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**CONSERVATION *EX SITU* OF *GALANTHUS* SPECIES WITH BULGARIAN ORIGIN**

**Key Words:** *ex situ* conservation, *Galanthus*, protected plants

Species from genus *Galanthus* L. (Amaryllidaceae) are distributed in Europe, Asia Minor and Near East (Davis 1999). Presented like harvests of the spring, they have economical interest like garden plants and for content of alkaloids with pharmacological activity.

Despite of hundreds of cultivars, bulbs form *Galanthus ssp.* are one of the most wild-collected in the world (Entwistle & al. 2002). That is the reason the trade, export and import of bulbs to be controlled in the World by the CITES (Convention on International Trade in Endangered Species) convention, that ensure the international trade with wild species.

Bulgarian species from genus *Galanthus* are *G. nivalis* and *G. elwesii* (Anchev 1992). *G. elwesii* is widely distributed on the territory of Bulgaria, while *G. nivalis* was found in several geographically distinct locations (Sidjimova 2008). Both species occurs aeries with high human pressure - forests and river values. There are taxonomical problems and wide morphometrical and phytochemical variability with those species (Sidjimova 2008). *G. nivalis* and *G. elwesii* are protected by Biodiversity law (2002). According to IUCN criteria, used for the new edition of Red Data Book of Bulgaria the two species are evaluated like “endangered species” with recommendation for *in situ* and *ex situ* conservation (Evstatieva 2011).

The experimental cultivation comprises 36 origins of *G. elwesii* and 11 origins of *G. nivalis* (Table 1). It has been carried out on over 250 m<sup>2</sup>, located within the Green House of Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences.

Table 1.

**Origins from *G. elwesii* Hook. f. and *G. nivalis* L., included in the *ex situ* collection.**

№	Sample code	Species	Native habitat
1	028a	<i>G. elwesii</i>	NE Bulgaria: Obrochiste village, by the road to Balchic town, oak forest, 219 m; E exposition, 20.02.2002.
2	029a	<i>G. elwesii</i>	NE Bulgaria: Tsarkva village near Dobrich town, oak forest, 117 m; E exposition, 21.02.2002.
3	045	<i>G. elwesii</i>	NE Bulgaria: slopes right of the road Omurtag–Turgovishte, W exposition,
4	0210	<i>G. elwesii</i>	NE Bulgaria: Shumensko plateau, <i>Carpinus orientalis</i> forest near the monument, 454 m; W exposition, 21.02.2002; 28.02.2004.
5	0211	<i>G. elwesii</i>	NE Bulgaria: Slopes right of the road Omurtag–Turgovishte, deciduous forest, 301 m; W exposition, 21.02.2002; 26.02.2004.
6	049	<i>G. elwesii</i>	NE Bulgaria: Penev Grob locality between Kyulevcha and Markovo villages, <i>Carpinus orientalis</i> forest, 343 m; NE exposition, 28.02.2004.
7	048	<i>G. elwesii</i>	NE Bulgaria: Shumensko plateau, oak forest at the beginning of Bunata Reserve, on the left of the road, 555 m; NE exposition, 28.02.2004.
8	0410	<i>G. elwesii</i>	NE Bulgaria: The plateau above Madarsky Konnik locality, <i>Carpinus orientalis</i> forest, 50 m before the stronghold, left of the road, 450 m; NW exposition, 29.02.2004.
9	0216	<i>G. elwesii</i>	NE Bulgaria: Tervel town, locality under the wall; NE exposition, 20.02.2002.
10	0218	<i>G. elwesii</i>	NE Bulgaria: Tervel town, Dan Kula locality; NE exposition, 20.02.2002.
11	047	<i>G. elwesii</i>	NE Bulgaria: Oak forest between Sirakovo and Surnino villages, right on the road to Surnino; NE exposition, 27.02.2004.
12	046	<i>G. elwesii</i>	NE Bulgaria: Deciduous forest between Kalinata and Vasilevo villages, Chernata Gora locality, 300 m; NE exposition, 27.02.2004.
13	0411	<i>G. elwesii</i>	NE Bulgaria: Before Koprivets village, right on the road, <i>Carpinus orientalis</i> forest, 265 m; SW exposition, 29.02.2004.
14	0213	<i>G. elwesii</i>	Danube valley: Vladimirovo village, near the Monastery, bushes, 350 m; NE

			exposition, 22.02.2002.
15	0412	<i>G. elwesii</i>	Danube Valley: The valley of Chernelka River between Kurtozhabene and Gortalovo villages, near bushes, 175 m; SE exposition, 29.02.2004.
16	021	<i>G. elwesii</i>	Danube valley: Levsky town, “Karamandol” locality, 2001.
17	062	<i>G. elwesii</i>	Danube valley: Levsky town, the bridge over Osam river by the road to “Karamandol” locality, 61 m, NE exposure, 26.02.2006.
18	031	<i>G. elwesii</i>	Forebalkan (east): Yoglav village, Kamuka locality, near the forest; N exposure, 26.02.2003.
19	0212	<i>G. elwesii</i>	Forebalkan (east): Sevlievo town, <i>Tilia</i> forest near the road to Momina Salza hut, 400 m; NE exposition, 22.02.2002.
20	044	<i>G. elwesii</i>	Forebalkan (east): Veliko Turnovo town, slopes near the main road from Veliko Turnovo to Turgovishte, near bushes, 295 m; NW exposition, 26.02.2003.
21	0418	<i>G. elwesii</i>	Stara planina Mts (west): Baba peak, in the forest, 1500 m; NE exposition, 01.02.2003.
22	024	<i>G. elwesii</i>	Stara planina Mts (east): Karnobat town, Markela locality, before the town, 219 m; E exposition, 18.02.2002.
23	023	<i>G. elwesii</i>	Stara planina Mts (east): Sliven town, near the road to Karandila, oak forest, 848 m; W exposition, 18.02.2002.
24	0221	<i>G. elwesii</i>	Sofia region: Kokalyane village, near the Urvich stronghold, 610 m; NW exposition, 05.03.2002.
25	0419	<i>G. elwesii</i>	Sofia region: Makotsevo village, Sinigerov Dol locality, <i>Carpinus orientalis</i> forest, 650 m; NE exposition, 18.03.2004.
26	032	<i>G. elwesii</i>	Znepole region: Trun town, Mogilata locality, 550 m; NW exposition, 25.03.2004.
27	042	<i>G. elwesii</i>	Mesta Valley: Hadzhidimovo village, St. Dimitar chapel, deciduous forest, 450 m; SW exposition, 19.02.2004.
28	041	<i>G. elwesii</i>	Pirin Mts: Musomishte village, Karacheto locality, 550 m; E exposition, 19.02.2004.
29	043	<i>G. elwesii</i>	Pirin Mts: Gospodinci village, “Trudovaskata chesma” locality, E exposition, 19.02.2004.
30	0413	<i>G. elwesii</i>	Sredna Gora Mts: Nivata locality near the river Mativir, Ihtiman town, 600 m; N exposition, 28.05.2004.
31	034	<i>G. elwesii</i>	Rhodopes (west): Belovo–Yundola road, over Yadenica River, 776 m; NE exposition, 19.03.2003.
32	035	<i>G. elwesii</i>	Rhodopes (central): Ruen village near Plovdiv city, deciduous forest near St. Ilia chapel, 600 m; NE exposition, 19.03.2002.
33	0222	<i>G. elwesii</i>	Rhodopes (central): Bachkovo village, near the road from Monastery to children's camp, 485 m; NE exposition, 09.03.2002.
34	0422	<i>G. elwesii</i>	Central Rhodopes: Trigrad, on the rocks over the gorge, 1500 m, NE exposure, 25.03.2004.
35	022A	<i>G. elwesii</i>	Tundza Hilly region: Topolovgrad, over the river, E exposition, 10.02.2002.
36	022	<i>G. elwesii</i>	Tundza Hilly region: Topolovgrad, near Vishegrad peak, 826 m, E exposition, 10.02.2002.
37	029	<i>G. nivalis</i>	NE Bulgaria: Tsarkva village near Dobrich town, oak forest, 117 m; E exposition, 21.02.2002.
38	028	<i>G. nivalis</i>	NE Bulgaria: Obrochiste village, by the road to Balchic town, oak forest, 219 m; E exposition, 20.02.2002.
39	025	<i>G. nivalis</i>	S Black sea coast: Pmorsko, right on the road, in bushes, 9.7 m, E exposition, 19.02.2002.
40	026	<i>G. nivalis</i>	Black sea coast (south): Primorsko, oak forest near field for cultivation of <i>Leucojum aestivum</i> , 5.8 m, E exposition, 19.02.2002.
41	027	<i>G. nivalis</i>	Black sea coast (north): Varna, Aladza monastery, 240 m, E exposition, 20.02. 2002.
42	061	<i>G. nivalis</i>	Black sea coast (south): “Arcutino” reserve, left of the road to Primorsko, 2 m, E exposition, 01.03.2006.
43	063	<i>G. nivalis</i>	SBlack sea coast: The road from Kiten to Primorsko, in bushes on sand 2 m, E exposition, 01.03.2006.
44	0420	<i>G. nivalis</i>	Forebalkan (west): Belogradchic town, “Veneca” locality, deciduous forest, 575 m, NE exposition, 23.03.2004.
45	033	<i>G. nivalis</i>	Znepole region: Trun town, Filipovci village, “Sekirica” locality, deciduous forest, 650 m, NW exposition, 25.03.2004.
46	051	<i>G. nivalis</i>	Strandza: Zubernovo village, “Tomova bachtcha” locality, 250 m, E exposition, 10.02.2005.
47	052	<i>G. nivalis</i>	Strandza: Gramaticovo village, “Katchul” locality, 250 m, E exposition, 10.02.2005.

For each origin 30-50 individuals were taken from their natural habitats during May, having care not to affect the population in the collecting localities. The adult plants were collected with the permission of Ministry of the Environment and Waters.

The plants were grown according to ordinary used agro techniques (Evstatieva 1999, Evstatieva & Stanev 2005). The special prefer to soil and climatic conditions were taken (Usta & Arslan 1992, Arslan & al. 1997, Davis 1999, Entwistle & al. 2002, Balabas & al. 1965, Lapin (ed.) 1983, 1984).

The adult individuals were planted singly in flower beds. The bulbs were placed over 7 cm deep and 10 cm one from the other. The oak tree and bushes over the flower beds shaded them between 8 am and 3 pm. During the whole vegetation season the collection was cleaned from weed (3-4 times a year). The watering was done if necessary once a week in the spring and every day from June to September.

The transplanting crisis affected most of the plants. Over 20% of the plants died, and the rest had only leaves during first vegetation season in the collection. During the second vegetation season over 30% of plants flowered and 2.8% fructified. After three years the planted individuals were normally developed, with flowers and over 20% produced fertile seeds. All plants were successfully multiplied vegetatively, forming daughter bulbs.

The development of plants from most of the origins was at the same time, but the origins from over 1500 m altitude (0422 - Trigrad and 0224 – Kozia stena, 0418 – Baba peak) cross different phenological phases 2-3 days later.

The beginning of vegetation in the conditions of Sofia was during the second half of December. Plants were under the snow during winter in phase of butonization. During the juvenile faze (middle of January – beginning of March) the leaves grew high. At the flowering time (middle of January – beginning of April) the leaves were completely developed, but the final development was in maturity (beginning of April – the end of June). According to Davis (1999) this process is connected with collection of reserve nutrients in the bulbs. The resting period is dependent on temperature and humidity. Began in the first half of July, after ripening of seeds and ends in mid-December to late January.

In the *ex situ* collection the flowering began 10-12 days before the flowering in natural habitats. This dependence is established from Maak & Storch (1997) in *G. nivalis*. According to Roetzer & Wittenzeller (2000) this is connected with high sensitivity to temperature amplitudes of plants in the beginning of flowering.

The assessment of success of introduction had shown that plants were well adapted to *ex situ* environments in the Green House of IBER, BAS. The mature individuals were dry-resistant during development (beginning of February – the end of June) and had regularly blossoming (Fig. 1, 2).



Fig. 1. Blossoming individuals from *G. elwesii* Fig. 2. Blossoming individuals from *G. nivalis*

According to environmental conditions and the origin some plants fructified and formed seedlings (Fig. 3).



Fig. 3. Seedlings from *G. elwesii* in the *ex situ* collection

Over 95% of seedlings hardly survived winter and did not develop during next growing season. All plants in the *ex situ* collection showed good vegetative regeneration. After a 3 year period we had clumps with 3-4 blossoming individuals (Fig. 4).



Fig. 4. Clumps in the *ex situ* collection

Morphometrical variability and alkaloid content were investigated for most of the origins in the collection (Sidjimova 2008, Berkov & al 2011, Sidjimova & al 2003, Berkov & al 2004).

The creation of the *ex situ* collection of *Galanthus* species with Bulgarian origin has some very important functions:

- It contains five perspective hemotypes from *G. elwesii* and two from *G. nivalis*. They have high percentage of alkaloids with pharmaceutical activity, but their populations are with limited distribution in Bulgaria. Including plants from this origins in *ex situ* collection will ensure their conservation and give the possibility for more phytochemical analysis.
- The *ex situ* collection is very important for future taxonomic revisions of Bulgarian *Galanthus* species. Taxonomic investigations of *Galanthus* species necessitate the study of living specimens, because taxonomically important features are lost after pressing and drying when plants are prepared as herbarium specimens (Davis 1999).
- The *ex situ* collection is very important for conservation of species from genus *Galanthus* and preservation of original morphotypes and chemotypes. Its creation is a part of planned activities for conservation of the species (Evstatieva 2011). During the years the treatments were connected with uncontrolled collection of bulbs from native habitats, used for phytochemical and pharmaceutical investigations. Today the treats are different. Human activity leads to destruction of of habitat and reduction in plant populations. For example, in

the past *G. elwesii* had been widely distributed over Sofia (Stoyanov 1966), but during last 50 years this species the species was not found at Vitosha and other populations near Sofia are with about 150-200 individuals.

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