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Reproduction of snowdrop (*Galanthus nivalis* L.) in west Slovakia

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Abstract: Our interest was oriented on vegetative and generative reproduction of snowdrop, mainly on the process of fertilization, microsporogenesis, megasporogenesis, embryo, endosperm, seed and fruit development.

Keywords: generative reproduction, snowdrop micropopulations.

Introduction

Recently some papers dealing with the population biology of snowdrop (*Galanthus nivalis*) in west Slovakia were published. These published results were obtained in the experimental forest in Báb near the town Nitra (ELIÁŠ & PAUKOVÁ 2010, MARINIČOVÁ & EIIÁŠ 2016). In connection with the results of the population biology the life cycles and the reproduction of snowdrop in the localities of west and south Slovakia were more deeply studied.

The aim of the work was to obtain more information about the process of fertilization, development of seeds and fruits and to solve the question of generative and vegetative propagation of snowdrops in „micropopulations"as the parts of whole population of snowdrop in the studied territories.

Material and methods

The snowdrops for the study of the life cycle of this plant come from two localities:

a) the humid forests in the locality of Žitný ostrov
b) oak- hornbeam deciduous forests in Malé Karpaty

In the beginning we decided to use some results of the microcinemato -graphical examination of the processes of fertilization and early endosperm and embryo development in the living embryo sacs of snowdrop. These observations were made in collaboration with the Institute of scientific Film in Göttingen (Film 1463, 1982).

For these observations we removed the outer integument in the apical part of the isolated snowdrop ovule by means of entomological pins and observed the processes in the living embryo sacs in optical microscope using the contrast of Nomarski. The observation is possible in the inert medium (silicone fluid) or in the solution of sacharose during 12 - 24 hours. In the same time the basal part of the ovules remains intact.

The control slides were made using the methods of fixation (FAA or Nawashin´ solution were used as fixative) and the sectioning by microtome in paraffin used in classical optical microscopy.

Results

The ovule of *Galanthus nivalis* is an anatropous one with relatively slightly developed nucellus in comparison with the other species of the family Amaryllidaceae. In the chalazal region of ovule the nucellar cells adjacent to the embryo sac differentiate in several-celled hypostase, directly adjacent to the antipodal cells.

In the studied territory only one species of the genus *Galanthus* is found – *Galanthus nivalis L.* Some scientists observed the occurrence of *G. nivalis subsp.imperatii,* which is characteristic by larger flowers, in the localities near Bratislava (Dostál 1950, Májovský & Murín 1987). This subspecies probably does not exist in this localities any more, because of building up of roads and some other constructs in the places where it was originally observed. In our territories this subspecies is not present

The snowdrop flowers have six tepals in two circles. In the external one there are three long white tepals and in the internal one three short tepals with a green pattern (Fig.1a,b). In addition, in all micropopulations some abnormal flowers having four tepals in each circle (Fig. 2) can be rarely found.

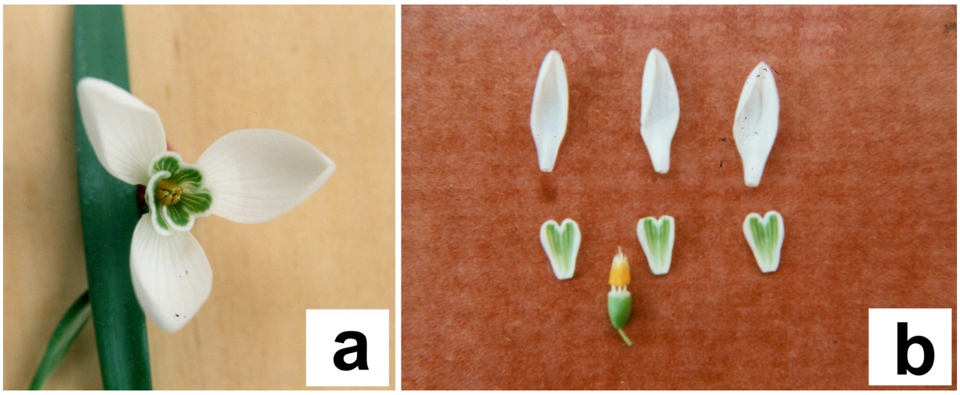

Fig. 1. a) Normal snowdrop flower; b) Parts of the snowdrop flower: 3 white tepals, 3 short tepals, ovarium with stamens, stylus and stigma.

Fig. 2. Abnormal flower with 4 tepals in each circle.

Snowdrop is a geophyte flowering very early in spring, often in February. Snowdrop flowers are adapted to this fact by termonastic and nyctinastic movements. The tepals protect the reproduction organs from a freezing damage.

The reproduction organs of snowdrops consist of six stamens and a pistillum consisting from ovarium, stylus and stigma. The anatomic structure of tepals is also adapted to the protecting role. The skin of tepals, consisting of one cell layer, is covered by cuticle. Under the skin there is a lysigene cavity which is full of polysacharidic slime (ŠČEPÁNKOVÁ & HUDÁK 2004) (Fig. 3). In the green part of petals there are ameboid chloroplasts. The chlorophyll content in tepals is lower than the chlorophyll content in snowdrop leaves, but the relation of the chlorophyll „a“ to chlorophyll „b“ is the same. It is 2:1. The chlorophyll content and the plastid structure may be connected with the slime production (WHATLEY 1978).

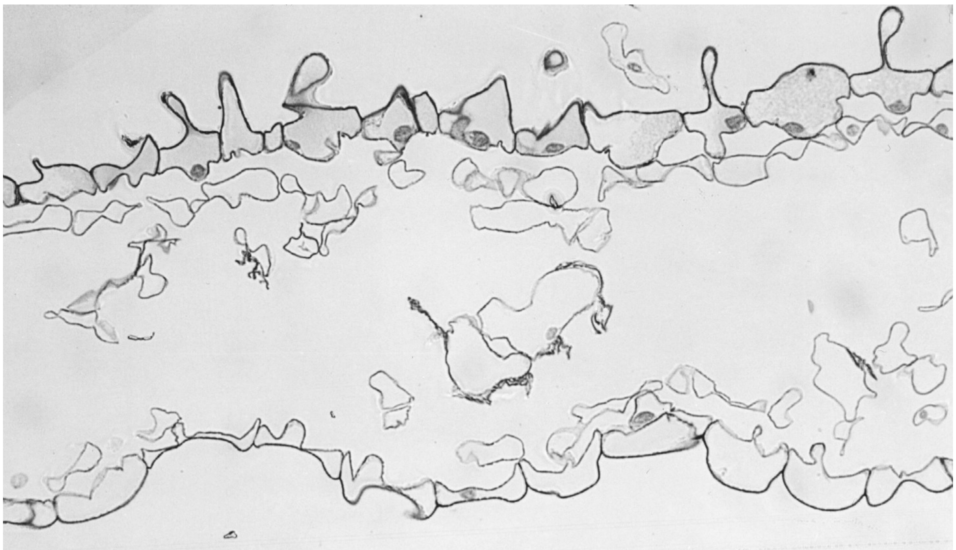

Fig. 3. Structure of white tepal with the lysigen cavity full of the polysacharidic slime.

The reproduction of snowdrop is vegetative by bulbs and generative by seeds.The reproduction by bulbs results in the formation of plant groups in one place which is interesting for cultivation on garden rocks.

In natural populations the reproduction of snowdrops is assured by the seeds in fruits which arise from the ovarium after the tepals fall off. Flower stem with the relatively heavy fruit on its top extends to 20 – 30 cm lying on the soil ground (Fig. 4). The extension is not connected with cell division.

Fig. 4. Fruit of snowdrop lying on the soil ground.

The seed structure was described by several scientists (NETOLITZKY 1926). Some of them suppose that the snowdrop seeds can arise without fertilization. Our observations of 100 fruits show the development of some seeds in each fruit. This factor also determinates the fruit dimension. Fruits with lower seed number were evidently smaller. For example, the fruit of the size 16 x 11 mm contained 13 seeds, the fruit of the size 8 x 4 mm only 1 seed.

When the material for cinematography was being prepared the snowdrop flower was pollinated by a pollen mixture from about 20 flowers. In all analyzed ovules some phase of fertilization could be observed. The growth of the snowdrop pollen *in vivo* and in *vitro* was described also by STEFFEN (1953).

CHUDZIK & SNIEZKO (2003) observed some snowdrop ovules without a well - developed embryo sac. These were sterile, in comparison with the fertile ovules having the well - developed embryo sacs. Somatic cells in the micropyle of the ovules show some secretory activity. Their exudates pass to the micropylar canal. The amount of the exudate increases after pollination. This exudate navigates the growth of pollen tubes to the embryo sac. This exudate is present neither in the sterile ovules without the well - developed embryo sacs, nor in the ovules which were not fertilized because of lack of pollen for pollination. Some ovules without embryo sacs were present also in our analyzed material (Fig. 5).

The lack of pollen may be connected with the lack of pollinators (wasps and bees) in very cold days of February. The majority of seeds in fruits is produced by the snowdrops which flower in March and April.

The snowdrop fruits ripen on the forest ground by the end of May. Up to this time the green fruits become yellow and their cover tissues crack. Small heaps of seeds are then found on the forest ground. The majority of them is interesting for ants, which eat the elaiosomes– fatty outgrows on the basis of each seed. At the same time ants deliver seeds farther from the mother plant and the micropopulation of snowdrops spreads relatively quickly. When we prevent the access of ants to the seed heaps by some mechanical obstructions, the seeds germinate. In autumn they have 1- 2cm long rootlets fixed in the forest soil.

The development of the flowering plant from the seed is gradual and needs some years, as it was described by TROLL (1928) and BUDNIKOV & KRICSFALUSY (1994).

The snowdrop plant prepared to flowering has two well - developed leaves and a stalk with flower. In its bulb there is another little young bulb from which one smaller leaf grows up (Fig. 6). This young bulb represents the initiation of the next year flower. In this bulb developing ovules are present. These ovules undergo microsporogenesis and megasporogenesis**.** The meiosis in megasporocytes takes place at the beginning of September. In the second half of September the ovules contain 1 – 2 nucleate embryo sacs (Fig. 7), at the beginning of October the 4-nucleate ones and towards the end of this months the 8 – nucleate embryo sacs predominate. This is immediately followed by the differentiation of the embryo sacs cells and by the fusion of polar nuclei, giving rise to the secondary nucleus. At this developmental stage the plants winter. In February or March of the next year the stem growth takes the flower above the soil surface.

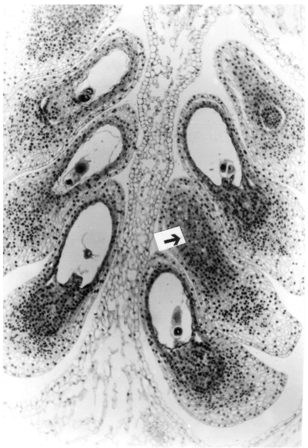

Fig. 5. Sterile ovule without embryo sac.

Fig. 6. Flowering snowdrop with the smaller leaf growing from the white young bulb as the initiation of the next year flower.

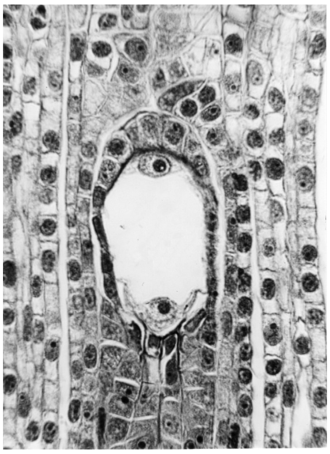

Fig. 7. Two-nucleate embryo sac.

Snowdrop belongs to the family Amaryllidaceae which has ameboid tapetum (PANKOW 1958).

In some populations we observed the tendency to form two functional megaspores during megasporogenesis (ERDELSKÁ 1975). In the course of megasporogenesis three apical megaspores from the tetrade normally degenerate and the basic one develops as the functional megaspore, from which the embryo sac arises. In the abnormal development two embryo sacs arise from two functional megaspores (Fig. 8a,b) Only one of them has a contact to micropyle and may be fertilized. The second one is later clasped by the developing one. In some populations this abnormality was found in about 2 % of plants. This tendency may be interesting from the point of view of microevolution.

KARAGIOSOVA (1970) described the rare possibility of formation of diploid snowdrop embryo sacs from the tetrade of megaspores. Probably she observed the same phenomenon as the above described two functional megaspores from one tetrade.

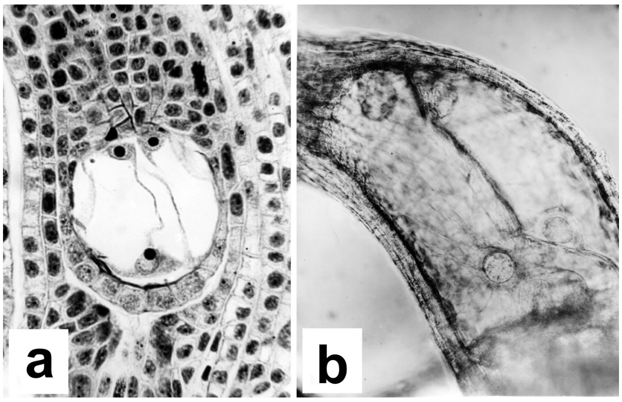

Fig. 8. a) Two embryo sacs arise from two functional megaspores; b) Two embryo sacs in one ovule later.

GVALADZE (1983) and GVALADZE & AKALCHADZI (1986) analyzed the ultrastructure of the embryo sacs of snowdrops in the end of vegetation period and in winter month in Georgia. In winter the authors could not observe any symptoms of life activity in the studied ovules.

SCHLIMBACH (1924) arranged seeds of the family Amaryllidaceae to several groups according to the water content and the type of reserve substances. The snowdrop seeds have the water content 39 – 60% and the reserve substances in the form of starch and cellulose.

In the publication „Comparative embryology of flowering plants V. (edit. by T.B. Batygina and M.S. Jakovlev, 1990) there is the summary of works of several scientists concerning the snowdrop, for example VORSOBINA & SOLNCEVA (1990), OSTAPENKO & CHVEDYNIČ (1987) and some others.

OSTAPENKO & CHVEDYNIČ (1987) studied the snowdrops cytofometrically with the aid of luminiscence microspectral analysis. They observed differences in the duration of the interphase of the nuclei, which are the precursors of the gamete nuclei in two- and four nucleate embryo sacs. The differences were detected in the prolongation of S-period and in the shortening of G2 period in 4- nucleate embryo sacs in comparison with the 2- nucleate ones. The lowest intensity of RNA and the highest quantity of bounded RNA was found in the female gamete.

Some authors observed that in the seeds of snowdrops, released from fruit, there are embryos which have not finished their development yet in this developmental phase (Fig. 9). It is in agreement with the relatively high water content in seeds (SCHLIMBACH 1924), which enables the embryo to continue its postharvesting development up to the end of the vegetation period.

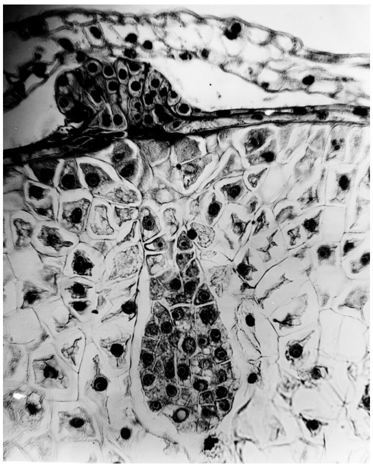

Fig. 9. Embryo in the time of its postharvesting development.

The snowdrop belongs to the species of the family Amaryllidaceae with nuclear endosperm. Several other species of this family have the helobial one.

The presence of some alkaloids in the snowdrop bulbs represents also an interesting information. The best known one is the nitrogenous heterocyclic compound - galantamin. This substance is used in the preparation of medicaments inhibiting the acetylcholine esterase and improving cognitive functions of brain. This is important for reducing the speed or stopping of the Alzheimer disease manifestation (HEINRICH & TEOH 2004).

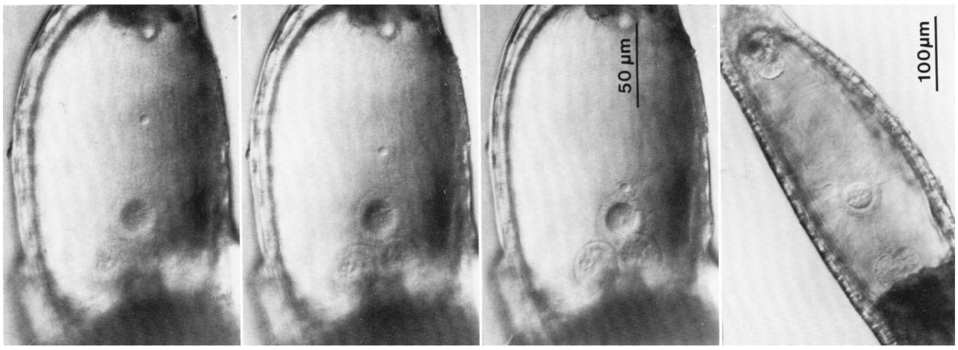

Fig. 10. Fertilization in isolated snowdrop ovules in the film which arose in collaboration with IWF Göttingen (1982).

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