Protection of rare and threatened seed plant species from Bulgaria in an *ex situ* collection

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Abstract. The article provides results achieved in an „*ex situ*" protection of 15 rare and threatened plant species. The collection comprises plant species of authentic origin, nine of which transferred live from their natural habitats, and six obtained from seeds, cuttings and seedlings. The process of collection-making has been traced out; data are presented on the source material of the different species, on some specific approaches applied during introduction of the plants into the collection, on the processes of plants adaptation and their development outside their natural environment.

Of the transferred live plants, good adaptive abilities and successful development have been observed on the part of perennial grass species and species with vegetative reproduction. About 80 % of the plants have reached their generative phase, flowering and seed-making. The specimens kept in the collection could serve as material for scientific research and/orcould be returned to their natural habitats, if necessary, in order to srtabilize the populations.

Key words: *ex situ* collections, rare and threatened species, protection

Introduction

The major normative documents in the domain of environmental protection in Bulgaria – the Biological Diversity Act (2002) and the Protected Territories Act (1998) – regulate the two main approaches for the protection of biological diversity: protection in the natural environment (*in situ*) and protection outside the natural environment under controlled conditions (*ex situ*). Application of these approaches to some particularly important elements of the biological diversity, such as the rare and threatened by extinction biological species, guarantees their survival in time.

Creation of an *ex situ* collection for the protection of rare plant species is a well established and ever wider applied practice, both internationally (Cohen & al. 1991, Dulloo & al. 2010) and nationally. The Institute of Biodiversity and Ecosystem Research with the Bulgarian Academy of Sciences has accumulated experience in the application of this conservation approach of long standing (Evstatieva & Koleva 2000, Evstatieva & Kunchev 2001, Gussev & al. 2003a,b, Evstatieva 2006 etc.) in the created and maintained collections of rare and medicinal plants. The „*ex situ*" protection approach has been applied to some of the species under the project "A Pilot Network of Small Protected Sites for Plant Species in Bulgaria Using the Plant Micro-Reserve Model". In the collection created within the framework of the Project, specimens have been preserved of some rare plant species represented in the country with limited-area and small-in-numbers populations that need presently or in the future some assistance in their natural environment.

Material and methods

The plants grown in the collection are authentic in origin and, according to their mode of introduction into the collection, are divided into two groups:
– live plants transferred from their natural habitats;
– plants obtained from seeds, cuttings or seedlings.

The infrastructure, spatial organization of the terrain and equipment of the collection rely on well-known agricultural techniques applicable to that type of activities (Trankov 1975; Penkov 1986; Dyakov 1993), as well as on the experience in *ex situ* growing of rare and threatened species of the Bulgarian flora (Koleva & al. 1989; Evstatieva & Koleva 2000; Evstatieva & Kunchev 2001; Gussev & al. 2003a,b; Evstatieva 2006; Kozuharova 2009; Gorgorov 2014; Uzundzhalieva 2014a,b). Reproduction of plants from seeds and their adaptation under controlled conditions were carried out in the vegetation house of the Institute of Biodiversity and Ecosystem Research, BAS, base 3. Data on the environmental status, biological type and mode of reproduction were taken from the articles published on these species in Vladimirov (2014).

Results and discussion

Infrastructure and collection equipment

The collection is set on an area of 150 m^2 adjoining the vegetation house of the Institute of Biodiversity and Ecosystem Research (Fig. 1). Preparation of the terrain includes double ploughing, clearing of weeds and rough materials, laying down a layer of loose soil, shaping out experimental plots, and setting of an irrigation installation.

Fig. 1. View from the collection.

Introduction of the plant species into the collection: specific approaches

Of the now existing 15 plant species in the collection (Table 1), nine were directly transferred from their natural habitats and six were obtained from seeds, cuttings and seedlings. In line with the biological peculiarities and ecological requirements of the different species, the following has been taken into consideration during their introduction into the collection:
– putting together in one and the same experimental plot species with kindred requirements in respect to soil, humidity and light;
– planting the specimens of tree and shrub species on independent plots with a view of their need in larger space for their development;
– planting the specimens of tree and shrub species separately from the photophylous species, so as to prevent the shadowing of the latter;
– matching the planting of specimens of tufaceous plants and plants with active vegetative reproduction to their need in more space for their subsequent development.

For successful adaptation and development of the plants, the soil composition for some of them has been additionally improved, according to nature of the soil substrate in the respective habitats. In most cases, during planting of the transferred live plants, soil from their habitats has been added to the basic soil of the collection. For species favouring calcareous soils, the place of their planting has been additionally treated with quicklime (for instance, for *Aethionema arabicum, Erodium absinthoides*). In order to ensure sufficient space for their further development, the young plants of *Eriolobus trilobata* and the rooted cuttings and seedling of *Amygdalus webbii* and *Spiraea crenata* were planted in the experimental plots in rows distanced 2 m from each other and at a distance of 1–1.5 m between the different specimens. The large-rosette plants (the *Verbascum* species), which form large tuffs (*Erodium absinthoides*, *Achillea thracica*), as well as the plants requiring more space owing to vegetative propagation (*Serratula bulgarica*, *Limonium bulgaricum*) were planted at a distance of 30–50 cm between the specimens.

For the *Amygdalus webbii* and *Eriolobus trilobata* species, the mulching technique was applied, so as to protect the young saplings from freezing in the winter months.

Table 1. Species in the *ex situ* collection.*

Species	Conservation status	Biological type	Means of reproduction	Source material for the collection
Achillea thracica Velen.	CR, BDA, BK, IUCN(R), Bulgarian endemic	Perennial	By seeds and vegetative reproduction by rhizome shoots	Living plants
Aethionema arabicum (L.) O.E. Schulz	CR, BDA	Annual	By seeds	Seeds
Amygdalus webbii Spach	CR, BDA	Shrub	Vegetative reproduction by sprouting	Sprouts
Centaurea finazzeri (Adamovič) Hayek.	CR, BDA, Balkan endemic	Perennial	By seeds and vegetative means	Living plants
Convolvulus holosericeus M. Bieb.	CR, BDA	Perennial	By seeds	Living plants
Eriolobus trilobata M. Roem.	CR, BDA	Tree	By seeds	Seeds
Erodium absinthoides Willd.	EN, BDA	Perennial	By seeds and vegetative means	Living plants
Limonium bulgaricum Аnцev	CR, BDA, Bulgarian endemic	Perennial	By seeds and vegetative means	Living plants
Matthiola odoratissima (M. Bieb.) R. Br.	CR, BDA	Perennial to semishrub	By seeds	Seeds
Serratula bulgarica (L.) Acht. & Stoj.	CR, BDA, IUCN(R)	Perennial	By seeds and vegetative means	Living plants
Spiraea crenata L.	CR, BDA	Shrub	By seeds and by root sprouting	Cuttings and sprouts
Verbascum anisophyllum Murb.	CR, BDA, Balkan endemic	Biennial to perennial	By seeds	Living plants
Verbascum purpureum (Janka) Huber-Morath	EN, BDA, BK, IUCN(R)	Biennial	By seeds	Seeds
Verbascum spathulisepalum Greuter & Rech. f.	EN, BDA, Balkan endemic	Perennial	By seeds and vegetative reproduction by rhizome shoots	Living plants
Verbascum tzar-borisii (Davidov ex Stoj.) Stef.-Gat.	CR, BDA, Bulgarian endemic	Perennial	By seeds	Seeds

* **CR** – Critically Endangered, **EN** – Endangered, **VU** – Vulnerable, **BDA** – Biological Diversity Act, **BK** – Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention), **IUCN(R)** – International Union for Conservation of Nature (Rare).

Plants transferred live from their natural habitats (Table 2; Figs. 2a, b)

In line with the specificities of the different species, the live plants transferred into the collection have undergone different periods of adaptation under the new conditions, with better adaptation abilities manifested by the perennial grasses and the species with vegetative propagation. In *Achillea thracica*, *Serratula bulgarica* and *Verbascum anisophyllum,* the adaptation period of transferred plants was short and they resumed their normal development soon after planting, reaching subsequently the generative stage. A longer adaptation period has been observed in *Limonium bulgaricum*, where for one year after planting the plants remained in the form of vegetative leaf rosettes, without forming flower-bearing stems.

In order to keep a larger number of specimens in the collection, without sacrificing their numbers in the natural populations, in *Achillea thracica* and *Serratula bulgarica* the rhizomes of the transferred plants from their natural habitats were successfully split. Owing to this, now two to three times more specimens of these species are grown in the experimental plots, as compared to those in the field. In *Centaurea finazzeri,* the

Table 2. Species transferred alive from their natural habitats.

Species	Number of transferred specimens/year	Number of specimens in the collection in 2014	Number of generative plants
Achillea thracica	2 – XI.2012; 3 – II.2013	5 + 13 (resulting from rhizome division in May 2013 and April 2014	18
Amygdalus webbii	10 – XI.2012 7 – IV.2013	14	0
Centaurea finazzeri	2 – VIII.2012	2 + 4 (self-seeding)	6
Convolvulus holosericeus	3 – IX.2013	3	3
Erodium absinthoides	4 – VII.2012	1	1
Limonium bulgaricum	3 – VIII.2013	3	0
Serratula bulgarica	9 – VII.2012	9 + 9 (resulting from division of rhizomes in May 2013)	18
Verbascum anisophyllum	4 – VII.2013	3	3
Verbascum spathulisepalum	8 – VII.2012	2 + 15 (by seed propagation)	17
Lathyrus pancicii	3 – VII.2012	none	
Astracantha thracica	2 – XI.2012	none	
Astragalus dasyanthus	3 – VI.2012	none	

Fig. 2a. *Erodium absinthoides* – transferred living plant.

Fig. 2b. *Verbascum spathulisepalum* – transferred living plant.

number of plants was also increased as compared to the initially planted, owing to self-seeding of the initially transferred specimens.

Observations of the transferred live plants have shown that the phase they were in at the time of their planting in the collection, and the state of their root system are both very important for their further development. Because of this and probably owing to unsatisfactory digging out of the plants from the field, or to cutting or damaging of their roots, there was an unsuccessful attempt at transferring into the collection *Astracantha thracica, Astragalus dasyanthus* and *Lathyrus pancicii* – all species with powerful and deep-reaching rhizomes. For these species, attempts have been made or are under way to reproduce them from seeds, so that they would be also preserved in the collection.

Plants obtained from seeds, cuttings and seedlings (Table 3; Fig. 3)

The seeds were collected from the plants in their natural habitats and sown for germination into pots in the vegetation house. After germination and reaching of a specified phase, they have been thinned out and put in a soil substrate (universal fertilized mixed soil with high peat content) and after a period of adaptation under the conditions of the vegetation house, the best developed were taken to the open-air experimental plots. In plants propagated from seeds, the best results have been obtained in the species with high germination capacity.

In *Matthiola odoratissima, Verbascum purpureum* and *Verbascum tzar-borisii,* it was established that their growing in a collection is possible only by seed

Fig. 3. *Eriolobus trilobata* – stages in the propagation from seeds.

Table 3. Species germinated from seeds, cuttings and shoots.

Species	Seed germination rate (%)	Number of sown seeds/year	Number of plants obtained from seeds	Period of adaptation under controlled conditions	Number of plants transferred to the collection	Number of plants present in the collection in 2014
Aethionema arabicum	5–20 %	70 May 2011 in the glass-house	13	12 months	0	0 (annual)
		20 November 2013 in the collection	1		1	1
Eriolobus trilobata	86 %	100 December 2010	56	4 months	25	6; 19 reintroduced in the native locality of the species
Matthiola odoratissima	16 %	100 May 2011	12	12 months	6	1
Verbascum purpureum	30 %	250 October 2013	71	1 month	30	10; 20 given to other collections
Verbascum tzar-borisii	35 %	100 February 2014	31	1 month	10	10
Spiraea crenata					12 cuttings – July 2012; 5 sprouts February 2014	17

reproduction; the attempts at transferring and keeping the live plants have proved unsuccessful. Of these species, the species of genus *Verbascum* have shown good adaptivity and have reached the generative stage. In *Matthiola odoratissima* and *Aethionema arabicum,* probably owing to their strict ecological and biological specificity, propagation from seeds and growing in the collection have proved very difficult.

Conclusion

Information have been achieved on an *ex situ* collection of rare species of higher seed plants distributed on the territory of Bulgaria in single or few habitats, with area-limited and critically low in numbers populations threatened by damage or extinction.

The collection comprises 15 plant species of authentic origin, nine of which transferred live from their natural habitats, and six obtained from seeds, cuttings and seedlings. Of the transferred live plants, good adaptive abilities and successful development have been observed on the part of perennial grass species and species with vegetative reproduction. About 80 % of the plants have reached their generative phase, flowering and seed-making. In plants propagated from seeds, the best results have been obtained in the species with high germination capacity.

The created collection not only ensures the protection of genetic stock from rare plant species, but also provides material for scientific researches, for enrichment of the live collections of rare species in the botanical gardens, as well as an opportunity for returning some of the plants into their natural populations for stabilization, if necessary.

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