ones.

Anther and development of the male gametophyte

In the studied populations of *Atropa belladonna*, tetrasporangiate anthers were present, being a typical feature for *Solanaceae*. Furthermore, formation of placentoids was observed that have also been reported for the anther characterization in different representatives of this family (Rodriguez 2000; Lyscovsky & al. 2009). The available information about the presence of placentoids in *Solanaceae* is still scarce, the systematic value of this feature being uncertain at present (Weberling 1992; D'Arcy & al. 1996). The development of the observed four-layered anther wall is in consistence with the Dicotyledonous type described by Davis (1966). Sharma & al. (1987) have also shown this developmental type for anthers of the genus *Atropa* L. In a study of the family *Solanaceae*, Carrizo Garcia (2002a) describes two principal types of the anther wall development of this family, namely "Basic" and "Dicotyledonous". Depending on the presence of subsequent divisions of the initial layers in each of these types, this author additionally makes the following subdivisions: "*stricto sensu*" and "*with subsequent divisions*". The above-mentioned pattern of the anther wall development observed by us in *A. belladonna*, gives us the reason to refer to it as the "*Dicotyledonous type with subsequent divisions*", after the classification of Carrizo García (2002a). In a further detailed study on *Solanaceae*, Carrizo Garcia (2003) gives four types of the anther wall development (named *sequences 1, 2, 3, 4*), according to the features of the cell divisions of the two secondary parietal layers, the outer and the inner, respectively. Our observations showed that the anther wall development of *Atropa belladonna* might be referred to Carrizo Garcia's "*Sequence 4*" of the Dicotyledonous type.

The cells of the initially one-rowed endothecium are one-nucleate, with rectangular shape, and typical for the *Solanaceae* family (Zhukova & Poddubnaya-Arnoldi 1987). In the Dicotyledonous representatives, the endothecium is commonly one-layered (Poddubnaya-Arnoldi 1976). In the populations of *A. belladonna,* studied by us, the cells of the initially one-rowed endothecium divided in a radial direction and this layer became 2–3-rowed on the outside, and multi-rowed towards the side of the connective tissue. As reported by Jain (1956), in *Lycium europaeum* L., a multilayered endothecium was observed in the area of the connective tissue of the anthers, while Zhukova & Poddubnaya-Arnoldi (1987) showed the formation of a two-layered endothecium in *Lycopersicon esculentum* Mill. There is scarce information about the endothecium thickenings in *Solanaceae*. As regards this feature in *Solanaceae*, Carrizo Garcia (2002b) studied 59 species of 44 genera, using a differential interference contrast microscope and observed the presence of four types of the endothecial thickenings, namely: annular, helical; reticulate-ribs type and palmatae baseplatae. For *Atropa belladonna* this author has reported the reticulate-ribs type of endothecial thickenings and, more precisely, Subtype 4 of that type.

During our study of *Atropa belladonna* we have observed that the tapetum cells towards the outer side of the anther wall and towards the connective tissue differ not only in the number of their nuclei but also in their morphological characteristics. Such difference between the cells of the inner and outer tapetum was also established at ultrastructural level in *Lycopersicon esculentum* (Polowick & Sawhney 1993).

The microsporogenesis and development of the male gametophyte ran normally. Only insignificant deviations were registered during the meiosis in MMCs. These observations corroborate the reports for other

representatives of the *Solanaceae* family, namely the genus *Lycopersicon* (Tourn.) Mill. and *Solanum tuberosum* L. (Chelak 1987).

At the time of shedding, the mature pollen grains of the representatives of *Solanaceae* are usually 2-celled (Davis 1966; Poddubnaya-Arnoldi 1982). In the anthers of the studied species, besides the three-colporate (often oblate-spheroidal), scabrate 2-celled pollen grains, 3-celled ones were also sporadically registered. The presence of three-celled mature pollen was also reported in *Solanum phureja* Juz. & Bukasov (Dnyansagar & Cooper 1960) and *Capsicum frutescens* L. (Davis 1966). According to the aperture number, exine pattern and pollen shape, Perveen & Qaiser (2007) recognized in the *Solanaceae* family six distinct pollen types and gave a key for their identification. Following this key, the pollen of *Atropa belladonna* might be referred to the *Datura fastuosa*-type.

The pollen grains of *Atropa belladonna* are starch-containing, which is a typical feature for *Solanaceae* (Poddubnaya-Arnoldi 1982) and in particular for the genera *Duboisia* R. Br. (Davis 1966) and *Lycopersicon* (Chelak 1987).

Ovule and development of the female gametophyte

The ovary of *Atropa belladonna* is tetralocular but plurilocular locular ovary was also described in the genus *Solanum* (Symon 1987), as well as in *Exodeconus maritimus* (Benth.) D'Arcy and *Nicandra physalodes* (L.) Gaertn. (Rodriguez 2000). Hunziker (1979) assumed that the plurilocular ovary in *Nicandra physalodes* arises from an increment of the carpel number. The observation gave us grounds to maintain that the tetralocular ovary in *Atropa belladonna* is formed very likely by false ovarian septa, which confirms the conclusion of Rodrigues (2000) for the two above mentioned species of *Solanaceae*.

For this family, anatropous, hemianatropous, amphitropous and campilotropous ovules were established (Davis 1966; Poddubnaya-Arnoldi 1982; Zhukova & Poddubnaya-Arnoldi 1987). Our observations show that the ovule in *A. belladonna* is anatropous and, according to a more detailed classification of the ovule types given by Shamrov (1999), it might be referred to the tenuinucellate type of "standard" (synpetal) variation. This type, characterized by a poor nucellus, is presented only by nucellar epidermis that decays before fertilization.

The integumentary tapetum and hypostase were shown as typical structures for the ovule of the representatives of *Solanaceae* (Poddubnaya-Arnoldi 1982), but during our study we found only an endothelium in *A. belladonna*.

According to our observations, the development of the female gametophyte in the ovules of *A. belladonna* followed the *Polygonum*- or *Allium*-type, both shown for representatives of the *Solanaceae* family (Davis 1966; Poddubnaya-Arnoldi 1982; Zhukova & Poddubnaya-Arnoldi 1987; Watson & Dallwitz 1992 onwards).

Our study confirms a longer preservation of the antipodal cells in the ES's of *A. belladonna* than it was reported by Poddubnaya-Arnoldi (1982), Zhukova & Poddubnaya-Arnoldi (1987) and Watson & Dallwitz (1992 onwards) for the *Solanaceae* family.

The literature data on endosperm development in *Solanaceae* (Dnyansagar & Cooper 1960; Ribchenko 1965), and in the genus *Atropa* in particular (Davis 1966), show that it is *ab initio* cellular. On the contrary, we found that the endosperm in *A. belladonna* initially passes a free nuclear stage, before its transformation into a cellular one. In this study, we have observed the formation of a lysis zone, as result of degeneration of the endosperm cells around and ahead of the young embryo, which has been also reported for different species of the genus *Solanum* (Tourn.) L. (Beamish 1955; Lee & Cooper 1958; Dnyansagar & Cooper 1960; Briggs 1993) and in *Nicotiana tabacum* L. (Erdelska 1985).

In *Solanaceae*, a reduced parthenogenesis, integumental and nuclear polyembryony (adventive embryony) were reported (Schnarf 1931; Johansen 1950; Gluschenko 1973). In *Solanum muricatum* Ait., Kopcińska & al. (2004) have also mentioned the presence of an additional embryo that is usually smaller than the legitimate globular embryo, without however showing its origin. We have observed the formation of illegitimate embryos in some ovules of the studied population from the Balkan Range. On the basis of its location (deeply within the endosperm) and some morphological characteristics (the absence of a suspensor), we consider these embryos to be of endosperm origin (endosperm embryos), which is a new feature not only for *A. belladonna* but also for the genus *Atropa* and the *Solanaceae* family.

As result of this study on *Atropa belladonna*, some embryological features, typical for the genus *Atropa* and the family *Solanaceae* were observed. These are:

tetrasporangiate anthers; Dicotyledonous type of anther wall formation; simultaneous microsporogenesis; two-celled starch-containing mature pollen grains; anatropous, tenuinucellate unitegmic ovule; unicellular female archesporium; *Polygonum*- and *Allium*-type ES development; *Solanad*-type embryogenesis and cellular endosperm. In addition, a new feature, namely endosperm embryo on top of the legitimate one has been also established. Some of the relatively plesiomorphic features, which were observed in the male generative sphere, such as a multiplied number of the anther wall layers (especially of the endothecium); fibrous endothecium; multilayered sporogenous tissue; two-celled pollen grains are not in correlation with the relatively apomorphic ones of the female generative sphere.

The greater plasticity established for the female gametophyte is expressed in the different type of embryo sac development (like basal *Polygonum*- and additional *Allium*-type); formation of an additional endosperm embryo, besides the legitimate one; and high viability and long preservation of the antipodals (even after the beginning of the embryogenesis and endospermogenesis). All these features, connected with some apomorphic peculiarities, provide a more successful realization of the reproductive potential of *Atropa belladonna* and increase its adaptivity.

In spite of the similar character of habitats and the degree of estimated pollen and embryo viability, the two studied populations differ in their size and density. Very likely that is due to the stronger anthropogenic influence on the population in Mt Lyulin, in an area which is part of an active tourist area near the city of Sofia.

The high pollen and embryo viability play an important role for maintaining the size and state of the populations of *Atropa belladonna* as a source of raw material for the pharmaceutical industry, as well as its status of endangered species in biodiversity preservation of the Bulgarian flora.

References

Beamish, K.I. 1955. Seed failure following hybridization between the hexaploid *Solanum demissum* and four diploid *Solanum* species.– Am. J. Bot., **42**: 297-304.

Briggs, C.L. 1993. Endosperm development in *Solanum nigrum* L. Formation of the zone of separation and secretion. – Ann. Bot., **72**: 303-313.

Carrizo García, C. 2002a. Anther wall formation in *Solanaceae* species. – Ann. Bot., **90**: 701-706.

Carrizo García, C. 2002b. An approach to the diversity of endothecial thickenings in *Solanaceae*. – Flora, **197**: 214-223.

Carrizo Garcia, C. 2003. Combination of sequences of cell divisions in the anther wall formation in Solanaceae species. – Flora, **198**: 243-246.

Chelak, V.R. 1987. *Lycopersicon* Mill., *Solanum* L. – In: **Yakovlev, M.S.** (ed.), Embryology of Cereals, Pulses and Vegetable and Food Cultivated Plants. Pp. 107-118. Shtiintsa, Kishinev (in Russian).

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

D'Arcy, W.G., Keating, R.C. & Buchman, S.L. 1996. The calcium oxalate package or so-called resorption tissue in some angiosperms anthers. – In: **D'Arcy, W.G. & Keating R.C.** (eds), The Anther: Form, Function and Phylogeny. Pp. 159-196. Cambridge Univ. Press, Cambridge.

Dnyansagar, V.R. & Cooper, D.C. 1960. Development of the seed of *Solanum phureja*. – Am. J. Bot., **47**: 176-186.

Erdelská, O. 1985. Dynamics of the development of embryo and endosperm I. (*Papaver somniferum*, *Nicotiana tabacum*, *Jasione montana*). – Biologia, **40**: 17-30.

Ganchev, G. 1995. *Atropa bella-donna* L. – In: **Bondev, I.** (ed.), Chorological Atlas of the Medicinal Plants in Bulgaria. Pp. 41-42. Akad. Izd. M. Drinov, Sofia (in Bulgarian).

Genova, E. 1984. *Atropa bella-donna*. – In: **Velchev, V**. (ed.), Red Data Book of the People's Republic of Bulgaria, Vol. I – Plants, p. 327. Publishing House Bulg. Acad. Sci., Sofia (in Bulgarian).

Genova, E. 2009. *Atropa bella-donna*. – In: **Petrova, A. & Vladimirov, V.** (eds), Red List of Bulgarian vascular plants. – Phytol. Balcan., **15**(1): 82.

Genova, E., Komitska, G. & Beeva, Y. 1997. Study on the germination of *Atropa bella-donna* L. seeds. – Bulg. J. Plant Physiol., **23**(1-2): 61-66.

Gluschenko, G.I. 1973. The Problems of Apomixis in Plants and Animals. Nauka, Novosibirsk (in Russian).

Hunziker, A.T. 1979. South American *Solanaceae*: a synoptic survey. – In: **Hawkes, J.G., Lester, R.N. & Skelding, A.** (eds), The Biology and Taxonomy of the *Solanaceae*, pp. 49-86. Academic Press, London.

Jain, T.C. 1956. The gametophytes of *Lycium europaeum*. – J. Indian Bot. Soc., **35**: 181-188.

Jankulov, J. 1961. Untersuchung über die möglichkeiten zum überwinden der langsamen und ungleichmabigen keimund der Samen von *Atropa belladonna* L. – Bull. Inst. Centr. Cult. Plant, **11**: 93-114 (in Bulgarian).

Johansen, D.A. 1950. Plant Embryology. Chronica Botanica, Watham, Massachusetts

Kopcińska, J., Łotocka, B., Kowalczyk, K. & Kobriń, J. 2004. Seed development in *Solanum muricatum* Aiton. – Acta Biol. Cracov. Ser. Bot., **46**: 121-132.

Lee, J.H. & Cooper, D.C. 1958. Seed development following hybridization between diploid *Solanum* species from Mexico, Central and South America. – Am. J. Bot., **45**: 104-110.

Liscovsky, I.J., Cosa, M.T. & Barboza, G.E. 2009. Flower vascularisation in *Solanaceae*: a particular pattern in *Metternichia* J. G. Mikan. – Adansonia, Sér. 3, **31**: 413-425.

Perveen, A. & Qaiser, M. 2007. Pollen morphology of family *Solanaceae* from Pakistan. – Pak. J. Bot., **39**(7): 2243-2256.

Peters, J. (ed.). 2000. Tetrazolium Testing Handbook. Contribution No 29 to the Handbook on Seed Testing Association of Official Seed Analysts (AOSA), Seattle – http://www.aosaseed.com/TZwebsite/2005pdf/2005Summary.pdf (update: 2005).

Petkov, V. 1982. *Atropa bella-donna* L. – In: Modern Phytotherapy, pp. 146-147. Medicina i fizkultura Press, Sofia (in Bulgarian).

Poddubnaya-Arnoldi, V.A. 1976. Cytoembryology of Angiosperms. Basics and Perspectives. Nauka, Moskva (in Russian).

Poddubnaya-Arnoldi, V.A. 1982. Chracteristic of the Angiosperms by Cytoembryological Features. Nauka, Moskva (in Russian).

Polowick, P.L. & Sawhney, V.K. 1993. Differentiation of the tapetum during microsporogenesis in tomato (*Lycopersicon esculentum* Mill.), with special reference to the tapetal cell wall. – Ann. Bot., **72**: 595-605.

Ribchenko, O.I. 1965. Cytoembryology of the Family *Solanaceae*. Naukova Dumka, Kiev (in Russian).

Rodriquez, I. 2000 Flower anatomy and morphology of *Exodeconus maritimus* (*Solanaceae*, *Solaneae*) and *Nicandra physalodes* (*Solanaceae*, *Nicandreae*): importance for their systematic relationships. – Adansonia, Sér. 3, **22**: 187-199.

Schnarf, K. 1931. Vergleichende Embryologie der Angiospermen. Gebruder Borntraeger, Berlin.

Shamrov, I. 1999. The ovule as the base of the seed reproduction in flowering plants: classification of the structures. – Biol. Žhurn., **84**: 1-35 (in Russian).

Sharma, R.C., Raghuvanshi, R.K. & Singh, D. 1987. Embryological studies in *Atropa* L. and *Hyoscyamus* L. – J. Indian Bot. Soc., **66**: 311–316.

Sundara, R.S. 2000. Practical Manual of Plant Anatomy and Embryology. Anmol Publ. PVT LTD, New Delhi.

Symon, D.E. 1987. Placentation patterns and seed numbers in *Solanum* (*Solanaceae*) fruits. – J. Adelaide Bot. Gard., **10**: 179-199.

Tognini, F. 1900. Sull' embryogenia di alcune *Solanaceae* di appunti lasciati. – Atti. Ist. bot. Univ. Lab. crittogam., Pavia, **6**: 109-112.

Watson, L. & Dallwitz, M.J. 1992 onwards. The families of flowering plants: descriptions, illustrations, identifications and information retrieval. Version: 14-th December 2000. – http://biodiversity.uno.edu/delta/.

Weberling, F. 1992. Morphology of Flowers and Inflorescences. Cambridge Univ. Press, Cambridge

Zhukova, G.Y. & Poddubnaya-Arnoldi, V.A. 1987. *Solanaceae*. – In: **Batygina, T.B. & Yakovlev, M.S.** (eds), Comparative Embryology of Flowering Plants. Vol. **4**, pp. 241-247. Nauka, Leningrad (in Russian).