Scientific Papers. Series B, Horticulture. Vol. LX, 2016
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

OPPORTUNITIES FOR *EX*-SITU CONSERVATION OF *CYCLAMEN COUM* MILL. IN SINITE KAMANI NATURAL PARK, EASTERN BALKAN RANGE, BULGARIA

Neli GROZEVA[1], Mariya GERDZHIKOVA[2], Galia PANAYOTOVA[2], Mima TODOROVA[2]

[1]Trakia University, Faculty of Agriculture, Department of Biology and aquaculture, Stara Zagora, Bulgaria
[2]Trakia University, Faculty of Agriculture, Department of Plant growing, Stara Zagora, Bulgaria

Corresponding author e mail: grozeva@uni-sz.bg

Abstract

The protected specie Cyclamen coum Mill. is found on the territory of Sinite kamani Natural Park in Ablanovo, Chukata and before Enyova bulka areas. Main threats for its populations in the Park are the anthropogenic impact and the difficult seedling regeneration. For their conservation and for stabilizing their state, according to the assessments made, it is necessary to include both in-situ and ex-situ conservation measures. The objective of the present study is to develop a technology for growing Cyclamen coum from ripe seeds under laboratory conditions. To implement the objective numerous literary sources have been studied and biannual terrain studies of the population in Sinite kamani Natural Park have been conducted. The developed technology is successful and totally compliant with the ecological conditions of the natural habitats of the species in the Park. By applying it the species has been successfully reproduced in the scientific laboratories of the Faculty of Agriculture at Trakia University from ripe seeds to replenish the populations. The technology can be used for replenishing other natural populations of Cyclamen coum in Bulgaria as well. To establish the effectiveness of the implementation of this measure for ex-situ conservation, we need to continue observations after introducing the plants grown in laboratory conditions in order to trace their adaptation and further development and, if necessary, to take care of their conservation and stabilization of their state.

Key words: *Cyclamen coum, ex*-situ preservation, Sinite kamani Natural Park.

INTRODUCTION

Sinite kamani Natural Park is situated on the southern slopes of Eastern Balkan range, Bulgaria. Regardless of its relatively small territory – 11,308.8 hectares, over 25% of the vascular plants in Bulgarian flora have found shelter in it. This study is part of a project, one of the aims of which is *ex*-situ conservation of protected and endemic species inhabiting the territory of Sinite kamani Natural Park. According to preliminary biannual investigations conducted, one of the species whose preservation in the park requires the application of *ex*-situ conservation measures as well, is *Cyclamen coum* Mill. Main threats to the populations are anthropogenic impact (trampling, unregulated picking of its beautiful flowers for bouquets, eradication of tubers for ornamental and medicinal purposes) and difficult seedling regeneration. The species is protected by the Biological Diversity Act in Bulgaria (2002) included in Appendix I of the Convention on the Conservation of European Wildlife and Natural Habitats - Bern Convention (1979) and Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora CITES (1973). *Cyclamen coum* was reported for the first for the territory currently comprising Sinite kamani Natural Park by Urumov (1906) in Chukata area. The field was confirmed by Andreev (1981), in relation to developing the forest management plan of the park. Grozeva et al., (2014) found in 2013 a total of 3 populations of the species in the natural park in Ablanovo, Cukata and before Enyova bulka areas and the best, according to the assessment of their state, is the one in Ablanovo area.

The objective of the present study is to develop a technology for growing *Cyclamen coum* from ripe seeds under laboratory conditions in order to replenish and stabilize the species populations in Sinite kamani Natural Park.

MATERIALS AND METHODS

The study was conducted in 2014-2015. To develop the technology for growing *Cyclamen coum* various literary sources have been used (Hayek, 1929; Yankulov, 1964, 2000; Medicinal plants, 2001; Grozeva et al., 2004) and the data from conducted observations and analyses related to the implementation of project "Restoration of habitats and preservation of biodiversity conservation in Sinite kamani Natire park, Sliven, Bulgaria". The morphological characteristics of the species conforms to the one given in Flora of the People's Republic of Bulgaria (Peev, 1982) and the data from the terrain studies conducted during the vegetation periods of 2014-2015.

Laboratory studies have been conducted in the scientific research laboratories of the Faculty of Agriculture at Trakia University – Stara Zagora.

Ripe seeds were taken from the species population in Ablanovo area after obtaining a permit from the Minister of environment and waters.

When preparing the soil for sowing the seeds the data from the conducted soil analyses for each population were used (Grozeva et al., 2015).

All activities in collecting ripe seeds, their growing in laboratory conditions and taking the plants grown back into the natural populations of the species in Sinite kamani Natural Park conform to the Protected Areas Act (1998) and the Biological Diversity Act (2002).

RESULTS AND DISCUSSIONS

Morphology and biology of the species

Perennial herb with tuberous rootstocks and fibrous roots (Figure 1). Tuber is round, smooth, fleshy, usually 3-5 cm in diameter. Leaves rounded to kidney-shaped, a wavy folded edge and unclearly serrated with 9-16 cm long, bare stems coming directly from the tuber. They develop before or during flowering. Leaf lamina 2-4.4 cm long and 3.7-6 cm wide, bare on the top, green, often with white concentric contour or stain, and brown grainy underneath. The flowers grow in spring. Pedicel long, usually coiling spirally in fruit. Calyx 5 lobed, brown grainy, 5-8 mm long and 1.5-2 mm wide. Corolla back ovate, bare, rolled back, pointed at the top and without ears at the base, red-purple, 6-23 mm long, 5-11 mm wide. Stamens 5, inserted at base of corolla. Staminal handles extended at the base, glandular fibrous; anthers rounded at the base. The fruit slightly elongated, bare, 7 to 12 mm long and 7 to 10 mm wide, opening with 5-8 short triangular teeth. Seeds light brown with a reddish tinge, ± extended; 1.4-2 mm long, 1.2-1.8 mm wide, slightly verrucose surface. The number of seeds in a box is between 14 and 26.

Phenology

Flowering from late January to March. Fruiting from March to April. Propagated by seeds and vegetatively - by daughter tubers.

Habitat

The species forms populations in the bushes and oak forests in North-Eastern Bulgaria, The Black Sea coast, The Balkan Range (Central and Eastern), Thracian Plane, The Tundja Hilly Plane, The Strandja (Peev, 1982; Delipavlov and Cheshmedzhiev, 2003; Asyov and Petrova, 2012).

Figure 1. *Cyclamen coum* Mill. – general view.

Requirements to environmental factors

Cyclamen coum according to data by Peev (1982), Asyov and Petrova (2012) prefers light deciduous forests at an altitude up to 500 m. On the territory of Sinite kamani Natural Park in Chukata area population of the species was registered at an altitude of 729 m. Frost-hardy plant. With stands low temperatures during the winter-spring period. The species is demanding

to soil moisture during flowering. In summer withstand drought. No special requirements to soil fertility as long as they have light mechanical composition with good permeability. Our studies (Grozeva et al. 2014, 2015) show that in Sinite kamani Natural Park *Cyclamen coum* develops on cinnamon forest and leached cinnamon forest soils with acidic to neutral reaction of the soil solution.

Activities in growing *Cyclamen coum* from seeds under laboratory conditions

To reproduce the species from seeds under laboratory conditions, at the end of flowering phenophase and early fruiting phenophase isolation bags were placed on well-developed, healthy and abundantly flowering plants from the population in Ablanovo area in order to collect ripe seeds (Figure 2A).

Due to the fragility of the flower handles the bags were tailored on site and were not tied but wrapped around the handles (Figure 2B).

Seeds were collected in full maturity, which in the conditions of Eastern Balkan Mountain, Ablanovo area at altitude of 537 m, they reached in the first half of April. The bags with ripe seeds were taken and transferred to the research laboratories of the Faculty of Agriculture, where the seeds are separated from the fruit, cleaned from mechanical impurities and stored in paper bags (Figure 2C,D).

To accelerate their period of rest, the seeds were placed in a refrigerator at temperature 2-5 °C for 28 days. At 5-day periods the packages were taken out and inspected. Seeds were reviewed in appearance: color, smell, glitter, etc. Their health status in relation to pest and disease attack was determined by using microscope equipment (Figure 2E).

During the rest of the seeds the soil for their sowing was prepared in the scientific research laboratories at the Faculty of Agriculture. Previously soil samples were taken from the three populations of the species. Two soil varieties were registered: Cinnamon forest soil in Chukata area and near Enyova bulka area and Leached cinnamon forest soil in Ablanovo area. According to data about chemical composition and reaction of the studied soils is was found out that *Cyclamen coum* develops in soil with reaction of the soil solution from acidic to neutral, with low to medium humus content, poorly stocked with absorbable forms of nitrogen, medium to lower stock of absorbable phosphorus and well stocked with potassium compounds.

Favorable environment for the development of the species are soils with light mechanical composition with good permeability and the presence of powdery structure or skeletal elements has no significant influence. Soil mixture of 2 parts beech leaves, 1 part peat and 1 part sand was prepared with a similar mechanical composition to the soil in the natural habitats. Although the species is not demanding in terms of soil fertility, putrid manure was also added to the prepared soil in soil fertilizer ratio 2:1. The so prepared manure soil mixture was sifted to remove mechanical impurities and larger lumps and poured into terrines (Figure 2F).

Seeds were sown on 20 May one by one into the prepared moist soil and covered with about 2-3 mm of the same soil, moistened and placed in a dark place at a temperature of 15-18 °C (Figure 3A). They were regularly watered with distilled water with temperature 2-3 °C higher than that of air. On the 25th day after sowing the first seeds germinated, on the 30th day - the last ones. After germination of all seeds, containers were placed in light (Figure 3B).

Care was taken at the beginning of their development the small tubers formed to be fully in the soil. In order to obtain healthy and vigorous plants, average temperature of 16-18 °C and air humidity around 50 % were maintained and the difference between the day and night temperature did not exceed 5 °C. Care for plants comprised regular watering to ensure moderate humidity, weeding, airing during the day in order to harden the plants, inspection for attack by diseases and pests.

Figure 2. *Cyclamen coum* Mill.: A,B-placing the isolation bags; C-separating the seeds from fruit; D-cleaning the seeds from mechanical impurities; E-checking the health condition of the seeds; F-preparing the soil for sowing

To supply the needs of plants for sufficient nutrients single foliar supplementation with combined NPK fertilizer was applied when first leaf was formed (Figure 3C,D). Economically significant diseases and pests were not found, but if necessary, appropriate insecticides and fungicides could be used. From late August until September 12, gradually all plants grown from seeds went into a period of rest. The soil was slightly moistened periodically, air temperature 20-23 °C was maintained.

The return of the plants grown into the territory of Sinite kamani Natural Park was done during the period 10-16 October. They were planted in selected appropriate sections of the populations in Ablanovo, Chukata and after Enyova bulka areas, the soil being very well treated and cleaned beforehand (Figure 3E). After planting the plants were watered and covered with mulch (Figure 3F). During the next vegetation period from the second half of January to 18 February development of 100 % of the plants imported in populations of Ablanovo area, 95 % of those imported in the population after Enyova bulka area and 92 % of those in Chukata area was recorded. These data give reason to believe that the used *ex*-situ measure for conservation of *Cyclamen coum* is successful. For complete stabilization of all three populations of the species it is imperative to continue the process of filling them with plants grown from seeds. The construction of a

scientific research laboratory on the territory of Sinite kamani Natural Park would help to promote the conservation of this and other protected rare and endemic plant species on the territory of the park.

Figure 3. *Cyclamen coum* Mill.: A-sowing the seeds; B-seed germination; C,D-forming a first leaf; E-soil preparation for the return of plants; F-planting the plants.

CONCLUSIONS

The developed technology for growing *Cyclamen coum* from ripe seeds until formation of young tubers in laboratory conditions is successful and can be used to replenish the natural populations of the species on the territory of Sinite kamani Natural Park, as well as other populations of the same species in Bulgaria. To establish the full effectiveness of the application of this measure for *ex*-situ conservation, at least 3-year observations of the plants imported in the populations should be made, trace their adaptation and further development and, if necessary, take care of their conservation and stabilization.

ACKNOWLEDGEMENTS

This work was financially supported by contract № OPOS2-36 / 24.07.2013 on project № 5103020-15-658 of Sinite kamani Natural park funded by contract № 5103020-C-002 OPERATIONAL PROGRAMME „ENVIRONMENT 2007-2013“.

REFERENCES

Andreev N., 1981. Development Plan of Sinite Kamani National Park. Botanical Characterization, *Agrolesproekt*, Sofia.

Assyov B., Petrova, A. (eds), 2012. Conspectus of the Bulgarian vascular flora. Distribution maps and floristic elements, 4 (In Bulgarian). BBF, Sofia.

Biological Diversity Act., 2002. Darzhaven Vestnik no. 77 from 9 August 2002, pp. 9-42. Amended in Darzhaven Vestnik no. 94 from 16 November 2007 (Bg).

Convention on International Trade in Endangered Species of Wild Fauna and Flora(CITES) http://www.cites.org/eng/disc/text.php 1973-03-03

Convention on the Conservation of European Wildlife and Natural Habitats - Bern Convention,http://iea.uoregon.edu/pages/view_treaty.php?t=1996-AmendmentsB-1979-EuropeanWildlifeNaturalHabitats.

Delipavlov D., Cheshmedzhiev I. (eds)., 2003. Key to the Plants of Bulgaria. *Acad. Press Agrarian University*, Plovdiv, 2003 (Bg).

Grozeva N., Todorova M., Gerdzhikova M., Panayotova G., Dohchev D, Tsutsov K., 2015. Studies On *Cyclamen Coum* In Sinite Kamani Natural Park, Bulgaria. 2nd International Symposium for Agriculture and Food, 7 - 9 October 2015, Ohrid, Macedonia – in press.

Grozeva N., Dohchev D., Gerdzhikova M., Tsutsov K., Todorova M., Panayotova G., Getova, N., 2014. New data for protected plants of Sinite kamani Natural park Sliven. Trakia Journal of Sciences, 12, 1, 13 - 20.

Grozeva N., Georgieva M., Vulkova M., 2004. Flowering plants and ferns. – In: Stoeva M (ed.), Biological diversity of Sinite Kamani Nature Park. – Kontrast, Bogomilovo, 9-112 (Bg).

Hayek A., 1929. Prodromus Fl. Penins. Balc. II. 281.

Medicinal plants. 2001. Fondation Bioselena, 2-20.

Peev D., Genus *Cyclamen* – In: Jordanov, D. (ed.), Flora Reipublicae Bulgaricae, vol 9, 316-317, Editio Acad. „Prof. Marin Drinov", Serdicae, (1982) (Bg).

Protected Areas Act., 1998. Darzhaven Vestnik no.133/1998. http://lex.bg/laws/ldoc/ 2134445060.

Urumov I., 1906. Nova adimenta ad floram Bulgaricae. Journal of BAS, 5:32.

Yankulov, 1964. Cultivation of essential oil and medical plants. CKS "Bilkoop"

Yankulov, 2000. Main medical plants. 19 modern technologies. Sofia. 225.