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AETHIONEMA ARABICUMANDRZ. EX DC (CRUCIFERAE) IN BULGARIA - *INSITUAND EXSITU* CONSERVATION

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Abstract. *Aethionema arabicum* Andrz. ex DC is extremely rare species ofthe Bulgarian flora. It is included in the Biological Diversity Act ofBulgaria and in the Red Book ofBulgaria with category *Critically Endangered.* The species is distributed in Balkan Peninsula (Bulgaria and Turkey), Southwest Asia (Asia Minor, Syria, Northern Iran) and the Caucasus. The only population of*A. arabicum* known in Bulgaria is situated in Eastern Stara planina floristic region, near Sotirya village, Sliven district. The population consists of only a few dozen individuals. This study provides information on the biology and ecology of the species, its population structure and the measures taken to increase the population. Protected area was designated to preserve this species.

Key words: endangered plant species, monitoring, long-term conservation, restoration

INTRODUCTION

The genus *Aethionema* R. Br. belongs to *Brassicaceae* family and includes 56 species with Old World distribution (http://www.theplantlist.0rg/l.l/br0wse/A/Brassicaceae/Aethionema/). Tlie territory ofTurkey is considered to be the center ofspeciation ofthe genus. There are over 40 *Aethionema* species, twenty ofwhich

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are endemics (Pinar et al. 2007). In the flora ofEurope the genus is represented by six species (Chater 1964). Two ofthem are distributed in Bulgaria - *Aethionema arabicum Andrz.* ex DC and A *saxatile* (L.) R. Br. (Vulev 1970). Recently, anew species was described from the Bulgarian part ofthe Rodopi Mts - *A. rhodopaeum* D. K. Pavlova (Pavlova 2007). Thus, there are three species ofthis genus in our flora.

The subject of this study is *Aethionema arabicum* - an extremely rare and protected taxon in the Bulgarian flora included in Appendix 3, Art. 37 of the Bulgarian Biodiversity Act and in the Red Book of the Republic of Bulgaria as *CriticallyEndangered(*Stanev 2015). It belongsto the category ofspecies forwhich regeneration and maintenance activities are foreseen in point V.7. ofthe National Biodiversity Conservation Plan 2005-2010 of the Ministry of Environment and Waters (MoEW) ofBulgaria. Because ofits high sensitivity and conservation value, it is subject to monitoring at national level included in the National Biodiversity Monitoring System ofMoEW.

The only population of*A. arabicum* known in Bulgaria is situated in Eastern Stara planina floristic sub-region, at *Laie bair* locality, near Sotirya village, Sliven district. Prior to this study, the species was not found for more than ten years, despite repeated attempts to do so. The taxon is generally distributed in part ofthe Balkan Peninsula (Bulgaria, Turkey) as well as Southwestern Asia (Asia Minor, Syria, Northern Iran) and in the Caucasus.

The aims ofthe present study are: 1) Exploration ofthe biology and ecology of this extremely rare species for Bulgaria; 2) Establishment of conditions for a long term monitoring; 3) Implementation of *in situ* and *ex situ* activities for long-term conservation and restoration ofthe species population.

MATERIALS AND METHODS

The survey was conducted between 2010 and 2014. The morphological description of the species is made on the basis of own observations, as well as analysis of the existing information from all the major literary sources (Hedge 1965; Takhtajan 1966; Valev 1970; Ketzhoveli 1979; Pinar et al. 2007) and the collections in the Bulgarian herbaria (SOM, SO, SOA).

To study the morphometric variability of the siliculae, eight plants derived from seeds collected from the natural locality ofthe species in 2011, were used. Three ofthe plants were grown under room conditions and five were grown in the Growing House ofthe Institute ofBiodiversity and Ecosystem Research (IBER). In 2012, 50 siliculae from indoor and outdoor individuals were collected to establish the variability of4 features ofthe generative sphere: Vari - length ofthe siliculae, Var2 - width ofthe siliculae, Var3 - concavity ofthe siliculae, Var4 - number ofthe seeds in the siliculae. For analysis ofthe results the statistical program Statistica 7

Figs. 1-5: 1- *Aethionema arabicum* Andrz. ex DC **-** habit; **2 -** Germination ofЛ. *arabicum* on dipped filter paper in Petri dish; **3** - Locality *Laie bair,* near to Sotirya village, Sliven district; **4** - Habitat ofЛ. *arabicum',* **5 -** The characteristic substrate ofthe population ofA *arabicum.*

was used (StatSoft, Inc. 2004).

For recording the germination, 75 seeds obtained from *ex situ* cultivated plants were placed on moistened filter paper in Petri dishes.

The monitoring of the population of the target species has been carried out for three consecutive years in accordance with the *Methodologyfor monitoring of vascularplants* (Gussev & Bancheva 2011), developed according to the instructions

of Ryttäri et al. (2003) and Harding & Williams (2010). A *Field form for monitoring ofvascularplants* ofthe Bulgarian Executive Environment Agency has been used, which has been supplemented and adapted for the particular species within a project LIFE08 NAT/BG/279 *A pilot network ofsmall protected sitesfor plant species in Bulgaria using the plant micro-reserve model,* funded by the EU s Program Life+ (LifeO8NAT/BG/279). All collected data are folly compatible with the National Monitoring System of Bulgaria ofthe Ministry of Environment and Waters. Two types ofindicators were collected: 1) Indicators for a single observation ofthe whole population, including: name ofthe target species, location, ecological and geographic characteristics, population boundaries, area, number and spatial structure ofthe population, land use, infrastructure elements close to the locality, *etc.;* 2) Indicators collected in different control plots: location of the plot using GPS coordinates, reporting unit, phenological phase, total projective cover ofthe vegetation, percentage cover ofthe control species, number ofthe individuals of the control species, phytocenological description, main threats fortarget species.

RESULTSAND DISCUSSION

Morphology andBiology

A. arabicum is an annual plant from *Brassicaceae* family, tertiary relict (Valev 1970). The stems are up to 10 cm, erect, and usually branched. The leaves are ovate, alternate, and naked. The flowers are small (sepals up to 4 mm long), white to pale violet. The fruits are siliculae, sub-spherical, bilocular with 1-4 seeds in each locus (Var4), opened by two valves; flattened and winged; densely crowded and imbricate; deeply concave to the top; the length ofthe siliculae (Vari) = 4,6-11 mm; the width ofthe siliculae (Var2) = 4-9 mm; the concavity ofthe siliculae (Var3) = 0,5-4 mm. The seeds are ovoid-elliptical, dark brown and very small (0.75x0.3 mm). The

average seed weight is 0.0001 g. The flowering period is from April to May and the fruit-bearing period lasts from late April to June **(Fig. 1).**

Seedproduction

In 2012 for the establishment of seed production, eight plants were grown in the Growing House of IBER, three of which were left to be grown under indoor conditions. Ofthe eight plants, a total of 718 seeds were obtained (about 90 seeds per individual). These results show that the potential seed production ofA *arabicum* is quite high.

Germination

The 75 seeds of*A. arabicum* placed on dipped filter paper in Petri dishes in February 2013 **(Fig. 2)** were harvested from *ex situ* plants grown in the Growing House ofIBER under natural conditions (40 individuals) and at room temperature (35 individuals). Within 3 months, 51 seeds germinated: 35 seeds obtained from

Figs. 6, 8-12: 6 - The monitoring of the population ofЛ. *arabicunr,* **8 -** Planted seeds of A *arabicum* at room temperature for *m-situ* actions; **9 -** *Ex situ* plants ofA. *arabicum* grown in the Growing House of IBER; **10 -** *Ex situ* collection ofA *arabicunr,* **11** - Marked plot for *m-situ* actions in the natural population ofA. *arabicunr,* **12 -** Germination of *ex-situ* produced seeds in the natural population ofA *arabicum.*

outdoorplants and 16 seedsfrom plants grown at roomtemperature. The germination rate was relatively high - 68%. These results provide additional information on the breeding abilities ofthe species and give reason to conclude that *A. arabicum* has the ability to self-pollination. The data from the Bulgarian literature, known before

the present study, suggest only pollination by insects (Stanev 2015). The self-fertilization is one of the most common evolutionary mechanisms in plants and has occurred in many taxonomic groups (Stebbins 1974; Barrett 2002). It is usually associated with peripheral and isolated populations (Stebbins 1957; Herlihy & Eckert 2005), such as the population of4. *arabicum* in Bulgaria.

Habitats, population structure and monitoring

The only population of*A. arabicum,* known in Bulgaria, is situated in Eastern Stara planina floristic sub-region, at *Laie bair* locality, near Sotirya village, Sliven district **(Fig. 3).** It belongs to the Protected site BG0000420 *Grebenets* of the NATURA 2000 ecological network. The species is generally distributed in part of the Balkan Peninsula (Bulgaria, Turkey) as well as Southwestern Asia (Asia Minor, Syria, Northern Iran) and the Caucasus.

A. arabicum is a pioneer and poorly competitive species. It grows on stony slopes

(36-40°) and screes, on limestone **(Fig. 4).** Soils are very poor, shallow, dry and eroded **(Fig. 5).** It participates in xerophilous herbaceous and open scrub communities together with *Achillea millefolium* L., *Ajuga chamaepytis* ssp. *chia* (Schreb.) Arcang., *Astragalus monspessulanus* L., *A. spruneri* Boiss., *Astracantha thracica* (Griseb.) Podl., *Berberis vulgaris* L., *Bromus mollis* L., *Carduus nutans* L., *Carpinus orientalis* Mill., *Centaurea diffusa* Lam., *Chrysopogon gryllus* (L.) Trin., *Colchicum bibersteinii* Rouy, *Convolvulus cantabrica* L., *Crocus flavus* West., *Crupina vulgaris* Cass., *Cynodon dactylon* (L.) Pers., *Dactylis glomerata* L., *Dasypyrum villosum* (L.) Cand., *Erodium cicutarium* (L.) L'Her., *Eryngium campestre* L., *Euphorbia amygdaloides* L., *Euphorbia myrsinites* L., *Euphorbia peplis* L., *Fraxinus ornus* L., *Helianthemum nummularium* (L.) Mill., *Hypericum cerastoides* (Spach.) N. Robson, *Jasminum fruticans* L., *Lamium amplexicaule* L., *Leontodon crispus* Vill., *Paliurus spina-christi* Mill., *Plantago lanceolata* L., *Poa bulbosa* L., *Rosa pimpinellifolia* L., *Sanguisorba minor* Scop., *Satureja montana* L., *Stachys recta* L., *Syringa vulgaris* L., *Teucrium chamaedrys* L., *Teucrium polium* L., *Thlaspi alliaceum L.* and *Viola kitaibeliana* Schult.

The population of*A. arabicum* occupies an area of 8 dka. The total projective coverage ofthe vegetation is 40% (35% grasses and 5% shrubs), while the coverage ofthe examined species is below 1%. The spatial distribution ofindividuals in the population is in groups. The monitoring **(Fig. 6)** includes all individuals of the population within a period of 4 years (2010-2013). In 2010, 37 individuals were counted, in 2011 - 55 individuals, in 2012 - 18 individuals, and in 2013 - 233 individuals. All plants are generative, fonn a large number offruits and are in good health. In 2011, we found that the average number of siliculae that an individual produced was 34. The average number ofseeds formed in one siliculae is two **(Fig. 7).** These results show that the potential seed reproductive capacity ofthe species is quite high. For example, with 55 individuals established in 2011, potential seed production would be 3740 seeds. Indeed, despite the high potential seed

reproductive capacity, *in situ* germinate and survive an extremely low percentage ofseedlings, due to a number ofreasons: small seeds that are difficult to retain on the eroded substrate, strong winds, *etc.*

Fig. 7. Metric data ofthe siliculae *ofAethionema arabicum;* Legend: Vari -siliculae height; V2 - siliculae width; V3 - concavity ofthe siliculae; V4 - number ofseeds ofthe siliculae.

Threats

The population of *A. arabicum* is subject to active direct and indirect anthropogenic activities, which, combined with the weak resemblance of the species, poses a real threat that can lead to its disappearance from the flora of Bulgaria.

Ex situ and in situ actions

In order to support the recreation capacities of the only population of *A. arabicum, in situ* and *ex situ* activities were carried out as follows: At the beginning of 2012, 70 seeds ofA. *arabicum,* collected in May 2011 from its natural population, were planted in soil **(Fig. 8). Of**these, only nine individuals sprouted and grew up **(Fig. 9).** Thousand and two hundred seeds were harvested from these plants. On 19 February 2013, one hundred ofthese seeds were planted in well-drained soil (Deno 1993) forthe creation ofan *ex situ* collection ofthe species at IBER. The first four plants sprouted after ten days. Within a period of 10 to 80

days, a total of 19 seeds germinated, 13 of them (13%) developing successfully **(Fig. 10).**

On 12 March 2013, another 200 seeds of the *ex-situ* produced plants were planted

in ten specially marked plots in the natural locality ofthe species **(Figs. 11, 12).** On 13 April 2013, 60 seedlings (30%) were found. During the monitoring in 2013, the highest number of individuals was recorded, 233 compared to all other years since the establishment ofthe species for the Bulgarian flora to the present day. We believe that these results are mainly due to our targeted *in situ* and *ex situ* activities. The number ofindividuals remains constant over the next years (2015 2016).

In order to ensure the success ofthe activities to protect, support and restore the population ofA *arabicum* in Bulgaria, at the initiative of our team in 2012, a Protected Site *Laie Bair* was designated (Order No. RD-937 of 20.12.2011 ofthe Minister ofthe Ministry of Environment and Waters), and in December 2014 an Action Plan forthe Conservation ofthe species was established (approved by Order No. RD-990 / 23.12.2014 ofthe Minister ofEnvironment and Waters).

CONFLICT OF INTERESTS

The authors declare thatthere is no conflict ofinterestsregarding the publication ofthis article.

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