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EX SITU CONSERVATION OF *SERRATULA BULGARICA* ACHT. & STOJ. IN THE REPUBLIC OF MOLDOVA

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Abstract

The study refers to an Asteraceae taxa – Serratula bulgarica Acht. & Stoj., a very rare species in the flora of the Republic of Moldova, reported only in the phytocenoses of Quercus pubescens Willd. in the vicinity of the Batîr commune in the Cimişlia district, whose actual number does not exceed 50 phytoindividuals. This is a Critically Endangered taxa, protected by law, included in the Red Book of the Republic of Moldova, 3rd ed., the 1997 IUCN Red List of Threatened Plants. For the conservation and multiplication of the Serratula bulgarica species in ex situ conditions, fragments of rhizomes collected in natural habitat in the autumn of 2022 were planted in the experimental plot. Ex situ the species develops well, goes through all ontogenetic stages, forms a vigorous habitus. The seeds are viable and the germination rate in laboratory conditions is about 90%. Vegetative propagation by plantlets from renewal buds on rhizomes is high.

Key words*: Serratula bulgarica Acht. & Stoj., rare taxa, conservation, Republic of Moldova.*

INTRODUCTION

Serratula bulgarica Acht. & Stoj. (= *S. caput-najae* Zahar., *Klasea bulgarica* (Acht. & Stoj.) Holub) of the Asteraceae family is one of the most endangered species in the flora of the Republic of Moldova and requires the application of urgent conservation measures (Ionita and Tofan-Dorofeev, 2015). Species protected by law (Environmental legislation..., 1999), included in the Red Book of the Republic of Moldova, 3rd ed. (Ioniță, 2015), as well as in the Red Book of Vascular Plants in Romania (Dihoru and Negrean, 2009), Red Book of Bulgaria (Tzonev, 2015), listed in the IUCN Red List of Threatened Plants (1997). Balkan sub-endemic. The general distribution of the species includes north-eastern Bulgaria, south-eastern Romania, southern Republic of Moldova and south-eastern Ukraine (Figure 1).
In the Republic of Moldova, *S. bulgarica* grows only in the phytocenoses of xerophilic *Quercus pubescens* Willd. with *Iris variegata* L. (Pînzaru et al., 2022) in the vicinity of Batîr commune, Cimislia district. Almoust 50 years after the first attestation of the species in the country's flora (Gochu, 1979), the state of its population is unfavorable, threatened with extinction, with a very small number of individuals.

Figure 1. *Serratula bulgarica* Acht. & Stoj. distribution worldwide powo.science.kew.org

In such cases, the only way to support the conservation of species is to create artificial populations, grown *ex situ* in Botanical Gardens for a long time (Gorbunov et al., 2008), with the aim of propagating and restoring population conditions *in situ*. At the same time, the collection of living plants serves as a base for in-depth study of the biological and ecological peculiarities of the species.

MATERIALS AND METHODS

The research was conducted during 2022-2024 according to the generally accepted method (Akeroyd et Jackson, 1995; IUCN Guidelines for Re-introductions, 1998; Gorbunov et al., 2008; Godefroid et al., 2011). This study is part of the *ex situ* conservation and *in situ* reintroduction program of endangered plant species, carried out within the "Al. Ciubotaru" National Botanical Garden (Chisinau). The materials for the creation of the collection of living plants under *ex situ* conditions of the species *Serratula bulgarica* were taken within its *in situ* study.

The plants for conservation, multiplication and seed production were obtained vegetative, from fragments of rhizomes collected in natural habitat in the autumn of 2022. In the 2023 growing season, *ex situ* plants developed well, bloomed and fructifies, and the collected seeds were used for germination capacity research and seedling cultivation for *in situ* actions. The seed germination ratio was tested by placing on filter paper in Petri dishes at 18-20°C and watered with distilled water and recording the proportion of germinated seeds. Subsequently, germinated seeds were transferred from Petri dishes to pots of 10 cm diameter, which contained a peat and soil mixture.

The population assessment of *Serratula bulgarica* species was carried out, the degree of threat was estimated and the endangered category established, according to the requirements of the International Union for Conservation of Nature (IUCN, 2012), at the same time limiting factors and proposing protection measures were identified. The color photos are original, taken by Olga Ioniță with a Nikon D 3100 digital camera.

RESULTS AND DISCUSSIONS

In the flora of the Republic of Moldova *in situ,* the population of the species *Serratula bulgarica* grows in the clearings and edge of a stand of *Quercus pubescens* Willd. near the village of Batîr, Cimislia district, which represents an unprotected forest sector, subject to anthropogenic pressures. The size of the population and its number of individuals is extremely low, about 30 specimens, and seed production is practically absent (Figure 2).

Figure 2. *Serratula bulgarica* in natural habitat

Although *Serratula bulgarica* has an increased capacity to reproduce vegetatively, in the natural habitat the species faces severe regeneration problems. Being threatened with extinction, *ex situ* conservation is the most suitable option for conserving the species.

Serratula bulgarica Acht. & Stoj. in Bull. Soc. Bot. Bulgarian. 5: 111 (1932) (= *S. caput-najae* Zahar., Bull. Sect. Sci. Acad. Roumaine 28 (Nouv. Esp. Compos. 9): 318 (1946); *Klasea bulgarica* (Acht. & Stoj.) Holub, in Folia Geobot. Phytotax. 12(3): 305 (1977).

Morphology. Perennial plant, 35-100 cm tall. Blackish-brown horizontal rhizome, branched. Stem erect, sparsely foliated. Basal leaves subleathery, elliptical to lanceolate-ovate, long-petiolate, acute or obtuse, irregularly toothed or entire, glabrous, only on short rough-ciliated edges. Stem leaves variable: the lower similar to the basal ones; the middle attenuated and gradually shorter petiolate, lanceolate, the lamina towards the base hard toothed to the penate-fidate; the upper narrow-lanceolate to linear, sessile, deeply penate-partite or whole. Anthodium solitary, globular or campanulate, 30-35 mm long and 16-18 mm in diameter.

Involucral bracts polyseriate, coriaceous, glabrous, green, at the tip with yellowish, membranous, wide-ovate, ovate-triangular or subround appendages, wavy on the edges. Flowers tubular, actinomorphic, bisexual, purplish to pinkish-purple, 20-25 mm long, Achene glabrous, compressed, with papus of dentate hairs (Cannon et Marshall, 1976, Ioniță, 2022) (Figure 3).

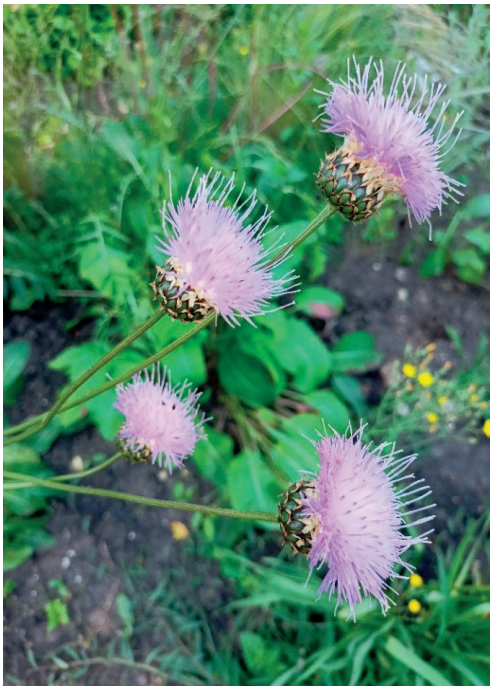

Figure 3. Flowering specimens of *Serratula bulgarica* in *ex situ* conditions

Biological and ecological peculiarities. Balkan hemicryptophyte. Mesophilous species. It blooms in May-June, fructifies in June-July. It propagates both generatively, through seeds, and vegetatively, through root rosette. An anemochore and zoochore plant. Decorative during flowering.

***Ex situ* conservation actions.** In August 2022, trips were made to the natural habitat of the *Serratula bulgarica* species, with the aim of both conducting floristic research and collecting the botanical material necessary for creating the collection of living plants preserved *ex situ*.

In the spring of 2023, 7 specimens sprouted in the experimental sector: 4 generative, floriferous and 3 vegetative (Figure 4).

Figure 4. *Serratula bulgarica* in experimental plot of the "Al. Ciubotaru" National Botanical Garden (I) (April, 2023)

Under *ex situ* conditions, the mature specimens of the *Serratula bulgarica* species have gone through all the phenological phases of the seasonal rhythm of development (Figure 3). In this way, frutify and produced viable seeds, which served as a basis for further research and the multiplication of the species generatively.

In the study, in March 2024 the ability to germinate seeds under laboratory conditions was tested.

To perform the experiment, 50 seeds were placed in the Petri dish on filter paper, moistened with distilled water, at a temperature of 18-20°C. On the fifth day from the beginning of the imbibition, the first seeds germinated, and germination was recorded over 5 days, until new germinations were not observed. According to the data obtained, the germination rate is high, about 90%, an important aspect for the propagation of endangered species that produce a low number of viable seeds (Figure 5).

Figure 5. *Serratula bulgarica* - seed germination

On the tenth day after the start of the experiment, the plantlets in the Petri dish were planted in pots with a diameter of 10 cm and transferred to the greenhouse for further growth and development (Figure 6).

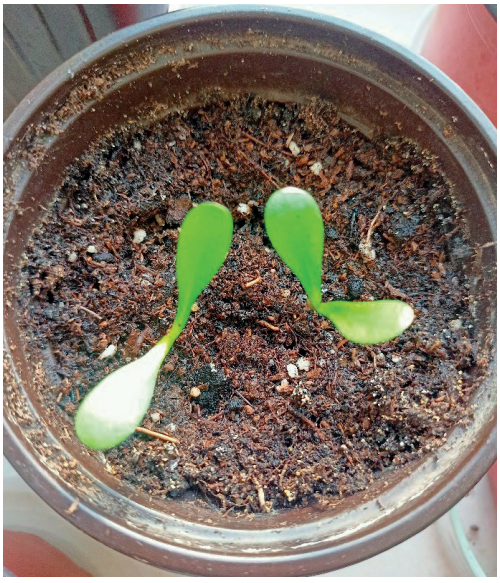

Figure 6. Plantlets in first week

After 4 weeks, the grown seedlings were transferred to the open space. Subsequently, after 5 weeks, in the second decade of May, the acclimatized seedling was planted directly in the soil, in open ground where they quickly adapted (Figure 7). Within 5 months of planting, significant increases were recorded. In October, the vegetative specimens obtained from seeds had rosettes of 15-20 cm high, with 9-18 leaves, 8-20 cm long and 3-7 cm wide. Well-developed root system, 10-15 cm long. Most plants, in addition to the main rosette, emitted from 2-3 to 6-7 seedlings from the root buds (Figure 8). As a result, for the first time, 40 vegetative specimens of the species *Serratula bulgarica* were generatively obtained, under cultivation conditions, multiplied in order to expand the population in the natural habitat, which is at a standstill.

Figure 7. Seedlings recently, planted in open ground (May, 2024)

Sozoological status. In the Republic of Moldova *Serratula bulgarica* is an endangered species, categorized, according to the guide developed by the International Union for Conservation of Nature as Critically Endangered [B2ab(ii,iii); C2a(i,ii); D], included in the Red Book of the Republic of Moldova, 3rd edition.

At the same time, *Serratula bulgarica* is a rare species in all the countries from which it has been reported so far, being included in the Red Book of Vascular Plants in Romania, as Vulnerable (Dihoru et Negrean, 2009) and in the Red Book of Bulgaria, within the Critically Endangered category (Tzonev, 2015). In Ukraine, where the species was most recently

discovered in 2001, it is also a rare taxon, proposed for inclusion in the Red Book of Ukraine (Kucherevskyi et al, 2009).

At the European level, *Serratula bulgarica* is included in the 1997 IUCN Red List of Threatened Plants, with category ”Rare” (Walter et Gillet, 1998).

As an additional conservation measure of the species, *S. bulgarica* is cultivated in the Botanical Garden of Kryvyi Rih, Ukraine (Kucherevskyi et al, 2009), the "D. Brandza" Botanical Garden in Bucharest, Romania (Dihoru et Negrean, 2009) and in the National Botanical Garden "Al. Ciubotaru" in Chisinau, Republic of Moldova.

As an efficient means of medium or long-term *ex situ* conservation, researchers from the Institute of Biology in Bucharest used *in vitro* culture systems, presenting for the first time efficient protocols for seed germination, *in vitro* seedling development, induction of somatic embryogenesis and complete regeneration, for the purpose of *ex situ* conservation of the endangered species *Serratula bulgarica* (Manole-Aiftimie et al, 2013).

Limitation factors. Small area, limited specific habitats, very small number of individuals. Weak natural regeneration. Inadequate forest management and destruction of individuals during forest improvement works. Anthropopression of forest sectors where the plant grows, mowing and grazing of forest glades. Ruderalization of habitats.

Necessary protection measures. Strict compliance with the protection regime for rare species. Monitoring the state of the population, size, number of individuals, growing conditions. Research of the biological and ecological peculiarities of the species, threats, presservation of germplasm in gene banks. To include in the Fund of State Protected Natural Areas of the Republic of Moldova the forest sector in the vicinity of Batâr commune, Cimislia district, the only place of growth of the species *S. bulgarica*. Developing an action plan aimed at identifying the measures and activities necessary to ensure the sustainable conservation of the population and habitat of the *Serratula bulgarica* species in the Republic of Moldova, an action plan that was successfully developed and implemented for this species in Bulgaria (Stoyanov et Goranova, 2014).

Figure 8. Seedlings at the end of the growing season (October, 2024)

CONCLUSIONS

Serratula bulgarica is an endangered subendemic species with a very restricted range, fragmented populations and a small number of individuals. Field research conducted in the natural habitat of the species has elucidated that the biological indicators of the populations are deteriorating more and more, and *Serratula bulgarica* is threatened with extinction in flora of Republic of Moldova. *Ex situ* conservation in the "Al. Ciubotaru" National Botanical Garden (I) is a meaningful way to preserve this endangered species, as well as to accumulate experience regarding its behavior in culture conditions.

As a result of this study, it can be concluded that in *ex situ* conditions the species manifests itself differently than *in situ*, mature specimens develop a vigorous habitus, bloom and fruit, producing viable seeds, with a high germination rate of 90%. Thus, it was possible to cultivate the plants generatively, the experience demonstrating very good results. Most of the plants, successfully reproduced *ex situ*, will be reintroduced into the natural habitat where the species grows for reinforcement and restore the *in situ* population, which is facing serious

propagation problems. This will represent an important stage in the efforts made to conserve the species on the territory of the Republic of Moldova.

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