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Research Article

On the reproductive biology of *Angelica archangelica* L. (Apiaceae)

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Abstract

Embryological study on *Angelica archangelica* L. has been carried out. The anther is tetrasporangiate. Its wall develops according to Dicotyledonous-type and consists of epidermis, fibrous endothecium, one middle layer and glandular tapetum. The simultaneous microsporogenesis leads to formation predominantly of tetrahedral and isobilateral microspore tetrads. The mature pollen grains are 3-celled. The ovule is anatropous, tenuinucellate and unitegmic with multicellular archesporium. The development of the embryo sac follows the *Polygonum*-type. The legitimate embryo and endosperm develop after double fertilization. The embryogenesis runs according to Solanad-type. Initially, the endosperm is nuclear.

Keywords: *Angelica*, embryology, male and female gametophyte

Introduction

In contemporary complex biosystematical studies of plant species, the data for their embryonal development along with those obtained with other methods, such as karyological, morphological, anatomical, are used to solve a wide range of problems of the systematic, phylogeny and evolution of plants. In other hand, the embryological data provides useful information about the state of the populations of medicinal, rare and threatened plant species and the possibility for their conservation and regeneration.

In this paper are present the results of the embryological study of *Angelica archangelica* L., a valuable medicinal and aromatic plant cultivated and used from the tenth century on (Greenwood, 1995). The drag is used as a calming remedy for treatment of hysteria, seizures, hypertension, rheumatism and biliary diseases. Have been proven also a cytostatic activity of the coumarins isolated from the fruit of *A. archangelica* on the growth of cancer cells (Gawron and Glovniak, 1987).

While the ginseng is considered the main herb as a male tonic in China, *Angelica* is considered the female analogue. The Chinese have long realized that regular use of *Angelica* by women provided them easy conception, no miscarriages, safe delivery and lack of menopausal complaints (Elinberg, 2013).

From the Middle Ages, *Angelica* is used to flavor liqueurs or aquavits (.g. Chartreuse, Bénédictine, Vermouth and Dubonnet), omellets and trout, and as jam. *Angelica* is unique amonst the Umbelliferae for its aromatic odor, a pleasant perfume entirely different from fennel, parsley, anise, caraway archervil. It has been compared it to musk and to juniper. Even the roots are fragrant, and form one of the principal aromatics of European growth. The fruits are used in the production of absinthe and other alcoholic drinks (Simonetti, 1990)

Although that it is believed that all parts of the plant help a wide range of diseases, the main curative part is the root (Ilieva, 2012) Therefore, the demand for the row material of this species is very high, which is solely met through harvesting of its wild populations (Bath et al., 2011). Market demand of the species for pharmaceuticals and ethno-medicinal utility, are met trough harvesting from wild populations. Due to unsustainable harvesting, habitat loss, and grazing pressure, this species has been assigned as endangered in the Himalayan region (Ved et al., 2003, Vashistha et al., 2006). *A. archangelica* is propagated by the seeds and vegetative parts. However, the existing report on seed germination is not reliable in view of its low germinability (Butola and Badola, 2004; Vashistha, 2006, Vashistha et al., 2009) and slow growth (Butola and Badola 2006). Initiation of large-scale cultivation of *A. archangelica* in suitable climatic zones, by using suitable tools, have been suggested as a conservation strategy and crucial step to ensure a sustainable supply of raw materials to the pharmaceutical industries (Butola and Badola, 2004; Vashistha et al., 2007).

Although *A. archangelica* has been traditionally used in mountainous and tropical region and appreciated for centuries, its biological properties are only in the beginning to be elucidated scientifically. Up to now, the species has been studied mainly phytochemically in connection with the use of the drugs and extracts of biologically active substances accumulated in plant parts. The embryological data on *A. archangelica* are scanty and fragmentary (Kordyum and Velednitskaya, 1964; Kordyum, 1967, Grevtsova, 1987).

Materials and Methods

As plant material were used flower buds and flowers at different stage of development collected from plant individuals grown on the experimental basis of the Institute of Biodiversity and Ecosystem Research in Sofia They are fixed in FAA mixture (formalin : glacial acetic acid : 70 % etanol in correlation 5:5:90 parts) and embedded in paraffin according to classical paraffin method (Sundara, 2000). The serial paraffin cuts with thickness 9-15 μm, made with rotary microtome, were stained with Heidenhain's haematoxylin (Sundara, 2000). The permanent slides were embedded in Enthelan. Observations and photography were carried out using an Olympus CX 21 microscope and digital "Infinity lite" Camera, 1,4Mpx.

Results and Discussion

The present embryological study carried out was confirmed already known features of male and female generative sphere of the reperesentatives of genus *Angelica* and family *Apiaceae* (Kordyum, 1967; Poddubnaya-Arnoldi, 1982; Yankova and Robeva, 2003; Yankova, 2004).

Anther and development of the male gametophyte

The examination of the peculiarities of anther structure and the development of male gametophyte in *A. archangelica* during the present study was established that:

The anthers are tetrasporangiates. The anther wall is four-layered and consists of: an epidermis, an endothecium, one middle layer and tapetum and develops according the I^{-st} type of the types of construction of anter wall given by Batygina and al. (1963).

The epidermis is composed of one row large, rectangular, tangentially withdrawn cells. The endothecium is also one-layered but its constituent cells are smaller in size compared with. the epidermal ones. They develop the typical for this layer in the representatives of family *Apiaceae* fibrous thickenings (Poddubnaya-Arnoldi, 1982, Grevtsova, 1987) after the formation of one-celled pollen grains in the anthers. Like established in our previous studies of *A. sylvestris* and *A. pancicii* (Yankova and Robeva, 2003; Yankova, 2004), in the studied species they are band-type from the reported by Grevtsova (1987) broushe- and band-shaped fibrous thickenings in the representatives of family *Apiaceae*.

The middle layer comprises morphologically heterogeneous in shape and size rounded cells. Like in *A. pancicii* (Yankova and Robeva, 2003), the middle layer in the studied species is not ephemeral and remains vital to the stage of one-nucleate pollen grains (Fig.1-5). Rests of this layer were observed in the stage of mature pollen grains (Fig. 1-6). This fact supports the opinion of Kordyum (1967) that the middle layer in *Apiaceae* family is not ephemeral.

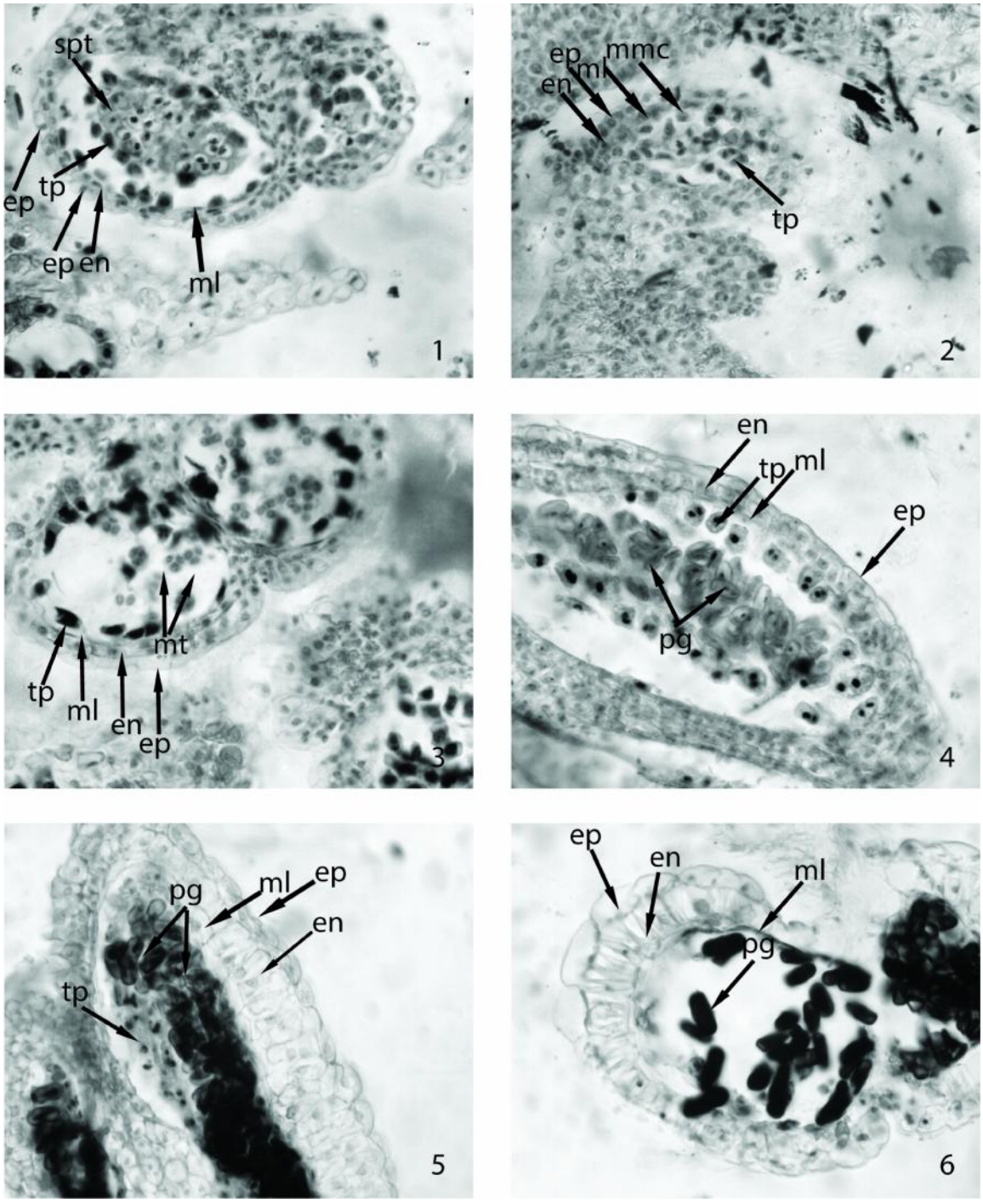

Fig. 1. Anther and development of male gametophyte: 1**)** Four-layered anther wall and sporogenous tissue; 2**)** Four-layered anther wall and MMCs; 3) Four-layered anther wall and microspore tetrads;4) One-celled pollen and anther wall with still nonfibrous endothecium; 5) One –celled pollen grains before their division to form the generative and vegetative cells, anther wall with fibrous endothecium and still preserved middle layer; 6) Mature pollen grains and anther wall consisting of epidermis, fibrous endothecium and rests from the middle layer. ep – epidermis, en – endothecium, ml – middle layer, tp – tapetum, pg – pollen grain. (magnification for Figs 1,2,3,6 – 100x and for 4,5 - 400x).

The tapetum is one-rowed, consisting of non-uniform in shape and size parenchymal cells. It is of glandular type, typical for the family *Apiaceae* (Kordyum, 1967; Grevtsova, 1987). The multiplication of the nuclei of tapetal cells (up to 2-4) (Fig. 1-4) described for number *Apiaceae* and *Angelica* species (Gupta and Gupta, 1964; Kordyum, 1967; Grevtsova, 1987; Yankova, 2004), was established in *A. archangelica* too. This multiplication was observed during the heterotipous division of meiosis in microspore mather cells (MMCs). During the ontogenesis of anther wall, the tapetum layer remain glandular without transformation into ameboid one that is typical for the *Apiaceae* species (Kordyum, 1967; Grevtsova, 1987). At about the time of one-nucleate pollen grains formation, the tapetum degenerates forming together unusual mass in which the individual protoplasts are clearly distinguished (Fig. 1-5). The tapetal cells degenerate completely when mature pollen grains form. At the stage of maturity, the anther wall comprises epidermis, fibrous endothecium and dark rest from the middle layer (Fig. 1-6).

The sporogenous tissue is multilayered (4-6 layers) (Fig. 1-1). The sporogenous cells differentiate directly into MMCs (Fig.1-2). Meiosis in they 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 that correspond to diploid state of the species. The microsporogenesis is of simultaneous type like in the majority *Apiaceae* species (Hakansson, 1923; Marano, 1954; Zenkteler, 1962; Gupta, 1964; Kordyum, 1967; Grevtsova, 1987). In result, tetrahedral and isobolateral tetrads form (Fig. 1-3) cited as typical ones for the family *Apiaceae* (Kordyum, 1967). The mature pollen is three-celled, three-colporate, with smooth and layered exzine and elongated oval form (Fig.1-6).

Lake in other *Apiaceae* species, in *A. archsngelica* the microsporogenesis and development of male gametophyte pass before the macrosporogenesis and development of the female gametophyte (Kordyum, 1967; Grvtsova, 1987). When the ovules was in the stage of archesporium, in the anthers was observed microspore tetrads. Therefore, it may be determine the studied species as a strongly proterandrous species .

Ovule and development of the female gametophyte.

On the basis of observations on the structure of ovule and development of female gametophyte in *A. archangelica* made during the present study, was established that:

In each locule of the two-loculate ovary two ovules, on parietal placentation, develop simultaneously. The well-developed ovule is anatropous, with poor nucellus and one integument and may be define as a medionucellar type according the latest classification of ovules types given by Shamrov (1999). In the still hemitropous ovule, a multicellular archesporium differentiates (Fig. 2-3). The differentiation of multicellular archesporium was announced as typical for the development of ovule in the representatives of family *Apiaceae* by Kordyum (1967) and Grevtsova (1987). Like in *A. pancicii* (Yankova and Robeva, 2003), in the studied species we also have observed the differentiation of single archespore cell in the nucellus (Fig.2-2). In the studied species, we observed the development of funicular obturator (Fig.2-1) shown as characteristic formation for the *Apiaceae* species by Kordyum (1967). This structure represents a growth of tissue sections of the funiculus towards the mycropile and according Poddubnaya-Arnoldi (1976), have a secretory role in growth, nutrition and penetration of pollen tube in the embryo sac.

From the specialized structures of the ovule, in *A. archangelica* we have established the presence of podium – cup like structure formed in the chalazal region of the nucellus (Fig.2-5) and postament - a tissue of nucellar origin occuring as a column below the antipodal cells (Fig.2-6). The primary function of both structures is involved in translocation of metabolites arriving from the hypostase to sporogenous and subsequently gametophytic structures and also to protect the mature seed supporting the water balance in it (Batygina, 2002).

During the study was established that the development of multicellular archesporium occurs as one megaspore mother cell (MMC) occupies a central position in the nucellus and surpasses others archesporial cells in its development. This type of development of the multicellular archesporium was established in *A. sylvestris* by Kordyum (1967) and Yankova (2004) and in *A. pancicii* by Yankova and Robeva (2003) and was considered by Kordyum (1967) as evolutionary more advanced type of multicellular archesporium then those in which all cells develop simultaneously.

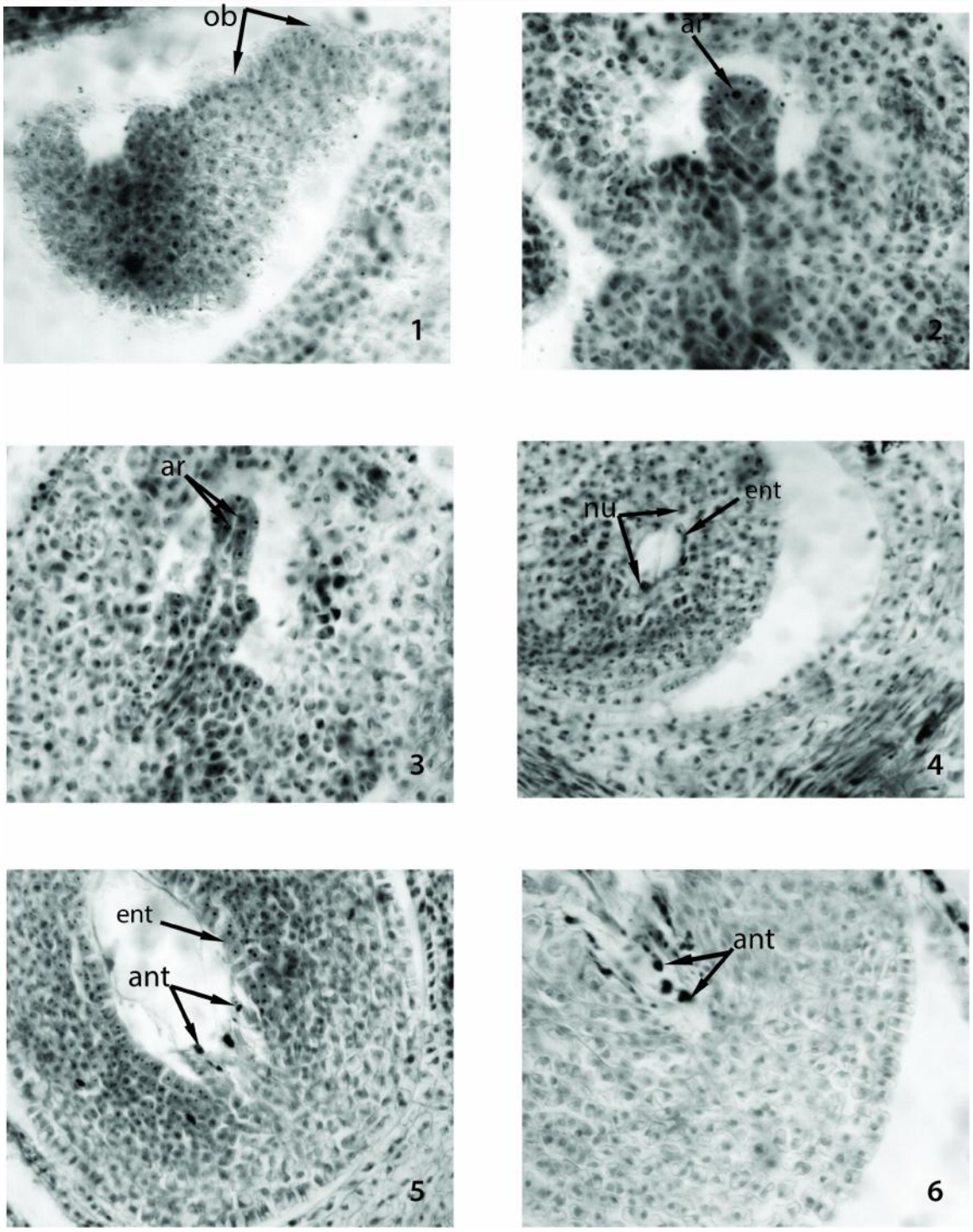

Fig. 2.Ovule and development of female gametophyte: 1)Anatrpopous ovule with obturator; 2) Unicellular archesporium in the ovule; 3) Two-celled archesporium in the ovule; 4) Two nuclear embryo sac and endothelium; 5) Antipodal cells located on the postament; 6) Antipodal cells located in the podium; ob – obturator; ar – archespor; nu- nucleus; ant- antipodal cell; ent – endothelium (magnification for Figs 1,2,3 – 100x and for 4,5,6 - 400x).

Regardless of the setting of unicellular or multicellular archesporium, only one archesporial cell undergoes further development and differentiates directly into megaspore mother cell (megasporocyte) without formation of parietal cells. In result of meiosis that take place in the megasporocyte, a linear tetrad of megaspores forms, from which chalazal megaspore the embryo sac (ES) develops after *Polygonum* type announced as basical for the majority representatives of *Apiaceae* family (Davis, 1966; Poddubnaya-Arnoldi, 1982; Grevtsova, 1987). The mature ES forms after consecutive mitotic divisions, and have the typical differentiation of elements in the *Polygonum* embryo sac – egg apparatus consisting of two sinergids and one egg cell; a big central cell formed after fusion of the two polar nuclei and usually situated close to the egg cell (Fig. 3-1), and antipodal apparatus consisting of three cells located in a horizontal or vertical row in the podium or the postament described above (Figs. 2-5 and 2-6). The antipodal cells are ephemeral and degenerated before fertilization, while the sinergids preserve after fertilization – when a number of endospermal nuclei are present in embryo sac cavity (Fig 3-2).

The endothelium, a structure that forms from the innermost layer of the integument and is characteristic for the ES in the *Apiaceae* species (Kordyum, 1967; Poddubnaya-Arnoldi, 1982; Grevtsova 1987), in *A. archangelica* differentiates in a stage of two nuclear ES (Fig. 2-4). The legitimate embryo and endosperm form after double porogamous fertilization. The first division of the 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 (Fig. 3-2). The direction of the cell wall cetting, during the first divisions of the zygote, show that the embryogenesis follows the Solanad-type (Fig. 3-3). This type embryogenesis Kordyum (1978) described for *A. archangelica* and was shown as a characteristic one for the family *Apiaceae* (Grevtsova, 1987). The endospermogenesis passes a free nuclear stage and differentiates into completely cellular at the globular embryo stage. The observed "vesicules" at the nuclear stage of endosperm in *A. pancicii* (Yankova and Robeva, 2003) and in other *Apiaceae* species (Gupta, 1964; Kordyum, 1967; Al- Atar Adnan, 1977; Grevtsova, 1987) were established in the studied species too (Fig. 3-4).

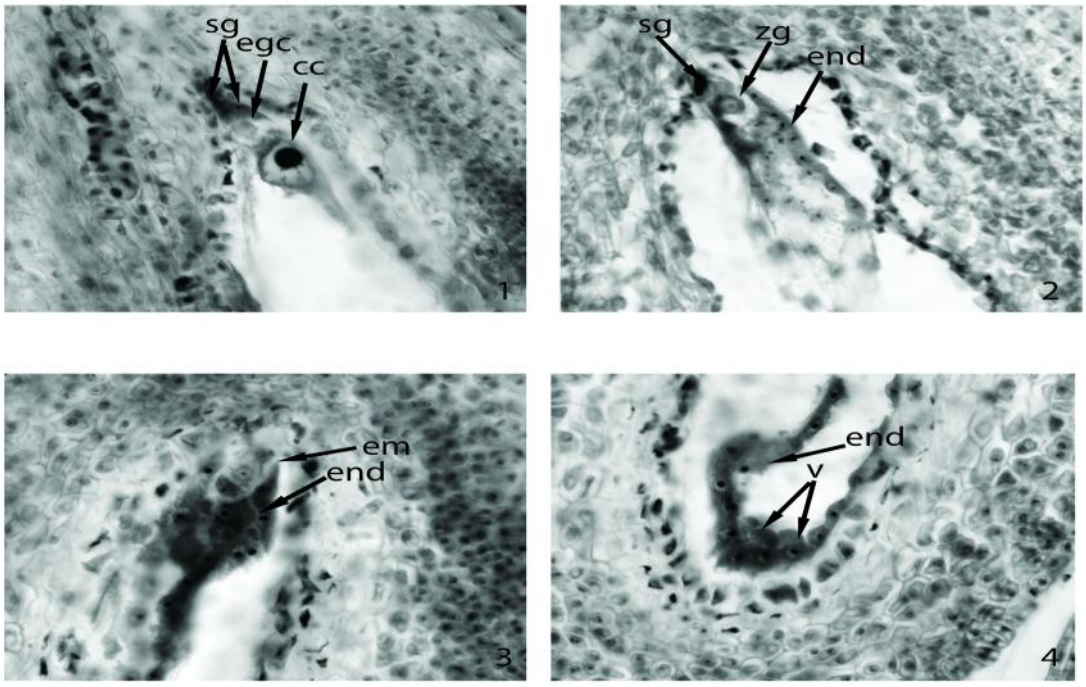

Fig.3 Ovule, female gametophyte development, embryo- and endospermogenesis: 1) Egg apparatus consisting of two sinergids and one egg cell and central cell in the embryo sac cavity; 2) zygote, two sinergids and nuclear endosperm in the embryo sac cavity**;** 3) three celled embryo and nuclear endosperm in the ebryo sac cavity; 4) vesicular endosperm in the embryo sac cavity**;** sg- sinergid; egc- egg cell; cc – central cell; zg- zygote; em – embryo; end – endosperm; v- vezicule; (magnification for all figs – 100x).

Conclusion

The results of the embryological study on *Angelica archangelica* carried out confirm data announced for other species of the genus *Angelica* and family *Apiaceae*: tetrasporangiate anthers; Dicotyledonous-type of anther wall formation; glandular tapetum; simultaneous microsporogenesis; tetrahedral microspore tetrads formation; two locular ovary; tenuinucellate and unitegmic ovule; multicellular archesporium, *Polygonum*-type of ES formation; porogamous fertilization, Solanad-type embryogenesis; nuclear endosperm.

The observed embryological features and absence of apomixis characterize the studied species as sexually reproducing taxon. The regularity of processes in the generative sphere provides a high reproductive capacity of the studied species that guarantee the conservation of size of its populations.

The obtained results also reveal and specify the knowledge of the characteristics of the reproductive system of this valuable species that is the basis of the establishment of strategy for its successful cultivation.

References

Al- Atar Adnan, A. 1977. Occurrence of non nucleate cytoplasmic vesiclesduring development of embryo and endosperm in *Torilis nodosa* and *Pseudorlaya pumila* (*Umbelliferae*). Proc. Indian Acad. Sci. India, 85 (5): 364-368.

Bath, Z.A., Kumar, D.,Shah, M.Y.. 2011. *Angelica archangelica* Linn. is an angel on earth for for the treatment of diseases. Int. J. Nutr. Pharmacol Neurol. Dis. 1: 36-50.

Batygina, T.B., Terehin, E.S., Alimova, G.K. and Yakovlev, M.S. 1963. Genesis of male sporangiums in Graminae and Ericaceae. Bot. Zhurnal. 48 (8): 1108-1120.

Batygina, T.B. 2002. Embryology of Flowering Plants. Vol. 1. Generative Organs of Flowers, Science Publishers Inc., USA.

Butola, J.S., Badola, H.K. 2004. Effect of pre-sowing treatment on seed germination and seedling vigour in *Angelica glauca*, a threatened medicinal herb. Cur. Sci. 87, 796-799.

Davis, G. 1966. Systematic Embryology of the Angiosperms, New York, London, Sydney, John Wiley & Sons, Ltd., 528.

Elinberg, J. 2013. *Angelica archangelica* L., Apiaceae. In: Quave, C. (ed). - Medical plant. Monographs. Pp. 58-66.

Gawron, A. and Glovniak, K.1987. Cytostatic activity of coumarins in vitro. Planta medica. 53 (6): 526-529.

Grevtsova, N. 1987. *Apiaceae.* In: Comparative Embryology of Flowering Plants, Leningrad, Nauka: 35-44 (In Russian).

Greenwood: Electronic version of A Modern Herbal. 1995. The medicinal, culinary, cosmetic and economic properties, cultivation and folk-lore of herbs, grasses, fungi, shrubs and trees with their modern scientific uses, by Mrs. M.Grieve, furst published 1931. Available in:http://www.botanical.com/botanical/mgmh/aneg1037.html.

Simonetti, G., 1990. Stanley Schuler, ed. Simon and Schuster's Guide to Herbs and Spices. Simon and Schuster, Inc.

Gupta, S.C.1964. The embryology of *Coryandrum sativum* and *Foeniculum vulgare* Mill. Phytomorphology. India. 14 (4): 530-547.

Gupta, S. and Gupta, M.1964. Some observation on the embryology of *Bupleurum tenue* Buch. Ham ex D. Don. Cur. Sci. 33 (18): 563-564.

Hakansson, A.1923. Studien uber die Entwickiungesgeschichte der Umbelliferen. Lunds. Univ. Arsskr. 18: 1-120.

Ilieva, A. Angelica archangelica L. (Archangelica officinalis Hoffm.) Available in: http://bilki.bg/encyclopedia-bilki/angelika-lechebna-pishtyalka.html

Kordyum, E. 1967. Cytoembryology of Family Umbelliferae. Kiev, Naukova Dumka (in Russian).

Kordyum, E. 1978. Evolutionary Cytoembryology of Flowering Plants. Naukova Dumla, Kiev, Ukraine.

Kordyum & Velednitskaya 1964

Marano, I. 1954. Lo sviluppo del gametofito feminile in Bupleurum spinosum L. Fil. N.G.Bot.Ital. 61: 720-721.

Poddubnaja-Arnoldi V. A. 1976. Cytoembryology of Angiosperms. Moskva, Nauka, 1976, 507.

Poddubnaja-Arnoldi V. A. Characteristics of the Angiosperms families on cytoembryological features. Moskva, Nauka 1982, 352.

Shamrov, I. 1999. The ovule as a base of the seed reproduction in Flowering plants: Classification of the structures. Bot. Zhurn. 84 (10): 1-35.

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

Vashistha, R.K. 2006. Ecophysiology and agrotechnology of two important Himalayan herbs Angelica glauca Rdgew and A.archangelica Linn. Srinagar, Garhwal, Uttarakhand India: Thesis submitted to H.N.B. Garhwal University.

Vashistha, R., Nautiyal, B.P. , Nautiyal, M.C. 2006. Conservation status and morphological variations between populations of Angelica glauca Rdgew and A.archangelica Linn.in Garhwal Himalaya. Curr. Sci. 91: 1537-1542.

Vashistha, R.K., Nautiyal, B.P. and Nautiyal, M.C. 2009. Pre-sowing treatments to improve seed germination in Angelica glauca Edgew: An endangered medicinal herb oif the western Himalayas. J. Herbs. Species Med. Plants. 15: 73-85.

Ved, D.K., Kinhal, G.A., Ravicumar, K., Prabhakaran, V., Ghate, U., Sankar, R.V. 2003 CAMP Report Workshop, Shimla, Himachal Pradesh, FRLHT, Bangalore, India.

Yankova, E. 2004. Comparative-embryological study on the bulgarian representatives of grnus *Angelica* L. (*Apiaceae*) and revealing the possibility of their in vitro cultivation. Phd Thesis. Sofia

Yankova, E. and Robeva, P. 2003. Embryological study of the Balkan endemic *Angelica pancicii* (*Apiaceae*). Phytologia Balcanica. 9 (1): 79-92.

Zenkteler, M. 1962. Microsporogenesis and development of tapetum in normal plants in carrots and in plants with male sterility. Am.J.Bot. 49 (4): 341-347.