საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 12, №2, 2018
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 12, no.2, 2018

Botany

Reproductive Strategy and ex situ Conservation of Locally Distributed Endangered Georgian Endemic Species *Campanula kachetica* Kantsch.

Nino Melia* **and Tinatin Barblishvili***

National Botanical Garden of Georgia, Tbilisi, Georgia

(Presented by Academy Member Giorgi Nakhutsrishvili)

ABSTRACT. As is known, reproductive strategy of the species greatly determines its survival in the wild. On the other hand, understanding of reproductive biology of endangered wild plant species is important for elaboration of protection measures. With this aim the following issues were studied in critically endangered species *Campanula kachetica* Kantsch.: flower morphology, peculiarities of flowering and pollination; the processes of development of the generative sphere; productivity of seed formation; capacities and terms of seed germination, seed storage behaviour via testing quality and germination capacity of seed, stored for four years in the seed bank, in conditions of long-term storage. In extremal environmental conditions an own survival mechanism developed in *Campanula kachetica*. As revealed by our research, capacity for regeneration by means of sexual propagation is quite high in *Campanula kachetica*. The species is characterized by biological plasticity, labile character of pollination - in particular the presence of facultative autogamy along with allogamy. This in our opinion points to the strong survival strategy of the species. Ex situ conservation of the species was carried out. Tested high quality seed of *Campanula kachetica* is deposited for long-term storage in the National Seed Bank in the National Botanical Garden of Georgia and duplicated in the Millennium Seed Bank of the Royal Botanic Gardens, Kew, UK. *© 2018 Bull. Georg. Natl. Acad. Sci.*

Key words: *Campanula kachetica*, pollination, seed, germination, propagation, conservation

Campanula kachetica Kantsch. (Family Campanulaceae, genus Campanula, sect. Saxicolae (Boiss) Charadze – kakhetian bellflower – is a narrow local endemic of Georgia [1, 2]. Its distribution range in Georgia is very limited. The species occurs in East Georgia, Kakheti, the environs of Dedoplistskaro (Fig.1), on limestone massifs of the Jurassic period dated back by160-170 thousand years (Fig.2a). Habitat disturbance and degradation that took place in recent years caused significant decline of the population of this unique species. Currently the species is listed as CR, among Top 50 National Conservation Priorities for Georgia [3] and there exists an urgent need of rescue and preservation of this local endemic.

To develop methods for species protection it is necessary to have understanding of its biological peculiarities and study its reproduction biology both in native distribution range and experimentally

Fig. 1. Location of a single population of *Campanula kachetica* in Dedoplistskaro, near Vashlovani Protected Areas.

– under laboratory conditions. According to the literary data biological aspects of species united in the genus Campanula are studied to different extent. Among these of great significance are issues of flowering, pollination and general reproduction of the genus. As the process of pollination is a very important chain in the reproduction cycle of a species, a very peculiar mechanism of pollination, existing within the genus, referred to as the secondary pollen presentation, attracts a special attention. In particular, this means that the pollen is not presented to pollinators on the anthers. The anthers shed their pollen onto the pollen-collecting hairs of the style and stigma just before the opening of the bud. After having deposited their pollen, the stamens wilt. Visiting pollinators come into contact with the pollen on the style. Numerous data on the function and role of pollen-collecting hairs are reviewed and analyzed by Shetler [4]. In addition, the hairs serve not only as pollen-collectors, but also as an important and efficient mechanism, controlling spreading of stigma and promoting cross-pollination [5, 6]. Species of the genus Campanula mainly are characterized by allogamy, as maturation of pollen precedes the maturation of ovules. There are data on the possible autogamy in several species of Campanula, when in one case the lobes of stigma disclose backwards and the own pollen germinates through the papillae of dorsal surface of the stigmatic lobes; or in other case if germinable pollen is preserved in heavily bent twisted flowers, self-pollination may take place [7]. According to the existing data the secondary pollen-presentation and partial protandry are differently manifested in different species of the genus Campanula and are dependent on a variety of factors. In the context of self-incompatibility, phenotypic variation in the ability to self-fertilize is thought to be advantageous in small populations and in environments with variable pollinator or resource conditions [8, 9]. In case of isolation 100% set of fruits was registered in *C. trachelium* and *C. latifolia* [10]. Studies of reproductive success in hand-pollinated *Campanula rotundifolia* revealed that seed production was reduced upon self- pollination in comparison with cross-pollination. Seed yield reductions upon hand self-pollination as compared with hand crosspollination were attributed to inbreeding depression into partial self-incompatibility in *Campanula rotundifolia* [11]. Studies of plants from a natural population of *Campanula rapunculoides* revealed that the flowers of most individuals are strongly self-incompatible when the

Fig. 2a *Campanula kachetica* growing in crevices of limestone rocks.

Fig. 2b *Campanula kachetica* in its natural habitat.

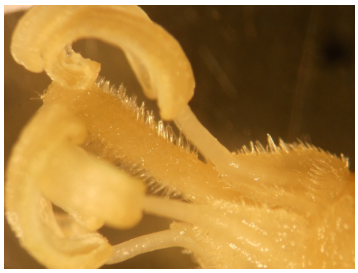

Fig. 3a Stamen and hairy stigma in the bud.

Fig. 3b Separated stigma lobes.

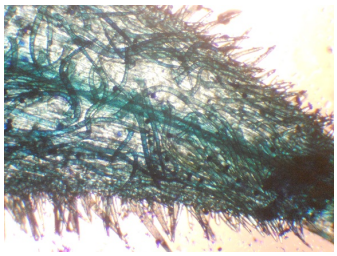

Fig. 3c Widened base of stamen with nectar drops.

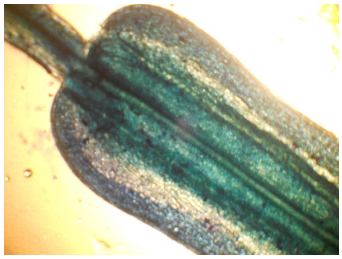

Fig. 4a Stamen.

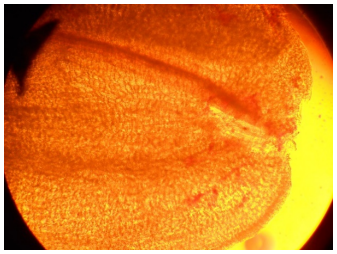

Fig. 4b Wall of the anther.

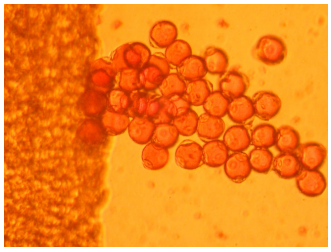

Fig. 4c Pollen grains.

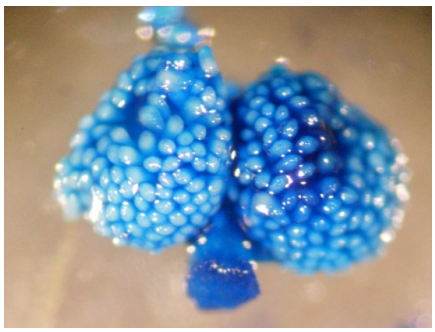

Fig. 5a Ovules extracted from the ovary locules.

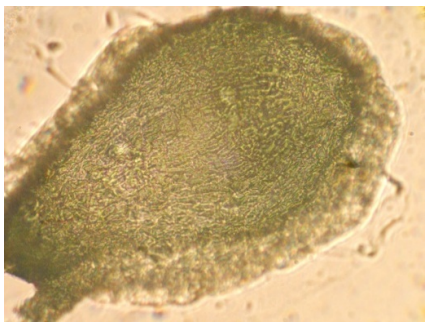

Fig. 5b Ovule.

the flowers age [12, 13]. In *Campanula alliariifolia, C. persicifolia, C. rapunculoides, C. trachelium* autogamy is excluded [14]. The ecological life of *Campanula americana* and germination characteristics show, that plants from seed that germinate in summer or autumn behave as winter annuals and those from seeds that germinate in spring act as biennials [15].

All the above mentioned and numerous other researches point to individual peculiarities of different species of Campanula. That shows the necessity and importance of the profound research of particular species of the genus Campanula.

For the complete study of reproductive peculiarities in *Campanula kachetica* the following issues were investigated flower morphology, peculiarities of flowering and pollination; embryology – the processes of development of the generative sphere; productivity of seed formation; biology of seed germination – capacities and terms of seed germination. Seed storage behaviour was studied via testing quality and germination capacity of seed, stored for four years both in the seed bank, in conditions of long term storage, and under room conditions.

Materials and Methods

Campanula kachetica is a perennial herbaceous plant, growing in crevices of limestone rocks (Fig. 2a). The plant is covered with dense greyish, thin, coarse hair. Stem is branched from the base, lignified and columnary ascending with old leaves. The plant is characterized by caudex. Rhisome is thick and branched. Stem is up to 15 cm high, leaves leathery, covered with coarse tomentum. Basal leaves are triangular-ovate-cordate, undulate serrate on margins. Upper leaves are almost ovate-lancetoid, attaining 25 mm length together with petiole. Flower bearing stems with low number of flowers are bristle with small coarse leaves. Corolla is light-rose-bluish (Fig. 2b), narrow bell-shaped, cut to 1/5 of its length, 17-19 mm long. Flowers are apical or nodal, borne in paniculoid-racemoid inflorescences. Segments of calyx triangular, appendages of calyx thin [1].

From 2010 to 2016 annually, twice a year, in July and September, field studies were carried out in environs of Dedoplistskaro, in Eagles' gorge, close to the Vashlovani Protected Areas.

Embryological study of the material was carried out using the methods accepted in cytoembryology [16, 17]. Different age buds and open flowers were fixated in Carnoy's fluid, FAA and FPA solutions. Pollen fertility was studied on temporary plates using staining with acetocarmine. Pollen germination capacity was studied by placing the pollen into different concentration saccharose solutions (6, 10, 12 and 15%). Embryological control was carried out using the light microscope (Carl Zeiss, Germany).

For studies of seed germination capacity in laboratory conditions each year seed was sown on Petri dishes in spring and in autumn in different conditions, in three variants: on 1% agar in the fridge, at +4°C; on 1% agar in the incubator, 21/14°C, 12/12 hours day/night and on a filter paper, in conditions of room temperature (20-23°C) twice a year. To create living collections, seed was also sown in soil – in pots.

To study peculiarities of pollinaton of the species, in particular, to reveal the possibility of autogamy, field studies were carried out in a native distribution range of a species in late spring in the phase of buds. The closed buds were isolated by wrapping them into cotton gauze. The isolated flowers were left on a plant until the maturation of fruits. In autumn, in the stage of fruiting, the isolated individuals were unwrapped for the analysis.

Results and Discussion

It is known that flower of *Campanula kachetica* is actinomorphic, bisexual, entomophilic, dichogamic. It is characterized by protandry. Pollen number is five, equal to the number of petals. According to our data in the phase of green buds length of the stigma and stamens, densely arranged around it are equal and make 6 mm. In unopened big size flower length of stamen and stigma still

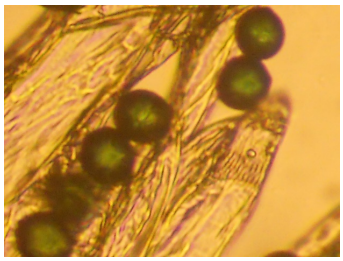

Fig. 6a Pollen deposited on pollen hairs.

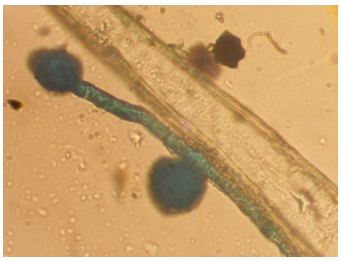

Fig. 6b Elongation of pollen tube towards the stigma papillae.

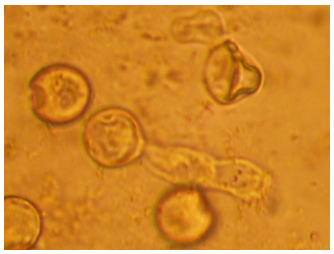

Fig. 6c Growth of pollen tube on the 12% saccharose medium.

Fig. 7a Mature seed.

Fig. 7b Seeds germinated on Petri dish.

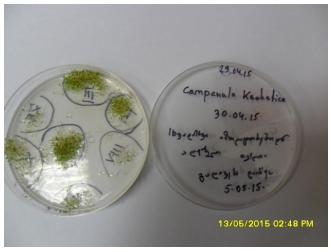

Fig. 7c Germination of seeds harvested from isolated individuals.

Fig. 8a Seedlings at a later stage of development.

Fig. 8b Generative phase *of Campanula kachetica.*

remain the same – 8 mm. Stigma is covered with dense hair (Fig.3a). In the process of further growth in a slightly disclosed flower stamen become twisted. Length of style together with stigma is 13 mm. In fully disclosed flower the style is prolonged, but the lobes of stigma are not separated yet, length of the style is 15 mm. In the disclosed flower, when the female phase starts and the three lobes of the stigma separate (Fig. 3b), the total length of style with stigma is 16-17 mm. Stamen are equal in size, filaments are free, significantly widened at a base and densely haired. The base, with which it is attached to the nectar disc is triangular by shape (Fig. 3c). Anther (Fig.4a,b) is four-locular; its wall consist of four layers – epidermis, double layered endothecium, intermediate layer and binuclear tapetum Tetrads are produced by simultaneous type. The majority of pollen in buds is mononuclear, though binuclear grains are observed too. Mature pollen grain is bicellular, three porous. In studies of pollen fertility, using the cytoembryological methods, the mix of pollen grains, taken from the same or different buds (up to10) was used. Almost 100% of pollen turned out to be fertile (Fig.4c). Ovary is lower, three locular, with numerous ovules in each locule(Fig.5a). Placenta is large. Location of ovules on placenta is superficial-lateral. Ovule is anatropic, tenuinucellate, with a very short funiculus (Fig. 5b). Embryo sac develops by

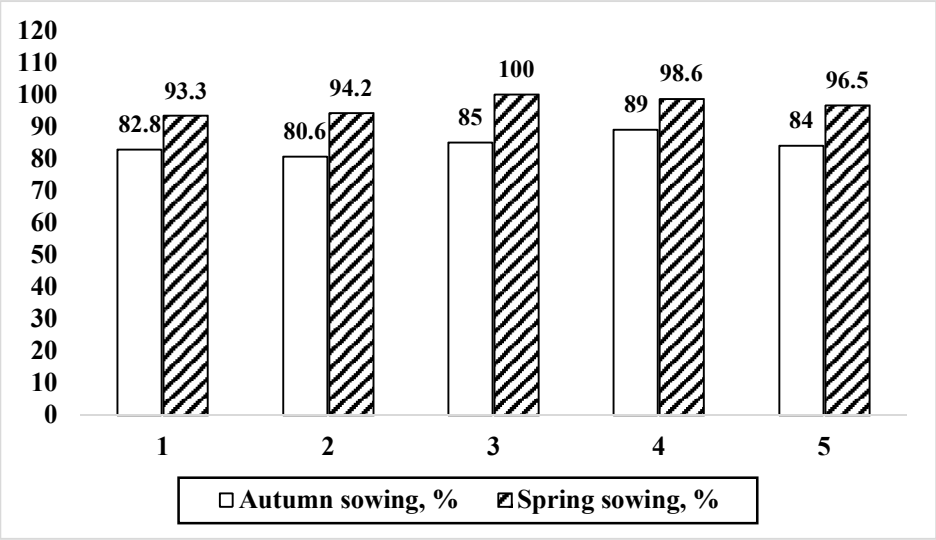

Fig. 9. Germination capacity of seeds of open pollinated individuals depending on time of sowing- **1 - 2010, 2 – 2011, 3 - 2012, 4 - 2013, 5 – 2014.**

polygonum type. Polar nuclei merge before the fertilization. Endosperm is cellular. The formed embryo is located in the central part of the mature seed. Tapetum cells of integument are big in size and on both sides of embryo sac are similar to the nucellar cells. Embryological issues of *Campanula kachetica* are given in our earlier works [18, 19].

Similar to other species of genus Campanula, the male phase starts with the dehiscence of anthers which already takes place in the flower bud.. Though we noticed, that in some cases anthers retained the integrity even in big,unopened flowers. On the following stages of development in the disclosed flowers the pollen attached to the hair is drawn out as a result of longitudinal growth of the style. Stigmal lobes are not yet separated by then. Hairs are abundantly covered with pollen grains (Fig.6a).

Female phase starts after the separation of stigmal lobes and the stigma is ready to accept the pollen (Fig. 6b). The process of fertilization was described by Charles Barnes in *Campanula americana* back in 1885 [20].

The inner surfaces of stigmal lobes are equipped with small papillae. The conductive tissue is located around the narrow central channel. Due to this pollen tube easily reaches the conducting tissue, where it penetrates through the channel between the cells of conductive tissue and passing through the placenta reaches the ovules. By our observations, in the longitudinal transect of the style cells of conductive tissue are represented by oblong cells. Great number of pollen tubes was fixed in the condictuve tissue of the style.

When placed into saccharose solution, growth of pollen grains was most intense and rapid in 12% solution. The 60% of pollen grains started germination after 3 hours. Length of pollen tubes was different. The longest was a quite big in size attaining several mm in length (15-17 mm). Spermiae are visible in the pollen tube (Fig. 6c.).

By the end of September flowering process in the population of Kakhetian bellflower is terminated and the plant is in the phase of fruiting. Fruit is a capsule, which dehisces with three valves. While studying reproduction biology of *Campanula sibirica* authors described presence of a kind of specialized organ – axicorn in this species, which facilitates opening of valves and dispersal of seeds [21].

To assess productivity of seed formation we counted the number of ovules in the ovary of unopened

flower. In up to 40 individuals number of ovules per flower in the phase of bud fluctuates at average from 500-to 600. Number of actually formed mature seeds per fruit is within 481-508; number of seeds per locule is from 180 to 217. Seed formation capacity, which represents ratio of actually formed seeds to the number of ovules in the ovary, calculated based on these data is quite high – from 80 to 96.2%. Ripe seed is brownish, its surface is mesh-like, walls of epidermis are thickened (Fig.7a). The embryo is located in the middle part of the seed. The embryo is differentiated, surrounded by the thick layer of epidermis. Seed is minute, weight per 1000 seeds 0.036-0.044 g.

Studies of seed germination capacity - negative result was obtained when sowing seeds on Petri dishes at temperature as low as +4°C. As concerns the rest two variants - 1% agar in the incubator, 21/14°C, 12/12 hours day/night and on a filter paper, in conditions of room temperature (20-23°C) - the result was good. Germination percent and percent of sprouting in the laboratory was almost 100% (Fig. 7b).

Results of germination of seeds, obtained as a result of open pollination in different years and different seasons (spring and autumn sowing) are presented in the Fig.9.

Period of germination in all variants varies from 4 to 7 days, and after 2 weeks number of sprouts reaches 80-100%. In flowers, under the isolation fruits have developed and significant number of seeds was obtained. Number of seeds, developed in isolated individuals in different years is shown in Fig. 10a.and 10b. Fig. 10a presents data on total and average number of seeds, developed on 6 individuals under isolation in 2013 and Fig. 10b shows data of total and average number of seeds, developed on 12 individuals in 2014.

Number of seeds in capsules in conditions of isolation is very variable. Their number is different and it varies from 53 to 350.

Germination capacity of seeds, collected from isolated individuals was tested on Petri dishes, on agar and filter paper in controlled conditions – in the incubator and under the room conditions. Results have demonstrated that germination of seeds takes 4 to 10 days after sowing .The percentage of seed germination and viability is high (85-100 %).(Fig.11). The seedling develops in two weeks.

Further observations on seedlings, developed from seeds from both - open pollinated and isolated (self-pollinated) individuals have shown, that the seedling attains the stage of the first leaf in 10-15 days. During this period the main root significantly grows in length and branches out. Seeds sown in autumn (01.10. 2014) under Greenhouse conditions (19-23°C) in soil in pots and containers of small volume started germination in 10 days, germination attained 100% and all seedlings reached the stage of the 3rd leaf.

Laboratory studies of seeds, collected from both open-pollinated individuals and isolated (self-pollinated) individuals in previous years, has shown, that at room temperature (+19-23°C) seed retains good germination capacity for three years and in 15 days germination reaches 92%

Results of germination and sprouting of seeds, collected from isolated individuals in incubator on 1% agar substrate are presented in Fig.7c.

Viability of seed, stored in the Seed Bank at – 20°C for 4 years was also checked. Seed started gemination (on agar in incubator 21/14°C 12h/12h) in 4 days and germination percent attained 90% in 10 days. When sowing in soil in 3 weeks one leaf emerged and in a month three leaves developed.

Seedling, developed from seeds sown in soil in pots in the glasshouse continue normal development and in 1.5 year reach generative period (Fig. 8b).

Conclusion

Based on our research we can conclude that like other species of Campanulaceae (*Campanula kemulariae and Campanula armazica*) [22, 23], generative sphere of *Campanula kachetica* develops without essential disturbances. Both male and female spheres (generative organs) develop

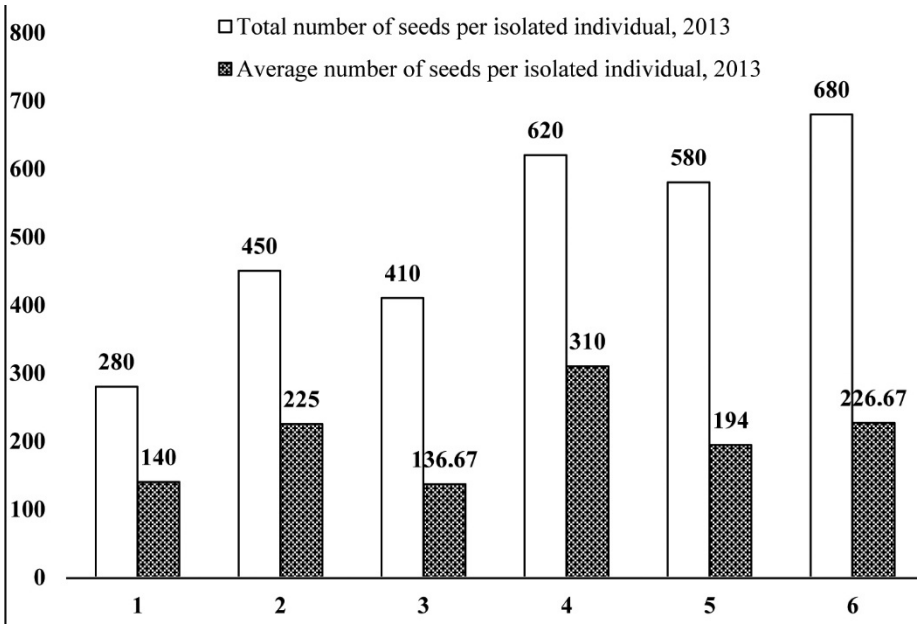

Fig.10a. Data of 6 individuals (2013)

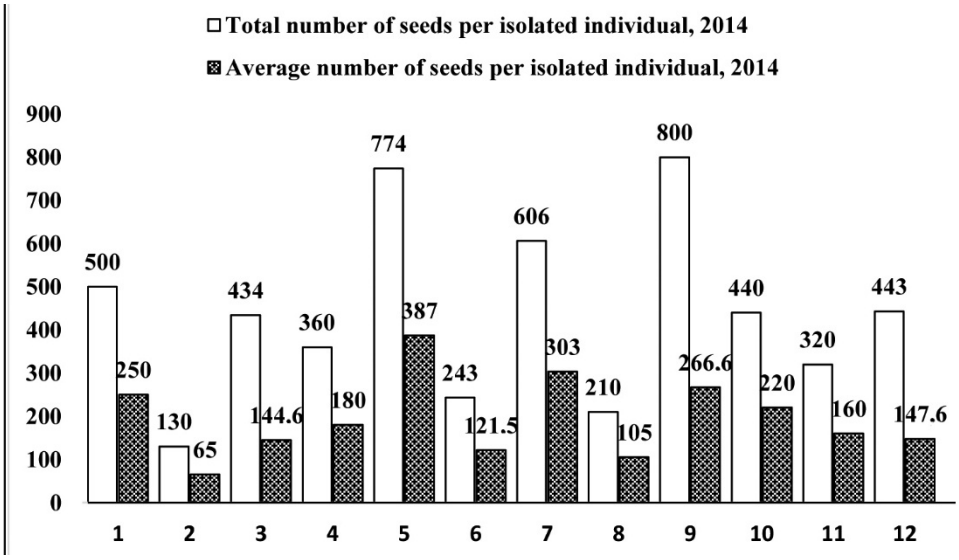

Fig.10b. Data of 12 individuals (2014)

normally. Formation of embryo sac, embryo and endospermogenesis proceed normally. Fertility of pollen is high (90-100%). Seed formation capacity is high (80-96%). This indicates that the species does not have problems in generative sphere and biological factor does not seem to cause the decline of the population.

Studies of self-regeneration capacity of the species revealed a quite high germination percent of seeds, yielded from both cross-pollinated and self-pollinated individuals of *Campanula kachetica* - 85-95%.

As it is known, plant reproductive success is affected by a number of factors, such as paternal environments and plant resource status during flowering and fruiting, and pollen origin in fertilization [8, 9, 24]. By our observations for propagation of *Campanula kachetica* by seed in the natural distribution range it is necessary for the seed to fall into favourable conditions. In extremal conditions

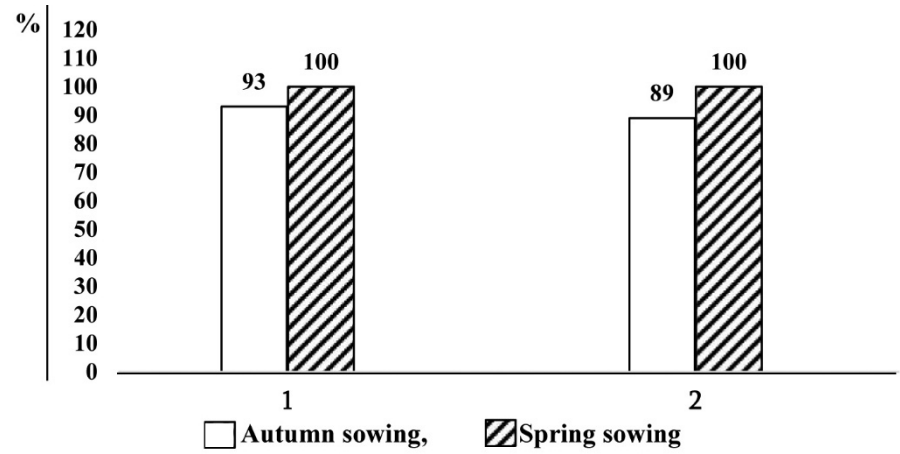

Fig. 11. Germination capacity of seeds of isolated individuals depending on the time of sowing
1 - seeds harvested in 2013, 2 - seeds harvested in 2014.

an own survival mechanism developed in *Campanula kachetica*. As revealed by our research, capacity for regeneration by means of sexual propagation is quite high in the studied species. *Campanula kachetica* is characterized by biological plasticity, labile character of pollination - in particular the presence of facultative autogamy along with allogamy. This, in our opinion, is an indication of the strong survival strategy of the species.

The data, obtained as a result of study of reproduction biology of the species along with other criteria are important for the evaluation of the species status in the wild and development of methods for species reintroduction.

Species ex situ conservation activities have been carried out. The tested high quality seeds of *Campanula kachetica* are deposited for long term storage in the National Seed Bank of the National Botanical Garden of Georgia and duplicated in the Millenniums Seed Bank of the Royal Botanic Gardens, Kew, UK.

ბოტანიკა

გადაშენების პირას მისული ვიწროლოკალური, საქართველოს ენდემური სახეობის *Campanula kachetica* **Kantsch.** -ს რეპროდუქციული სტრატეგია და **ex situ** კონსერვაცია

ნ. მელია და თ. ბარბლიშვილი

საქართველოს ეროვნული ბოტანიკური ბაღი, თბილისი, საქართველო

(წარმოდგენილია აკადემიის წევრის გ. ნახუცრიშვილის მიერ)

პირველად კომპლექსურად არის გამოკვლეული კავკასიის მცენარეთა წითელი ნუსხის ვიწროლოკალური, მაღალი საკონსერვაციო ღირებულების მქონე სახეობის, საქართველოს ენდემის კახეთის მაჩიტას - ***Campanula kachetica*** **Kantsch.** ბიოლოგიური თავისებურებები, კერძოდ: ყვავილობისა და დამტვერვის ბიოლოგია, გენერაციული სფერო, თესლის პროდუქტიულობა, აღმოცენების უნარი და ვადები განსხვავებულ გარემო პირობებში, აგრეთვე თესლის ბანკში ოთხი წლის განმავლობაში შენახული თესლის სიცოცხლის-უნარიანობა თვითგანახლების შესაძლებლობის ტესტირების გზით. შეფასდა კახეთის მაჩიტას ბუნებრივი პოპულაციის არსებული მდგომარეობა. შესწავლილ კრიტერიუმებზე დაყრდნობით გამოვლინდა, რომ ***Campanula kachetica***-ს რეპროდუქციული შესაძლებლობლობები საკმაოდ მაღალია. სახეობას ახასიათებს ბიოლოგიური პლასტიკურობა, დამტვერვის ლაბილური ხასიათი. ბუნებრივ არეალში სახეობის თესლით გამრავლებისათვის აუცილებელია თესლის ხელსაყრელ გარემოში მოხვედრა და ამ პერიოდისთვის საჭირო კლიმატური პირობების თანხვედრა. ექსტრემალური პირობების შექმნისას კახეთის მაჩიტას თვითგადარჩენის თავისებური მექანიზმი გამოუმუშავდა. კერძოდ, ალოგამიის გარდა საკმაოდ წარმატებული ავტოგამიის შესაძლებლობაც, რაც ჩვენი ვარაუდით მიუთითებს ამ სახეობის თვითგადარჩენის ძლიერ სტრატეგიაზე. აღნიშნული კვლევა შესაძლებელია გამოყენებული იყოს ბუნებრივ არეალში სახეობის რეინტროდუქციის მეთოდების შესამუშავებლად.

ჩატარდა საკონსერვაციო სამუშაო - ლაბორატორიულად დამუშავებული და შემოწმებული მაღალი ხარისხის თესლი გრძელვადიანი შენახვისათვის განთავსდა საქართველოს ეროვნული ბოტანიკური ბაღის თესლის ბანკში და კიუს სამეფო ბოტანიკური ბაღის ათასწლეულის თესლის ბანკში (დიდი ბრიტანეთი).

REFERENCES

1. Flora of Georgia (2001) XIII: 189-190 (in Georgian).
2. Gagnidze R. (2005) Nomenclatural Checklist of Georgia, Tbilisi.
3. 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 109-47: 125-126. Missouri Botanical Garden Press.
4. Shetler S.G. (1979) Pollen-collecting Hairs of Campanula (Campanulaceae). *Historical review.* Taxon, **28**, 1 2/3: 205-215.
5. Nyman Y. (1992) Pollination mechanisms in six campanula species (Campanulaceae). *Plant Systematics and Evolution*, 181: 97-108.
6. Nyman Y. (1993) The pollen-collecting Hairs of Campanula (Campanulaceae) II.Function and adaptive significance in relation to pollination. *American Journal of Botany,* 80: 1427-1436.
7. Carolin R.C (1960) The structures involved in the presentation of pollen to visiting insects in the order Campanulales. *Proc.Linn Soc.*, 84: 197-207. New South Wales.
8. Lloyd D.G., Schoen D.J. (1992) Self and cross fertilization in plants I. Functional Dimensions. *Int. J Plant Sci,* 153: 358-369.
9. Uyenoyama M.K., Holsinger K. E.,Waller D.M (1993) Ecological and Genetic Factors Directing the Evolution of Self-fertilization. Oxford Surveys in Evolutionary Biology.
10. Antonova (1972) Sezonnaia i sutochnaia ritmika tsvetenia rastenii shirokolistvennogo lesa.. *Ekologia*, 4: 73-79 (in Russian).
11. Nuortila C. (2007) Constraints on Sexual Reproduction and Seed Set in Vaccinium and Campanula. Acta Univ. Oulu, Finland.
12. Richardson T. E., Stephenson A.G. (1989) Pollen removal and pollen deposition affect the duration of the staminate and pistillate phases in *Campanula rapunculoides*. *Am j. Bot.* 76: 532-538.
13. Richardson T. E., Hrincevich A., Kao T H, Stephenson A. G. (1990) Preliminary studies into age-dependent breakdown of self-incompatibility in *Campanula rapunculoides*: Seed set, pollen tube growth, and molecular data. PI *Cell Incompat. Newslett*., 22:41-47.
14. Fomina T. I. (2011) Biologia tsvetenia nekotoryx vidov kolokolchikov. *Vestnik OGU,* 4, (123): 94-97 (in Russian).
15. *Baskin J. M., Baskin C. C.* (1984) The ecological life cycle of *Campanula Americana* in horthcentral kentucky. *Bulletin of Torrey Botanical Club.* **III**, 3: 329-337.
16. Pausheva Z. P. (1988) Praktikum po tsitologii rastenii. M. (in Russian).
17. Herr J. M (1971) A clearing-squash technique for the study of Ovule development in angiosperms. *Am. J. Bot.* 58: 785-790.
18. Melia N., Barblishvili T., Jgenti L. (2014) Study of self–reproduction capacity and ex situ conservation of endemic species *Campanula kachetica* Kantsch.. *Proceedings of the Third International Symposium on the Biology of Rare and Endemic Plant Species* (Biorare -2014) p.83, Antalya, Turkey.
19. Melia N, Barblishvili T. (2015) Study of reproduction capacity and ex situ conservation of the species of primary conservation status *Campanula kachetica* Kantsch. Included in the Red List of Endemic Plants of Caucasus Region. *Materials of I Scientific Conference of National Botanical Garden of Georgia.* pp.68-71.
20. Barnes C. (1885) The Process of Fertilization in *Campanula Americana* L. *Botanical Gazette,* **10**, 9/10: 349-354.
21. Miroshnichenko,N. N., Shevchenko S.V. (2014) Nekotorye Osobennosti Reproduktivnoi Biologii *Campanula sibirica. Zhivye i biokosnye sistemy*. vipusk 7: 1-8 (in Russian).
22. 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: 96-104.
23. Kobakhidze L., Barblishvili T. (2017) Study of reproduction capacity and ex-situ conservation of *Campanula armazica* Charadze (Campanulaceae). *Bull. Georg. Natl. Acad. Sci,* **11**, 3: 119-125.
24. Galloway L.F. (2001) The Effect of Maternal and Paternal Environments on Seed Characters in the Herbaceous Plant *Campanula Americana*. *Am.. J. Bot*., 88, 5: 832-840.

Received February, 2018