საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 11, №3, 2017
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 11, no. 3, 2017

Botany

Study of Reproduction Capacity and Ex situ Conservation of *Campanula armazica* Charadze (Campanulaceae)

Lia Kobakhidze* and Tinatin Barblishvili*

**National Botanical Garden of Georgia, Tbilisi, Georgia*

(Presented by Academy Member Giorgi Nakhutsrishvili)

ABSTRACT. Reproduction biology of species of primary conservation concern – *Campanula armazica* Charadze, included in the Red Data List of the Caucasus Plants, was studied in order to establish the reasons of the decline of wild population and conservation strategy development. Current state of its wild population was evaluated and issues, such as: phenological phases of development (vegetation, flowering, fruit seed and maturation), processes of development of female and male generative spheres, peculiarities of pollination, fertilization, potential for seed formation and germination connected with elaboration of the proper conservation strategy were researched. The results of our studies proved normal course of seed formation processes. The species is well adapted to the conditions of its natural habitat. Thus, disappearance of the species from the previous distribution areas is not due to biotic factors.

Conservation ex situ, in particular, preservation of seed stock in the Caucasus Regional Seed Bank of the National Botanical Garden of Georgia and duplication of seed collections in the Millennium Seed Bank of the Royal Botanical Garden, Kew, and in situ artificial sowing of seeds in places of the former distribution of *C. armazica* and long-term monitoring of the site are offered as the means for the protection of the studied species. *© 2017 Bull. Georg. Natl. Acad. Sci.*

Key words: *Campanula armazica*, seed, reproduction, pollination, male and female generative spheres.

Recently, in conditions of increased influence of natural factors and human impact on the environment, threat of decrease of plant diversity and complete disappearance of particular species became especially urgent. This requires elaboration of complex protection measures to prevent species extinction.

Necessary conditions for implementation of the above measures is the availability of the distribution data of natural populations of particular plant species in the wild, their occurrence, viability of populations, range of accompanying species in the habitats, biological peculiarities of the species and processes of seed formation.

Georgia is an important centre of formation and occurrence of a number of species, belonging to the family Campanulaceae. 66 endemics of the Caucasus and Georgia can be found here [1].

Fig. 1. Habitat of *Campanula armazica*.

Fig. 2. Cusion like group of individuals of *Campanula armazica*.

Materials and Methods

Object of our research was a relict species, endemic to Georgia – *Campanula armazica* Charadze, which is included in Red Data List of Caucasus [2]. Current status of the species and its reproduction biology have not been researched so far.

Necessary data were obtained by means of field expeditions and laboratory trials. Field studies embraced the following activities: Current state of populations in places of occurrence was evaluated.

Materials were obtained for the study of consecutive phenological phases of development (vegetation, flowering, fruit seed and maturation) and the calendar dates and peculiarities of their course were established; Observations on pollination process were carried out in order to establish the means of pollination and the type of the pollination process.

The following issues were studied under laboratory conditions using the methods, accepted in structural and experimental embryology [3]: development of female and male generative spheres; fertility, productivity of seed formation, germination capacity and terms of germination according to Rabotnov [4]. Share of sexual and vegetative propagation in the maintenance of the studied plant population has been established.

Places of occurrence of *Campanula armazica* indicated in literary sources and herbarium materials [1, 5] were visited (environs of Mtskheta, Monastery of St. Nino, basin of river Algeti, mount Birtvisi; Armazi gorge). Population was registered only in Kvemo Kartli region, on the territory of mount Kldekari.

Results and Discussion

Mount Kldekari is situated on Trialeti range, in 12 km north-west of Manglisi, GPS coordinates: N41.74183; EO 044.20551; altitude above sea level - 1933 m. Accompanying species: *Thymus rariflorus, Astragalus incertus, Astragalus caucasicus, Poa caucasica, Coeleria caucasica, Colpodium variegatum* and others.

Habitat is rocky declivity, sloping, poor in soil (Fig.1). Radiation level is high, amplitude of difference between daily and night temperatures is quite big. Air is dry and winds are frequent in this area. In the given habitat the species exists in the form of cushions (Fig.2). Caudex of *C. armazica* spreading deeply in rock crevices and low height of the plant ensure tight attachment of the plant to the substrate, preventing it from the wind. Densely situated stems in patches create a special microclimate, due to which moisture is preserved in conditions of high summer temperature and dryness. During negative winter temperatures the same arrangement ensures preservation of warmth. Cushion life form is a clear example of species adaptation to the environment. Cushions are unevenly distributed on the studied territory. For example, on $50m^2$ 4 cushions were registered, on $12m^2$ – 24; on $30m^2$ area – 30, on $3m^2$ – 7. On isolated/remote

Fig. 3. Flowering *Campanula armazica.*

Fig. 4. Mass flowering of *Campanula armazica.*

rocks of 45m^2 area 35 cushions of the target species were found. Size of cushions is also variable from 15x17cm to 4x5 cm. Almost all populations are viable, blooming. Senile specimens were not registered.

C. armazica starts vegetation at the end of February. In the central part of stems, developed during the previous year, leaves start to develop, though remains of the sprouts of the previous years are preserved as scales. Their number gives an approximate representation on the age of plant.

In the beginning of May the species enters the phase of budding and in May-June intensive flowering takes place (Fig. 3). Up to 25 flowers develop on grown plants. Flowers are bell-shaped, 3.1-3.3 cm in size, developed on the quite long and coarse flower stems above the population level and situated on its peripheral parts.

Life-span of a single flower is 5-7 days. Thus flowering period is quite prolonged and lasts nearly for a month.

In the period of mass flowering *C. armazica* flowers are often visited by Hymenoptera insects, which transfer the pollen from one flower to another (Fig.4). Transfer of pollen by air-flow is also not excluded. Flower of *Campanula armazica* is also used by insects for rearing the generations.

Male and female spheres of *C. armazisa* developed normally, but their maturation is segregated in time.

Male generative sphere of *C.armazica* is represented by stamens of equal size. Pollen grains are 5-porous (Fig. 5), bicellular, in contrast to several species of family Campanulaceae [6, 7].

Pistil contains numerous ovules, intensely pubescent ovary (Fig. 6), short style, and long stigma, covered by bristles as described in other representatives of genus Campanula [6, 8].

Stamens open already in the bud and the pollen diffuses on the bristles of the style and is used by insects to pollinate other flowers. Empty stamens are

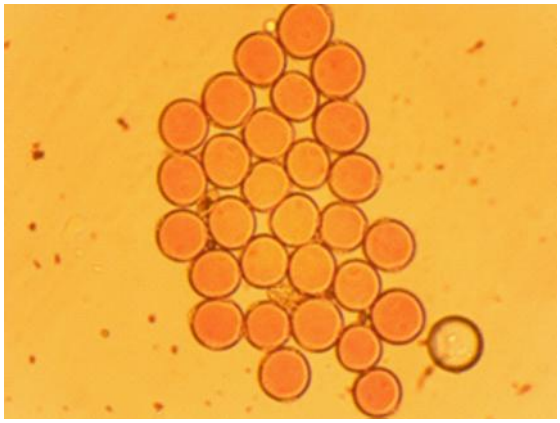

Fig. 5. Bicellular pollen grains of *Campanula armazica.*

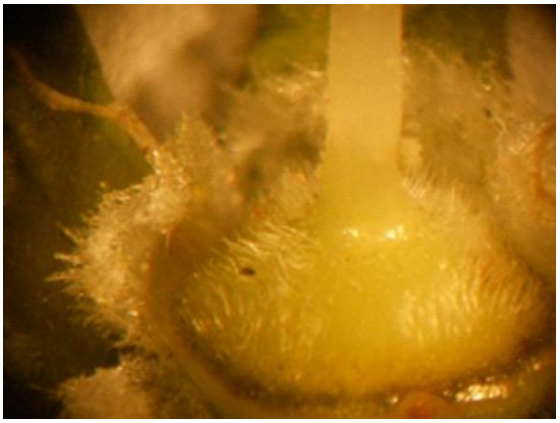

Fig. 6. Pubescent ovary *Campanula armazica*.

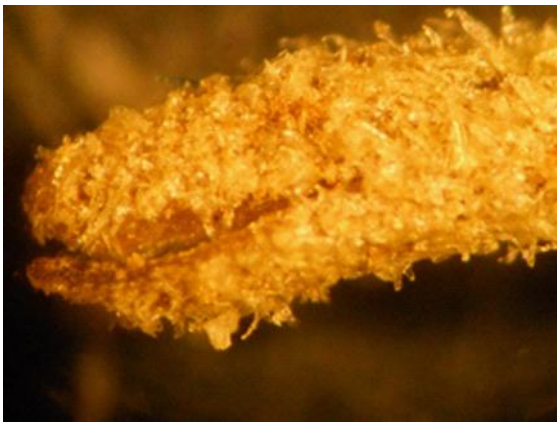

Fig. 7. Stigma of *Campanula armazica* with divided lobes.

Fig. 8. Mature seeds of *Campanula armazica*.

deposited on the surface of stigma. This is the end of the male phase and after this the development of the female phase starts.

During the above described period the lobes of the stigma are tightly attached to each other. With the beginning of female phase the lobes gradually separate from each other (Fig. 7). Simultaneously the style starts longitudinal growth and the open flower is ready for pollination. Thus the species under study is characterized by the clearly pronounced protandry. As a result, cross-pollination seems to be a priority way of pollination for *C. armazica*. Though, by the end of flowering, when the lobes of the stigma become maximally bent apart and downwards, they occasionally touch the pollen, deposited on the bristles of pistil thus making precondition for the autogamy. The fact is confirmed by several researchers [9-10]. Such episodic cases, in our opinion should be regarded as the supplementary way of pollination, which substitutes allogamy, in case when it is dropped-out.

Seed maturation takes place during July. Maximum number of seeds in fruits is 117.

Generative phase of the target species takes 3 months, and the whole life cycle lasts 8 months.

As a result of shaking of tall, lignified flower stalks, the seeds are the mature capsules. Dispersed form of seeds are transparent, light (Fig. 8). Weight of 1000 seeds makes 0.005 g. Seed is encircled with a fringe, which makes it winged. Light-weight seed is easily dispersed by airflow in different directions and thus distribution of the species is ensured. Part of seeds, remaining within the range of mother plants, contribute to the sustainability of the populations.

In order to establish seed germination capacity

and best terms for sowing, seeds were sown at different times on artificial media and in soil (Fig. 9). The best result was obtained when sowing was performed in autumn. Seeds are characterized by non-simultaneous germination and long dormancy period, due to which they create the so-called "soil seed-bank", which provides for gradual use of seeds and thus favors the steadiness of the species.

Cushions of the target species are vegetatively static. Because of this propagation by seed is leading for the species renewal.

In conclusion it can be said that *C. armazica* is well adapted to environmental conditions of its natural habitat.With this aim it developed cushion lifeform.

Normal development and functioning of generative organs is a precondition for the development of high-quality seed. Considering this, we think that disappearance of the species from the former places of distribution should be conditioned by factors of abiotic nature.

The following can be recommended on the basis of carried out research:

At the current stage *Campanula armazica* can be secured via ex situ conservation, by means of long-term storage of seeds in the Caucasus Regional Seed Bank of the National Botanical Garden of Georgia and duplication of seed collections to the Millennium Seed Bank of RBG, Kew (UK).

Fig. 9. Seedsof *Campanula armazica* germinated on artificial medium.

As follows from our experience, individuals of *C. armazica* do not adapt well to conditions of cultivation. Restoration of species in places of its former occurrence should be done considering best terms of sowing, by adding seeds to the natural sites and the long-term monitoring of the restoration site.

ბოტანიკა

არმაზის მაჩიტას (*Campanula armazica* Charadze (Campanulaceae)) რეპროდუქციული წარმადობა და ex situ კონსერვაცია

ლ. კობახიძე* და თ. ბარბლიშვილი*

**საქართველოს ეროვნული ბოტანიკური ბაღი, თბილისი, საქართველო*

(წარმოდგენილია აკადემიის წევრის გ. ნახუცრიშვილის მიერ)

პირველად არის შესწავლილი კავკასიის წითელ ნუსხაში შეტანილი პრიორიტეტული საკონსერვაციო სტატუსის მქონე სახეობის *Campanula armazica* Charadze (Campanulaceae) რეპროდუქციული ბიოლოგია მისი პოპულაციის შემცირების მიზეზების გარკვევის და კონსერვაციის სტრატეგიის შემუშავების მიზნით. შეფასდა ბუნებრივი პოპულაციის მდგომარეობა, შესწავლილია ის პროცესები, რომლებიც საფუძვლად დაედება კონსერვაციისათვის საჭირო სტრატეგიის შემუშავებას (განვითარების ფენოლოგიური ფაზები, მდედრობითი და მამრობითი გენერაციული სფეროს განვითარების პროცესები, დამტვერვის თავისებურებანი, თესლის წარმოქმნისა და გაღივების პოტენციალი). აღმოჩნდა, რომ თესლწარმოქმნის პროცესები დარღვევების გარეშე მიმდინარეობს. სახეობა კარგად არის შეგუებული საარსებო გარემოს პირობებთან. ამდენად, პირვანდელი გავრცელების ადგილებში *C. armazica*-ს გაქრობა ძირითადად ანთროპოგენული ზემოქმედებით უნდა იყოს განპირობებული.

სახეობის გადარჩენის გზად უპირველესად მისი ex situ კონსერვაცია (თესლის გრძელვადიანი შენახვა კავკასიის რეგიონულ თესლის ბანკში და მისი დუპლიკაცია ათასწლეულის თესლის ბანკში) მიგვაჩნია, ხოლო in situ, ბუნებრივ პირობებში თესლის ხელოვნური შეთესვა და ხანგრძლივი მონიტორინგის განხორციელება.

REFERENCES

1. Charadze A., Serdyukova L. (2001) Flora of Georgia, XIII. Tbilisi, 143-208 (in Georgian).
2. Schatz G. E., Shulkina T., Solomon J. (Eds.) (2013) Red List of the Endemic Plants of the Caucasus: Armenia, Azerbaijan, Georgia, Iran, Russia and Turkey St. Louis, US: Missouri Botanical Garden Press, 109-47.
3. Pausheva Z.P. (1988) Praktikum no tsitologii rasteniM.(in Russian).
4. Rabotnov T.A. (1960) Polevaia Geobotanika, M., - L. 2: 20-40 (in Russian).
5. Makashvili A. (1953) Flora of Tbilisi environs. II. Tbilisi, 140 (in Georgian).
6. Miroshnichenko N.N., Shevchenko S.V. (2014) Nekotorye osobennosti reproduktivnoi biologii *Campanula sibirica*. Zhivye I biokosnye sistemy #7 (in Russian).
7. Gabedava L. (2015) Study of structural peculiarities of generative sphere and ex situ conservation of Georgian endemic plant *Campanula kemulariae* Fomin included in the Red List of the Caucasus. *Bull. Georg. Natl. Acad. Sci.* **9**, 2:98-104.
8. Melia N., Barblishvili T., Jgenti L. (2014) Study of self-reproduction capacity and ex-situ conservation of endemic species *Campanula kachetica* Kantsch. included in the Red List of Endemic Plants of Caucasus Region. *Proceedings of the Third International Symposium on the Biology of Rare and Endemic Plant Species* (BIORARE-2014 April 19-23, 2014, Antalya, Turkey, Abstract Book, p 12.
9. Vogel S. (1975)*Campanula rotundifolia* (Campanulaceae). Pollination durch Apismellifica (Hymenoptera), Melittophilie - In: Encyclopaedia cinematographica E. 7 p.
10. Fegri K., Van der Peil L. (1982) Osnovy ekologii opyleniia. M. 275 s. (in Russian).

Received March, 2017