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SOME MORPHOLOGICAL AND ANATOMICAL DESCRIPTIONS OF SEED IN *GALANTHUS WORONOWII* LOSINSK. FROM WESTERN GEORGIA

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Abstract. Morphology and anatomy of seeds of *Galanthus woronowii* Losinsk. from Ajara and Guria (Western Georgia) regions were studied in laboratory conditions. It was found out that shape of fruits changed from round (big fruits) to longish (small fruits). Weight of mature seed was 0,0075 g. By the end of June elaiosome disappears and green hollow develops on its place. Gas bubbles were emitted from green tissues; emission was intensive in August, when embryo was already well developed. Greenness was localized mainly on cell walls. It is permissible that on cells walls located aerobic oxygenic phototrophic bacteria. In early August an embryo in seeds was well developed, reserve fat transformed to starch around of embryo. Germination of seeds began by the end September very slowly. Results of the work will be important for *ex situ* conservation of *G. woronowii*, also for expedient to plant industrial plantations.

Key words: *Galanthus woronowii*, seed, morphology, anatomy, development

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Introduction

The bulbous ephemerid Woronow's snowdrop (*Galanthus woronowii* Losinsk., Amaryllidaceae) is widely spread in Colchic forests of Western Georgia. It grows everywhere on western, north-western or northern exposures of Adjaria-Guria mountains, up to 500-800 m a.s.l. from the Black Sea shore, rarely up to 2000 m a.s.l., mainly in lower and medium belts of forest, in damp river ravines, on red or yellow forest ash-grey soils, with pH varying within 4.2-5.6 (Kemularia-Natadze 1947; Gagnidze 1991, 2005; Zazanashvili 1999; Kikvidze & Ohsawa 2001). Due to high content of alkaloids in bulbs (Proskurina & Ordzhonikidze 1953; Proskurina *et al.* 1955; Berkov *et al.* 2012; Takos & Rook 2013) and the decorative value, these species is intensively collected in natural areas of Western Georgia (CITES 2008) what causes misbalance in its local relict habitats. In 2004-2006 export quotas for *G. woronowii* were 18 million bulbs per year. In 2007 it was reduced to 15 million on the base of recommendations of CITES Secretariat and Georgian Scientific Authority (CITES 2008). In spite of the fact that natural stock of the plant in Western Georgia is about 300 million bulbs (CITES 2008) and the species is not included into the Red Book of Georgia (Red Book... 1982), we think that it is expedient to plant industrial plantations. For development of effective agrotechnical technologies and *ex situ* conservation of snowdrop (Sgenti & Khutsishvili 2013) it is necessary to study its biological peculiarities on all stages of ontogenesis. The results of morphological and anatomical study of bulbs of Woronow's snowdrop have shown that the mentioned species in Guria and Adjaria regions has populations, which differ by size and mass of bulbs. Anatomical peculiarity of a bulb gives an idea about storage of nutrients in it, water requirement and susceptibility to fusarium disease (Chkhaidze *et al.* 2013).

The results of study related to some morphological and anatomical peculiarities of seeds of snowdrop growing in Colchic forests of Western Georgia are presented in the paper.

Fig. 1. Blossoming of *Galanthus woronowii*: **A** – Adjara (27.02.2013); **B** – Guria (27.02.2013).

Fig. 2. Shape of fruits of *Galanthus woronowii* in laboratory condition (22.03.2013).

Fig. 3. Ripening of fruits of *Galanthus woronowii* from Adjaria region (10.05.2013).

Material and methods

The materials have been obtained as a result of the research expedition carried out in March-May 2013 in the forests of Guria and Adjaria regions. The plants in Adjaria region are spread on the altitude 100 m a.s.l., whereas in the mountains of Guria – on diapason of 350-400 m a.s.l. The plants and seeds of ripened fruits were placed in clay pots in laboratory conditions together with soils from the areas of distribution; soil surface has been covered with mosses and grasses, which are spread in natural places (Fig. 7). The process of development of embryo in seeds was checked periodically. Gas emission by seed and embryo was well seen after placing them in water. Anatomical sections have been prepared manually, and stained by

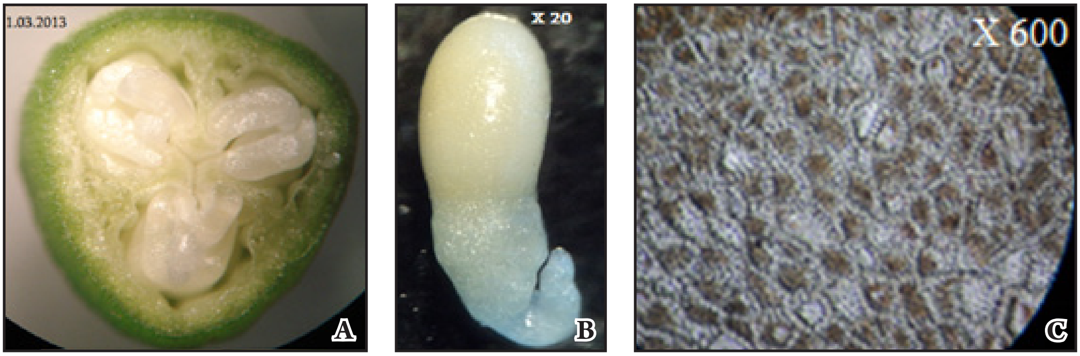

Fig. 4. *Galanthus woronowii*: **A** – fruit of with three locules, development of seeds; **B** – seed with elaiosome; **C** – nutrient substances of a seed are fats, cytochemical reaction with Sudan III (29.05.2013).

Table 1. Morphological parameters of fruit and seed of *Galanthus woronowii* from Adjaria (30.04.2013).

Parameter	Size of capsule		
	Big	Medium	Small
Length of fruit, cm	1.35±0.048	1.30±0.068	1.18±0.020
Width of fruit, cm	1.28±0.045	0.93±0.061	0.78±0.049
Length: width ratio	1.05	1.4	1.5
Weight of fruit, g	1.03±0.056	0.44±0.046	0.24±0.038
Average number of seeds in fruit	14.82±1.583	6.14±0.769	5.00±2.025
Weight of seeds in fruit, g	0.55±0.064	0.13±0.027	0.08±0.017
Weight of seed, g	0.037	0.021	0.016

Sudan III and J+Jk. The microscopes МБЗ-1 and МБО-9 were used. Photos have been made by the digital camera Sony (12.1 megapixels).

Results

In the conditions of Western Georgia Woronow's snowdrop blossoming (Fig. 1) in February-March, dependly from the altitude above sea level. Seeds develop in trilocular fruits of various forms (Fig. 2). Up to 30 seeds may develop per each fruit. Fruits ripen in May (Fig. 6). By that time the aerial parts of plant begin to turn yellow, fruits burst and seeds disperse on a surface of damp soil, covered with vegetation. Firstly seeds are of light brown color, and then darken (Figs. 3, 4). Size of fruits and seeds vary, which might be caused by phenotypic and genotypic variability. The

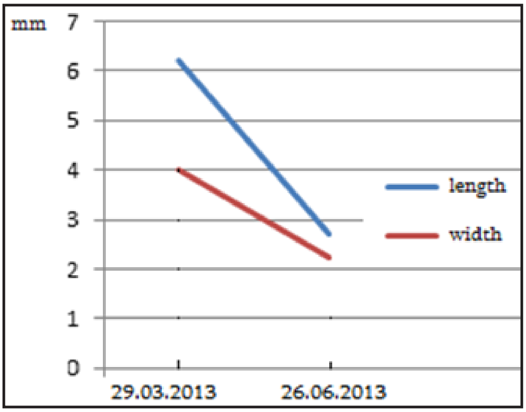

Fig. 5. Change of seed sizes of *Galanthus woronowii* in laboratory conditions.

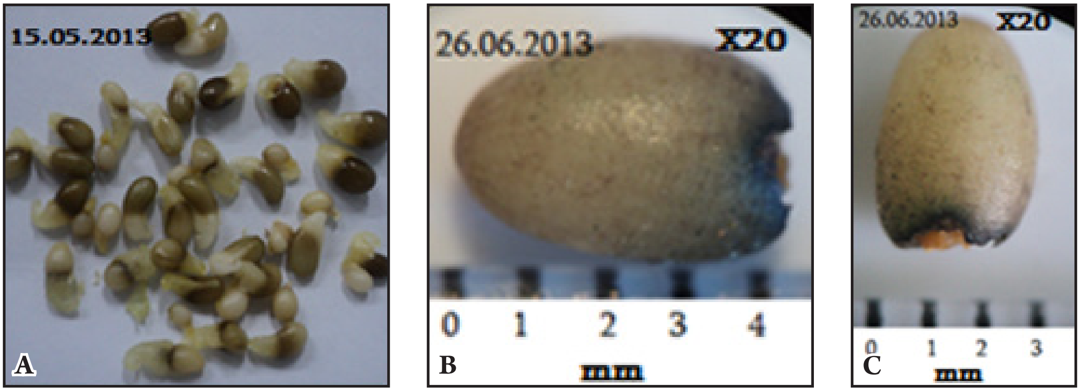

Fig. 6. Seeds of *Galanthus woronowii*: **A** – in natural size; **B, C** – multiplied in 20 times.

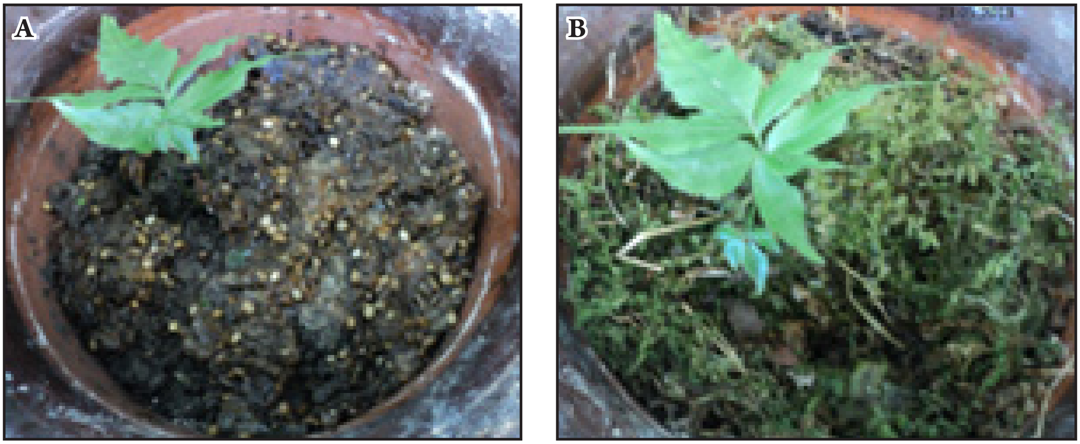

Fig. 7. Sowing of *Galanthus woronowii* seeds in pots (**A**), and covering them by mosses (**B**).

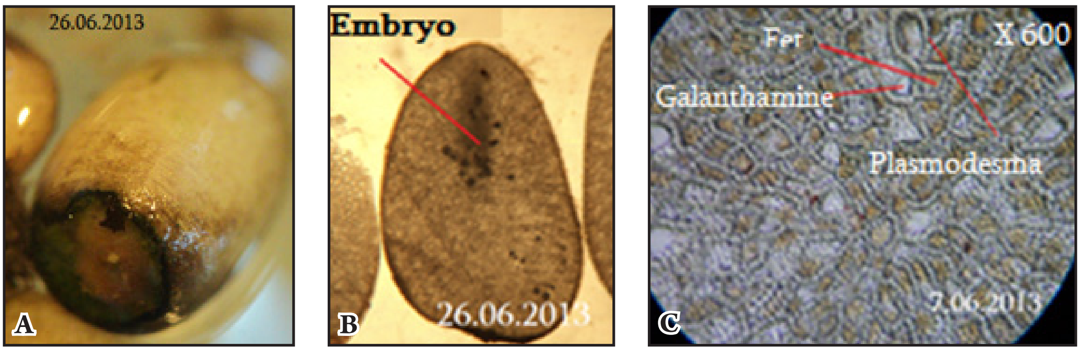

Fig. 8. *Galanthus woronowii*: **A** – seed without elaiosoma; **B** – development of embryo; **C** – reserve fats and plasmodesmas of seed.

variability coefficient of morphological features in the genus *Galanthus* is quite high (Budnikov & Kricsfalusy 1994). The shape of a capsule changes depending on its size (Tab. 1). The length and width of big capsules are equal; with decreasing of size the width increases; weight, number of seeds in it and average weight of one seed decrease too. When size of fruit decrease a number of empty cells increase (Tab. 1). When seed ripens, its weight decreases to 0.0075 g. The

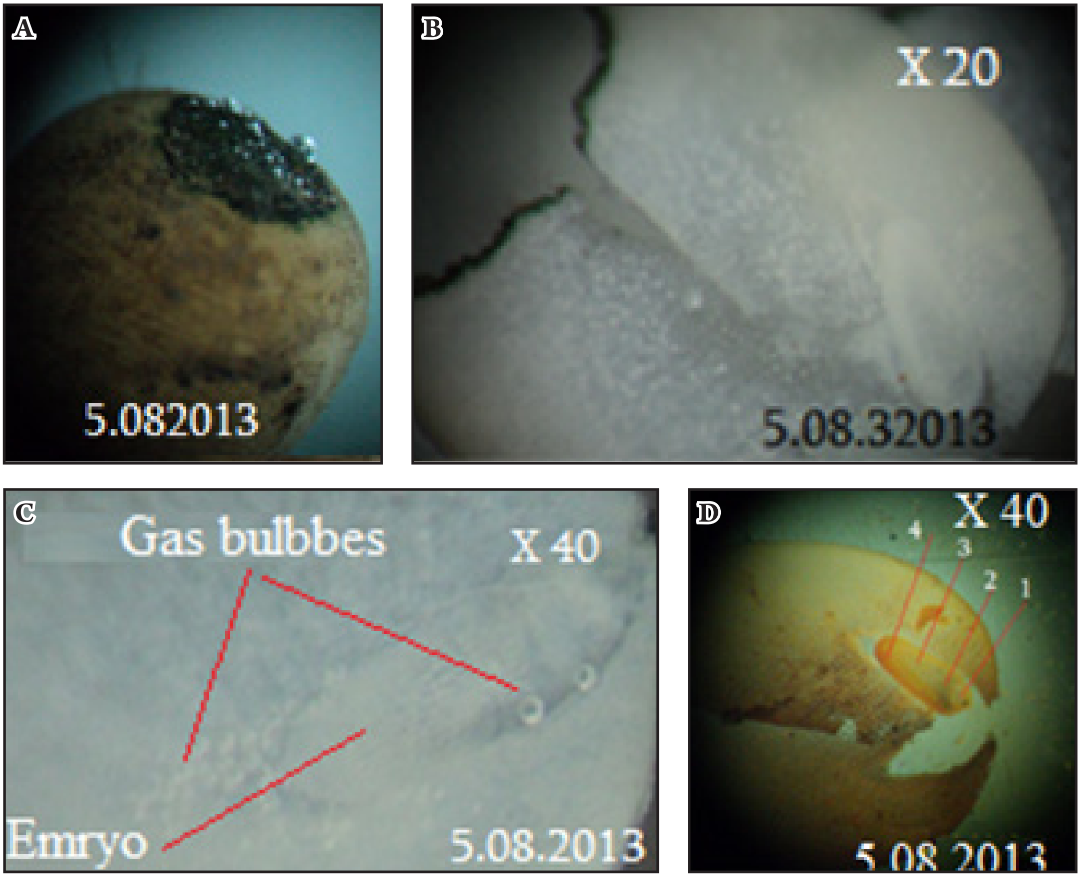

Fig. 9. Development of *Galanthus woronowii* embryo: **A** – seed respiration (natural size); **B, C** – embryo respiration; **D** – reserve substances (for 1-4 see explanations in text).

weight of a seed may vary within 0.007-0.012 g, depending on species, population and degree of ripeness (Budnikov & Kricsfalusy 1994; Sgenti & Khutsishvili 2013). After three months from opening of capsule, the length of seed decreased in 1.55 times, and width – in 1.20 times (Fig. 5).

By the end of May elaiosomes were well developed on the seeds, on the opposite side to embryo. By the end of June they disappear and green hollows develop on their places. Green spots were mentioned in other places of a seed as well. Gas bubbles of unknown composition were emitted from the mentioned green tissues. Emission of bubbles was intensive in August, when embryo was already well developed (Fig. 9).

Greenness was localized mainly on cell walls, as a result cells were highlighted by green contour. In some cells greenness was mentioned in a protoplast as well; cells of violet and dark blue coloration were also mentioned (Fig. 10).

In the first decade of May seeds contain fat-like substances in a big amount; by August they relatively decreased. There are cells in seed, which are not stained by Sudan III. They supposedly contain galantamine. Walls of seed's cells are thick; great number of plasmodesms is well seen in it. In the late May and early June embryo in a seed was not discerned. Its first contours become visible by the end of June (Fig. 8). Such seed does not contain elaiosome.

In early August an embryo is well seen on the cross- section of seeds. It was well stained by J+Jk and several zones has been highlighted: a) white, of elliptical form; b) of slightly conical form, blackish (reaction with J+Jk), in which starch should accumulate; c) lengthened

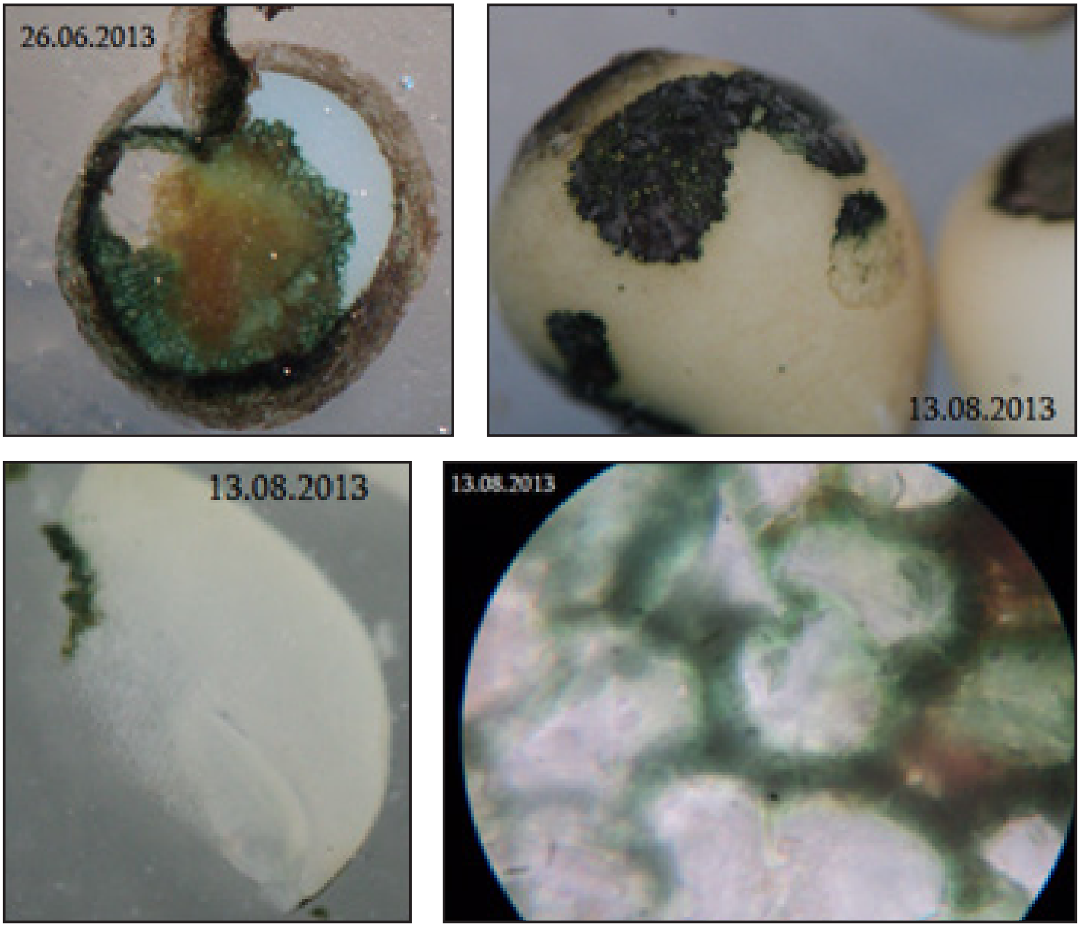

Fig. 10. Green tissues in the depth and on the surface of *Galanthus woronowii* seed.

formation of white color placed in yellow contour; d) yellow color capsule, in which three described structures are placed. Starch layer originated in the endosperm near to embryo. The tissue on the side of top of embryo has more crumbly structure; gas bubbles are well seen (Fig. 11).

Germination of seeds in the laboratory conditions began by the end of September and the process is very slow. By the end of October the length of roots was 2-3 times more than the length of seeds; aerial organs were not visible yet. It is to be mentioned, that by that time roots of bulbs placed in the pots together with seeds were developed as well.

Discussion

G. woronowii is a monocotyledon allogamous honey-bearing plant. In the conditions of Western Georgia it takes 3.5-4 months from fertilization till ripening of a fruit. On the early stage of development endosperm is rich in starch (Budnikov & Kricsfalusy 1994); active cytological and biochemical changes occur in line with development of seed; weight of seed increases at the expense of intensive assimilation of water and nutrients. It becomes very strong sink in respect of assimilates synthesized in the plant (Polevoi 1989; Copeland

Fig. 11. *Galanthus woronowii*: **A** – germination of seed in pots began; **B** – development of a root; **C** – development of stalk.

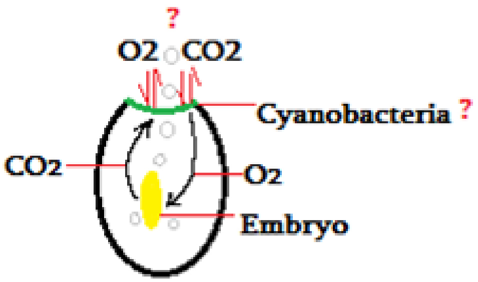

Fig. 12. Hypothetical scheme of gas exchange of the embryo with environment.

& McDonald 2001; Finkelstein 2004; Newtoni *et al.* 2013). In the later period of embryogenesis the metabolic processes and growth process cease; water content decreases to minimum (Copeland & McDonald 2001; Finkelstein 2004). Decreasing of weight of air dry seeds of *G. woronowii* may be explained by their dehydration. As opposed to seeds of the majority of monocotyledon plants, the reserve substance of *Galanthus* is fat instead of starch. Synthesis of fats and alkaloids in seed proceed, possibly simultaneously. The cytochemical reaction shows that about ¾ of cells of endosperm present a store of fats. Very developed plasmodesmic structure indicates active metabolic changes proceeding in a seed that is necessary for final formation of embryo. Formation of embryo takes approximately 5 months. After short pause germination and emergence of seedlings began. In the artificial conditions, germinability of seeds decreases mainly due to intensive loss of water. The closer are conditions of keeping the seeds to natural ones, the higher is the percent of germinability (Newtoni *et al.* 2013). In natural conditions in autumn the germinability of seeds is more than 80% (Sgenti & Khutsishvili 2013). Such peculiarities of seeds of *Galanthus* cause problems for *ex situ* conservation (Newtoni *et al.* 2013) and in-depth study is necessary in this direction. Seeding in open ground must be carried out just after abscission of fruits. The seeds are to be ensured with optimal hygrothermal regime that may be achieved by mulching of surface of soil and periodical watering, if natural precipitation is insufficient.

Formation of embryo is very energy-requiring process. It is accompanied by enhancement of intensity of respiration and synthesis of ATP. By the end of August fats begin transformation into starch in the places adjacent to the embryo; the products of hydrolysis of starch are consumed in aerobic respiration, and in synthesis of proteic substances. The tissues are relatively crumbly in the shortest direction from the embryo to the surface of seed; concentration of gas bubbles is mentioned in this place and around the embryo, as well as on the surface of the seed, where hollow and green colorings are developed.

Taking into consideration our results and literary materials we think to be acceptable, that aerobic oxygenic phototrophic bacteria in abundance provided with carbon dioxide emitted from the seed form a green coloring on the surface of a seed, and in turn create a medium rich in oxygen around the seed (Fig. 12). The green layer may have protective function as well, in order to withstand a colonization of seed by pathogen organisms. However, the mentioned point of view needs an experimental validation.

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