საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 18, №3, 2024
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Botany

Peculiarities of the Reproductive Biology and *ex situ* Conservation of Two Endangered Georgian Endemic Species *Dianthus azkurensis* Sosn. and *Dianthus ketzkhovelii* Makaschv. (Caryophyllaceae)

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(Presented by Academy Member Giorgi Nakhutsrishvili)

Several aspects of reproductive biology have been investigated in complex in two wild growing endangered species of the genus *Dianthus* included in the Red List of Plants of Georgia: *Dianthus azkurensis* Sosn. (EN) and *Dianthus ketzkhovelii* Makaschv. (EN) (family Caryophyllaceae). These aspects include seed formation capacity (potential and actual), seed germination under laboratory conditions and in the ground, the stages of ontogenesis. In the population of one of the target species, *Dianthus azkurensis* Sosn., the phenomenon of sexual dimorphism – gynodioecy or female dioecy, also a low occurrence of ginomonoecy was established, which has not been reported for this species so far. This phenomenon was clearly expressed under cultivation conditions when studying seed propagation capacity of this species. Some signs of advantage of functionally female individuals over the bisexual ones have been described. The peculiarities of individuals from two geographically remote populations (located in East and West Georgia) of *Dianthus ketzkhovelii* Makaschv. were compared. The viability and germination capacity of seeds of the target species preserved for eight years in the Seed Bank at -20°C was tested. *Ex situ* living collections of the target species are set up in the conservation plot of the Department of Plant Conservation at the National Botanical Garden of Georgia (NBGG). Theoretical and practical materials are prepared ensuring successful reintroduction of these species if needed. *© 2024 Bull. Georg. Natl. Acad. Sci.*

Dianthus azkurensis, *Dianthus ketzkhovelii*, ontogenesis, gynodioecy, seed set, conservation

Human impact, climate change and many other factors negatively influence natural flora, making the preservation and restoration of wild growing endangered and especially endemic species increasingly urgent. Investigation of biological characteristics of endangered plant species is one of the important preconditions for the species conservation and restoration of biodiversity. That enables to estimate the reasons, due to which the species became rare and to develop the propagation methods for their further reintroduction.

There are two main directions in the conservation of endangered plant species: population genetics and reproductive biology. One of them, in

particular, investigation of the reproductive biology reveals the possible restrictions in the process of sexual reproduction of the species, that often becomes the main reason underlying the decline in the population viability and threatening their survival in nature. The results of similar studies of a number of plant species promoted the planning of efficient conservation and management programs [1, 2]. The present work deals with a complex five-year study of biological peculiarities of two wild-growing endangered species of the genus *Dianthus*, having conservation status and included in the Red List of Plants of Georgia *Dianthus azkurensis* Sosn. (EN) and *Dianthus ketzkhovelii* Makaschv. (EN) (family Caryophyllaceae). The current state of natural populations of the target species, some aspects of reproduction biology – structural peculiarities of the male and female generative sphere, peculiarities of pollination, seed formation capacity (potential and actual), seed germination capacity, successive stages of ontogenesis have been studied at different stages of development. The population of *Dianthus azkurensis* Sosn. is fragmented on certain locations. According to the literary sources, this is an indicator of habitat loss and extinction risk [3]. In this population, the phenomenon of sexual dimorphism – gynodioecy or female dioecy was observed.

The sexual structure is one of the significant biological characteristics of the flowering plant species [4]. The data on sexual structure of population should be considered during the introduction or reintroduction of endangered species. Thus, studying the reproductive strategy of the species is of great importance and has become the main direction of our research. Populations of *Dianthus ketzkhovelii* are characterized only by bisexual flowers. The peculiarities of the individuals of two geographically remote (Eastern and Western Georgia) populations of *Dianthus ketzkhovelii* Makaschv., seed productivity in the wild conditions and under cultivation are compared to each other.

Materials and Methods

This work covers the data of experimens carried out in 2015-2020. A small population of the species *Dianthus azkurensis.* (EN) (genus Dianthus L., Sect. Leiopetali (Boiss.) Schischk.) can be found in separate locations in the Samtskhe-Javakheti region, in the vicinity of Vardzia and the village of Tmogvi, where it grows on the rocks in the middle

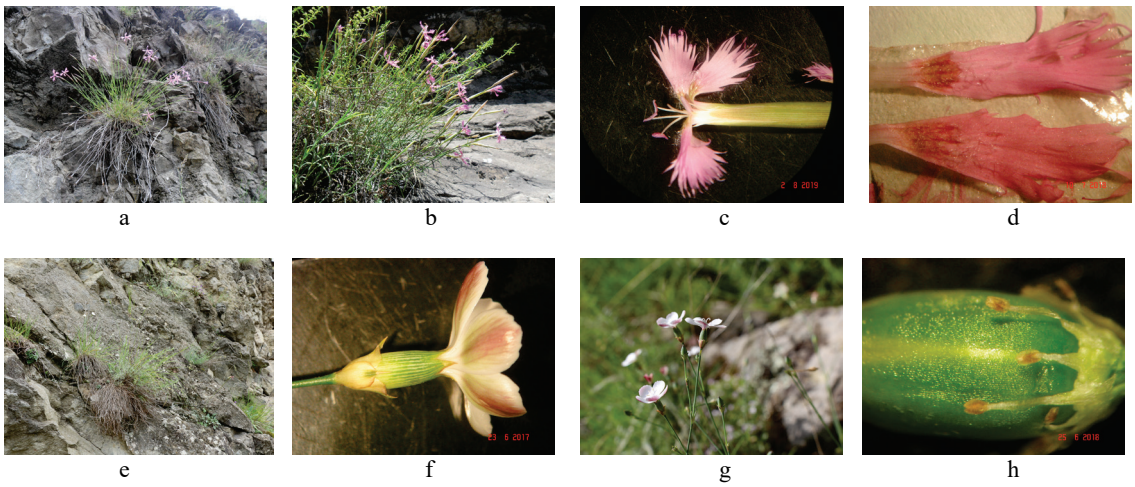


Fig. 1. Plants in their natural habitat. a – *Dianthus ketzkhovelii* Abastumani population; b – Ajara population; c – flower; d – corolla petals with dark spot at the base (Abastumani population); e – *Dianthus azkurensis*; village Tmogvi population; f – flower; g – bisexual and female flowers in nature; h – rudimentary stamens on the immature fruit.

mountain belt. The plant is perennial, with numerous, 20-40 cm tall stems emerging from the branched rhizomes [5]. The color of the petals varies from pinkish to whitish (Fig. 1-e, f, g). The ratio of different sexual forms in the population of *Dianthus azkurensis* was determined during the mass flowering of the species from the total number of plants, which was considered 100%.

Dianthus ketzkhovelii (EN) (genus Dianthus L., Sect. Fimbriatum F. Williams.) was described by A. Makashvili from Adjara. Later the species was found to be common in the western part of Meskheti. It grows on the rocks, in the lower forest zone. The study material was collected from the populations spread in the valley of the Acharistskali (West Georgia) and from the populations located on the steep slopes in the vicinity of the township Abastumani (East). (Figures 1a, b, c, d). The corolla petals are purple-red, fringed, with a dark spot at the base of bending and covered with white fur [6].

The material for the embryological control was collected from natural populations and processed using the classical methods accepted in embryology [6, 7]. Seed germination and viability were studied in laboratory conditions on 1% agar in Petri dishes by selecting different sowing regimes; seed viability was checked by the tetrazolium test (staining the samples in 1% solution of 2,3,5-triphenyl tetrazolium chloride for 24 hours). Petri dishes were placed in an incubator, where *day/night* temperature was +21/14°C; illumination regime 12 hrs day/12 hrs night. In addition, the viability and germination ability of the seeds of the target species stored in the seed bank at -20°C for 8 years were tested. In order to create ex situ collections, seeds were also sown in pots and outdoors in the conservation collection plot of the National Botanical Garden.

Results

A phenomenon of sexual dimorphism – gynodioecy (female dioecy) and a small share of gynomonoecy (simultaneous presence of bisexual and functionally female flowers at the same individual) was stated in the population of *Dianthus azkurensis* which was clearly demonstrated in cultivation while studying the possibility of the species for the self-renewal by seeds. Sexual dimorphism is characteristic of representatives of various families, including Caryophyllaceae family and also noted in some species of the genus Dianthus (*Dianthus versicolor, D. volgicus*, *D. repens*, *D. acicularis*, *D. Campestris*) [8]. In the studied population of *Dianthus azkurensis* bisexual individuals comprise the majority, while functionally female forms range from 16 to 40%. However, this ratio in the population was not the same in different years. In 2018 and 2020, in one part of the population, the number of female forms prevailed (79%-55.5%). There was also a small number of gynomonoecious individuals in the population, which share varied from 1% to 16% (Fig. 2).

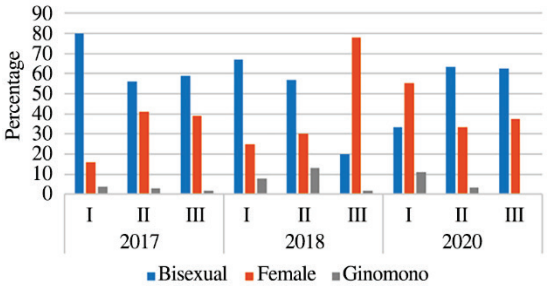
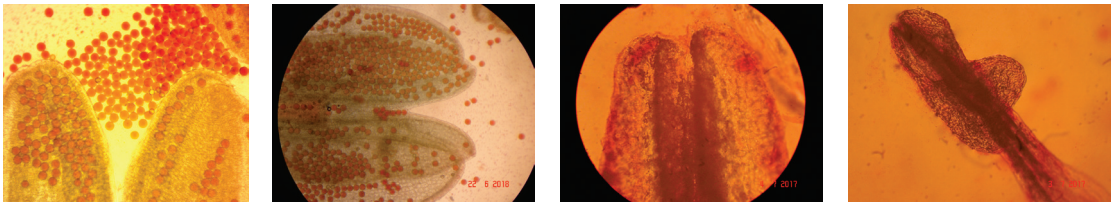


Fig. 2. Sexual structure of the *Dianthus azkurensis* population in different years.

Unlike the population of *Dianthus azkurensis*, the both populatons of *Dianthus ketzkhovelii* – in Abastumni and Adjara were comprised of only bisexual individuals. Comparison of morphometric characteristics of the generative sphere of individuals of these populations showed us that they only slightly differ from each other.

Bisexual flowers of the both studied species are characterized by sharply expressed dichogamy in the form of protandry. Fertility of the pollen is quite high – in *Dianthus azkurensis* – 83% of pollen grains is fertile. Better growth of the pollen tube



a b c d

Fig. 3. a – Anther wall of *Dianthus ketzkhovelii* (Abastumani population); b – Anther wall of the bisexual flowers of *Dianthus azkurensis*; c-d anther wall of *Dianthus azkurensis* female flower, wrinkled anthers.

was observed in 10% sucrose solution. In both populations of *Dianthus ketzkhovelii* 73-76% of pollen grains is fertile. Better growth of pollen tube was observed in 12% sucrose solution. Embryological research revealed almost no abnormalities in bisexual individuals of both target species *Dianthus azkurensis* and *Dianthus ketzkhovelii*. Microsporogenesis goes successfully (Fig. 3. a, b). Megasporogenesis occurs asynchronously in ovules. The embryo sac develops as polygonum type. Endosperm is nuclear, later it becomes cellular. At an early stage the endosperm is completely used for the embryo. On the next stage, the perisperm surrounds the dicotyledonous embryo which occupies almost the entire perimeter of the seed.

Functionally female flower of *Dianthus azkurensis* is slightly smaller in size compared to the bisexual one. The peak of flower opening, both in nature and in culture, starts around 2 PM and lasts till the evening. At night the flower closes. Flowering begins directly from the stigma phase (3-4 days). The male phase is dropped out. By the beginning of the flowering of the plant, the stigma is already quite raised, its papillae are elongated. Receptivity of the female flower stigma coincides with the male phase of bisexual flowers, when the pollen potential is quite high. Cytoembryological studies have shown that at the initial stages of male phase the formation of sporogenous tissue proceeds without deviation from the norm. Serious disorders occurs in the prophase of the first division of meiosis in microsporocytes which finally leads to complete sterilization of the androecium. All ten stamens become rudimentary, dwarf (Fig. 3. c, d, 1h).

Table. Assessment of seed productivity under natural and introduced conditions

	Dianthus azkurensis		*Dianthus ketzkhovelii*	
	bisexual	female	Abastumani	Adjara
Potential number of the ovules	80 to 105	89 to 105	40 to 90	40 to 85
% seed set in the nature	4 to 50	2 to 70	32 to 45	20 to 44
% seed set under the condition of introduction	4 to 33	2 to 54	1 to 6	1 to 8
The average weight of 1000 seeds	0.55 g	0.62 g	0.63 g	0.64 g.

The potential and actual ability for seed formation of the investigated species are different in the wild and under cultivation (Table). The rate of seed germination for both studied species was almost the same when sown under laboratory conditions on Petri dishes, agar medium or filter paper. The seed do not show primary dormancy. The 50% of the freshly harvested seeds germinate in 2-3 days (T-50) (Fig. 4). In both cases, the final rate of germination is high and reaches 95-100% in one week. Immature seeds ripen in 2 weeks at room temperature (+21-23°C) and germinate normally. The seeds are well stored at low temperature. The seeds stored in the seed bank for 7-8 years at -20°C germinate on the agar medium and filter paper also in 3 days and retain a fairly high germination capacity – 95% for *Dianthus azkurensis* and 74-75% for *Dianthus ketzkoveli*.

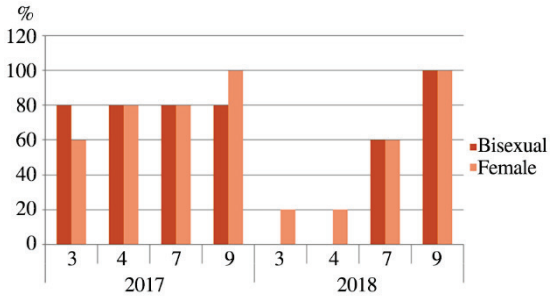


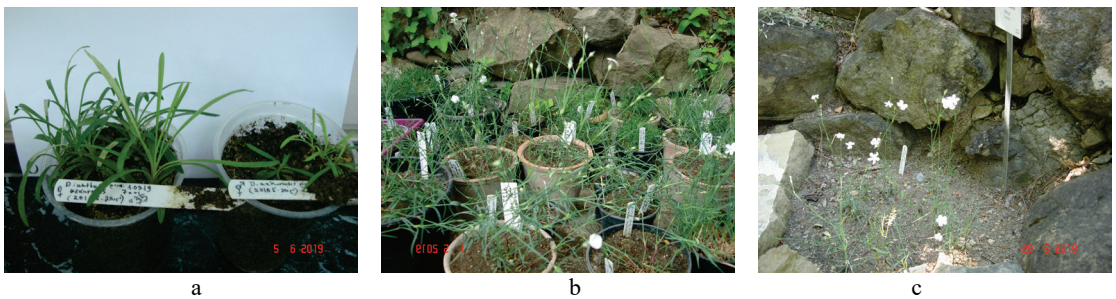
Fig. 4. Seed germination dynamics on Petri dishes(by days) (*Dianthus azkurensis*).

Seed germination of *D. azkurensis* after spring sowing indoor in the pots starts in 6-7 days, true leaf develops in 18 days, generative period starts in 1.5 years. It was also noted, that the process of growth and development of female individuals is noticeably faster than in case of bisexual plants (Fig. 5). The rhythm of seasonal development of plants grown from seeds, harvested from the natural populations of *Dianthus ketzkoveli*, is almost the same at the initial stages of ontogenesis. Germination starts in 4 days in spring and within one or two weeks in autumn. In the first year after sowing, the plant goes through all phases – from embryonic to the adult vegetative state with a well-formed above-ground part and the root system. Intensive growth and formation of reproductive organs begins in the second year of its development.

Discussion

The analysis of the sex ratio (Fig. 2) in nature showed that the bisexual individuals comprise the majority in the population of *Dianthus azkurensis*. The number of functionally female individuals is more or less maintained at a certain level, although in some parts of the population it considerably exceeded that of bisexual forms. The seeds of the target species are not characterized by the period of dormancy. Plants of both investigated species are propagated both by seeds and vegetatively, from the branched rhizome. Functionally female individuals have certain advantages over the bisexual ones. The advantage was expressed in the accelerated rhythm of growth and development, earlier flowering, the prolonged stigma stage, annual excessive flowering and in some cases in a higher fertility (both in nature and under cultivation). Some researchers [9] based on the theoretical analysis of the literature data on gynodioecious species, conclude that the ability of female individuals to germinate better is still not a determining factor that ensures the establishment of female individuals in the gynodioecious population.

When propagated by seeds in conditions of cultivation, all phases of ontogenesis in the target species proceed approximately in the same way during 1.5 years. Some authors, while studying the ontogeny of *Dianthus acicularis*, noted that under conditions of cultivation the plant goes through the pre-generational period faster and enters the generative period in the second year of development, while under natural conditions the plant only achieves it on the seventh or eighth years [10]. This particular issue in our case requires further study. Female individuals of *Dianthus azkurensis* compared to bisexuals strongly reacted to environmental conditions and developed better in conditions of



a b c

Fig. 5. Plants in the pots – a – the femals is ahead of bisexuals in growth; b – flowering plants; c – in the conservation collection plot.

excessive humidity. Our further studies have shown that from seeds of both – bisexual and functionally female individuals plants of both sexes are produced at different proportions in subsequent generations.

Compared to the wild growing plants, the individuals of both populations of *Dianthus ketzkhovelii* have difficulty in seed production when they grow under culture conditions, the number of perfect seed, set in fruits frequently reached – 5-6%. As shown by the embryological control, in most cases all stages of the formation of generative organs were within the norm. According to our observation, *Dianthus ketzkhovelii* is not resistant to biotic interactions (pests, fungi, pathogens), which causes damage to the fruits of the plant both in nature and under conditions of cultivation. In addition, the lack of pollinators , i.e. the lack of pollen during reproduction under culture conditions, leads to a decrease in the reproductive performance of the plant, which is also noted by some researchers [11, 12]. When flowers are isolated, seeds do not develop, autogamy is restricted by dichogamy. Therefore, under the conditions of cultivation *Dianthus ketzkhovelii* needs artificial pollination with compatible pollen to obtain an increased number of perfect seed sets.

Living collections of target species were created on the collection plots of Plant Conservation of the National Botanical Garden, and based on the obtained data, an appropriate material was prepared for the species further reintroduction in case of need.

The theoretical results of the research work can be used for the further evaluation of the populations of the target species and elaboration of efficient measures to save the species. The practical results of the research can be taken into account when the investigated species are introduced and reintroduced. In accordance with the international methodology [13-16] the tested, perfect seeds of the target species were processed and deposited for the long term storage in the National Seed Bank, operated at the National Botanical Garden of Georgia and also duplicated to the Millennium Seed Bank of the Royal Botanical Garden, Kew.

ბოტანიკა

საფრთხის ქვეშ მყოფი საქართველოს ენდემური სახეობების *Dianthus azkurensis* Sosn. და *Dianthus ketzkhovelii* Makaschv. (ოჯახი Caryophyllaceae) რეპროდუქციული ბიოლოგიის თავისებურებები და *ex situ* კონსერვაცია

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(წარმოდგენილია აკადემიის წევრის გ. ნახუცრიშვილის მიერ)

კომპლექსურად არის შესწავლილი საქართველოს მცენარეთა წითელ ნუსხაში შეტანილი პრიორიტეტული საკონსერვაციო სტატუსის მქონე ველურად მოზარდი გვარის *Dianthus* L. ენდემური, გადაშენების პირას მისული სახეობების *Dianthus azkurensis* Sosn. (EN) და *Dianthus ketzkhovelii* Makaschv. (EN) (ოჯახი Caryophyllaceae) რეპროდუქციული ბიოლოგიის ზოგიერთი ასპექტი: ყვავილების მორფოლოგია, დამტვერვის თავისებურებები, ციტოემბრიოლოგიური კვლევები, თესლწარმოქმნის (პოტენციური და რეალური) შესაძლებლობები, თესლის აღმოცენებისუნარიანობა როგორც ლაბორატორიულ, ასევე ღია და დახურული გრუნტის პირობებში, ონტოგენეზის ეტაპები. დადგენილია გვარ *Dianthus*-ის ერთ-ერთი საკვლევი სახეობის – *Dianthus azkurensis* Sosn-ის პოპულაციაში სქესობრივი დიმორფიზმის მოვლენა – გინოდიეცია, ანუ, მდედრობითი ორსახლიანობა, აგრეთვე მცირე რაოდენობით გინომონოეციის არსებობა (ერთსა და იმავე ინდივიდზე ორსქესიანი და ფუნქციურად მდედრობითი ყვავილების ერთდროული თანაარსებობა), რაც აქამდე ამ სახეობაში არ იყო აღნიშნული. ეს მოვლენა მკვეთრად გამოვლინდა კულტურაშიც, სახეობის თესლით თვითგანახლების შესაძლებლობის შესწავლისას. აღწერილია ორსქესიანებთან შედარებით ფუნქციურად მდედრობითი ინდივიდების უპირატესობის ზოგიერთი ნიშანი. შედარებულია *Dianthus ketzkhovelii* Makaschv.-ის ორი, გეოგრაფიულად დაშორებული პოპულაციის (აღმოსავლეთი და დასავლეთი საქართველოს) ინდივიდების თავისებურებები, თესლის პროდუქტიულობა ბუნებასა და კულტურის პირობებში გამრავლებისას. ამასთანავე, შემოწმებული იყო თესლის ბანკში მინუს 20 გრადუსზე 8 წლის განმავლობაში შენახული სამიზნე სახეობების თესლის სიცოცხლისუნარიანობა და აღმოცენების უნარი. შექმნილია *ex situ* კოლექციები საქართველოს ეროვნული ბოტანიკური ბაღის მცენარეთა კონსერვაციის განყოფილების საცდელ ნაკვეთზე. მიღებული მონაცემების საფუძველზე მომზადდა როგორც თეორიული, ასევე პრაქტიკული მასალა საჭიროების შემთხვევაში აღნიშნული სახეობების წარმატებული რეინტროდუქციისათვის ველურ ბუნებაში. საერთაშორისო სტანდარტებით შემოწმებული სამიზნე სახეობების თესლის

კოლექციები განთავსებულია გრძელვადიანი შენახვისათვის საქართველოს ეროვნულ თესლის ბანკში და დუბლირებულია კიუს სამეფო ბოტანიკური ბაღის ათასწლეულის თესლის ბანკში.

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