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Botany

Study the Self-Reproduction Capacity of *Aquilegia colchica* Kem.-Nath. Included in the Red List of the Caucasus by Seed and *ex situ* Conservation

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(Presented by Academy Member Gia Nakhutsrishvili)

The present state of the natural populations of *Aquilegia colchica* kem.-Nath. with the conservation status, its reproductive biology and ontogenesis was studied. The ontogenetic spectrum and self-reproduction capacity were defined on several plots of the pinpointed area of the population. The seeds collected from the natural habitat were processed in the laboratory, the quality of their emergence and design viability was determined, *ex situ* conservation work was conducted, seed bank and documented live collection were created for the further *in situ* reintroduction. In 2020 on the right bank of the Jruchula River and the environs of the Katskhi Monastery Complex from the base of the live collection was planted 104 plants were to restore the population. The monitoring is carried out every year to protect reintroduced plants. 21.4% of transplanted plants are viable. © *2022 Bull. Georg. Natl. Acad. Sci.*

Aquilegia colchica, conservation, pollen grain

Aquilegia colchica is included in the Red List of endemic species of the Caucasus as EN endangered species (B1ab (iii, v) + 2 ab (iii, v)) [1].

The species is described by Kemularia-Natadze from Imereti: Lectotypus: „Georgia, Prov. Kutais, distr. Schorapani, fauc. Djrutcha, propped pag. Darkwethi, ad rupes calcareas, 28. VIII.1928, Kemularia – Nathadze" [2].

In the literature (Barnabishvili), the specified area (The river. Kvirila valley, running donkey) of *Aquilegia colchica*'s population is no longer observed.

The species was recorded on the limestone cliffs between the village of Darvkveti and Sachkhere in the valley of the Kvirila River by L. c. Kemeraria-Natadze from Imereti. It grows in the middle mountain belt. The population of *Aquilegia colchica* was no longer observed in the area indicated by Barnabishvili (Kvirila River valley, Viris kencha).

Aquilegia colchica is currently distributed in the Imereti region, within the Chiatura municipality, on the right bank of the River Jruchula, on the rocky slope of the South-West exposition, and immediately on the river bank, on alkaline soil.

Materials and Methods

The experimental site was monitored in two different habitats (the Jruchula River Valley and the collection plot of the Conservation Department of the National Botanical Garden) in 2015-2020. Two experimental sites (25 m^2 and 16.5 m^2) were identified in the natural habitat where the species is spread (2017). In the selected experimental plot we studied the number and age range of individuals, soil pH. In 2019, the ontogenetic spectrum (percentage of pregenerative and generative individuals) were defined in several plots of the pinpointed area of the population, due material for reproduction research was recorded, herbarium samples and seeds were collected (5000). Material for embryological research has been fixed by the Carnoy's fixing [3]. The preparations were studied and photographed by the microscope Carl Zeiss (Germany). The quality and viability of the seeds were determined by the method adopted by international law (ISTA, 2020) [4].

ment of pollen sac walls passes like in dicotyledonous plants while others [6] consider that the process goes on as in main type of plants. The microscopic study showed that the primary parietal layer is divided periclinally into two secondary periclinal layers the cells of which are still divided and as a result endothecium, two intermediate layers, and binucleate secretory type tepetum are formed, which is similar to the development of the main type. The intermediate layer is ephemeral. In the microsporocytes, the meiosis passes normally with formation of 7 closed bivalents 2n = 14. The insignificant derangement in the anaphase and telophase of the second meiotic division does not carry regular character and does not cause a violation of the normal course of meiosis and eventually the viable pollen grains are obtained. Formation of tetrad is performed simultaneously. Arrangement of microspores in tetrad is tetrahedral. The matured pollen grain is binuclear (Fig. 1).

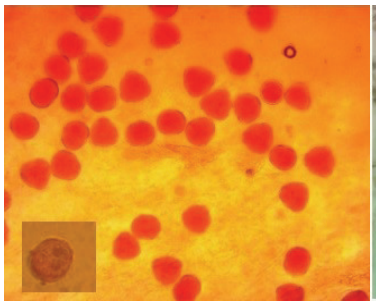

Fig. 1. Binuclear pollen grain.

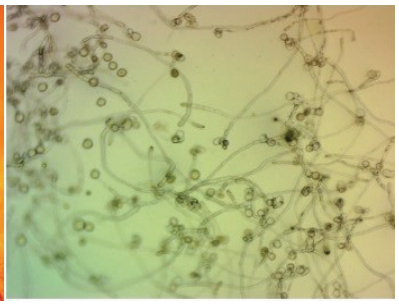

Fig. 2. Pollen Tubes.

Fig. 3. Sperm cells in the pollen tube.

Results and Discussion

The biological characteristics of *Aquilegia colchica* have been studied in the course of the experiment in order to develop the protection and conservation measures. It is known that the study of reproductive biology is a priority for the conservation work as it is impossible to develop a conservation measure without it. During the research process, we have successively studied the female and male phases of blossom. According to some authors [5] develop-

Monocyphonic, spheroidal, tricolpate, the aperture surface is prominent, and the exine is relatively thick. The pollen grains maintain vitality for 7-15 days. The optimum atmosphere for germinating a pollen grain is 7% sucrose, 0.1% boric acid, 1% agar and 21°C (Fig. 2, 3). Defective pollen grains are small in size. The fertility is high (97%), which is a pre-condition for a successful harvest. Pollen grains are physiologically active in the half opened colored budding phase. Female sphere is also developed normally (Figs. 4-6).

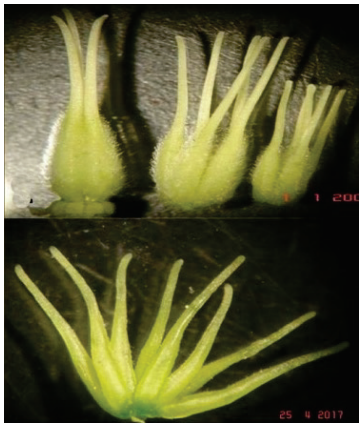

Fig. 4. Pistilate gynaeceum.

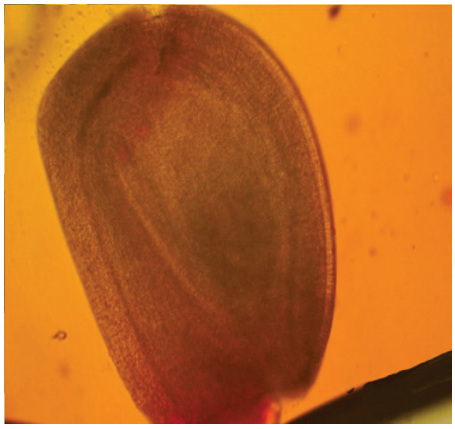

Fig. 5. The ovule.

Fig. 6. Ovules are located in two row.

The flower mainly consists of 4-5 pistils, rarely 6, and sometimes as an exception there may be 8 pistils (Fig. 4). The downy ovary is elongated. The style is hairless; the stigma is covered with thin mucous cells. It is physiologically active in the phase of full blossom. The ovule is anathropic, with two crassinucellate integuments (Figs. 5, 6). The number of ovules inside the ovary is about 18-22. Aquilegia kolchicaplant is an entomophilous plant. Though protandry is characteristic of the plant, autogamy is also expected that is confirmed by development of viable seeds from the isolated flowers. It is known that entomophilous pollination is an ecological problem. Successful pollination requires a combination of fertile pollen grains, physiologically active buds, positive abiotic factors, and sufficient number of insect pollinators. Pollination efficiency was unhomogeneous during our research. The number of seeds is relatively small during frequent rains. *Aquilegia colchica* is characterized by high productivity (average 1260 seeds per individual), which is conditioned by the number of generative shoots, flowers and seeds in the flower, high fertility of pollen grains and rapid development of shoots. The observations upon growth and development of the plant showed that the plants obtained from seeds are characterized by complete ontogenesis that involves four periods: (latent, pregenerative, generative, senile) and seven subperiods (seedling _P, juvenile_ J, immature _im, virgin _V, generative _G). In the latent period the plant is present as a seed. Seeds homogeneous form, with angular, glossy black surface. The germ is small in size.

The seeds are perfect, characterized by a high degree of emergence and viability, making seed propagation possible.

Table 1. Influence of seed storage terms on germination time and percentage

Immediately after collecting seeds	After 3 months	After 6 months	After 1 year	After 2 years
92	89%	85%	80%	76%

As shown in Table 1, the duration of seed storage causes a decrease in emergence energy. The percentage of seed emergence is gradually decreased with the growth of storage period. The seeds retain ability to emerge for 3 years. Seeds are orthodox. The pre-generative period begins with the development of sprouts. The indicators of the sub-period of a seedling (p) are formation of cotyledons and the first leaf. The root of the germ emerges from the seed and then a short hypocotyl is formed from the seed. At first the hypocotyl is arc-shaped, and then it straightens.

The stem of cotyledons is covered with loose long white down. The cotyledons are green, oval shaped, entire on margins, hairless with slightly

expressed veins. In two leaf phases the volume of cotyledons increases, in 4-5 th leaf phases the cotyledons turn yellow and fade. In the 6th leaf phase the cotyledons fall down. The first leaf is developed 7-9 days after a seed is formed. The leaf is three lobed. The tomentous lobes are wedge-shaped at the bottom. The main root is 6 cm long and 20-23 lateral roots are equally arranged on it. The indicators of the juvenile (j) sub-period are: development of 3-5 leaves, increase of the main root diameter and formation of the third-level fibrous roots. From the juvenile sub-period the plant passes on immature period. Within this period the number of leaves increases (7-9). In the virgin sub-period the number of sprouts increases (10-14). The vegetation continues until the end of November. New vegetation begins at the end of March or early in April. The first generative sprout is developed 2 years later, massively 3 years later. The amount of sprouts depends on the age of the plant. At first the top bud of the main generative sprout opens in the basipetal direction and the flowers of the secondary generative sprout open in acropetal direction. The flowers from the third-degree sprouts are often undeveloped. The numbers of sprouts vary from 1 to 14. The number of flowers on one sprout is 3-10. A separate plant blooms at the end of April, intensively at the beginning of May. Aquilegia kolchica is characterized by morphologically expressed 5 phases of blossom (green bud, semi-colored bud, colored bud, semi-opened colored bud and open flower phase). The green bud phase lasts for 11-18 days. In this phase the bud is small (0.4-0.8 cm). The two-day bud is undifferentiated. Its thin-walled cells are small. The wall of the 7-day pollen bag is already formed. The inner stamens turn into white membranous stamonodia, its wavy edges are rugose and hairless.

The size of the staminodia and the degree of the rugose edges are the specific feature. Gynandrophore (receptacle) is calyx. The excrescence between the stamen and the pistil is noticeable. The phase of colored bud begins on the 5^{th}-25^{th} day from the bud formation and lasts for 4-7 days. The bud is 0.8-1.3 cm. Some coloring is characteristic for the outer surface of the nectarines. The pollen sac is green. The stamens mature unevenly. The pollen grain is mononuclear. The stamens are longer than the curved nectar. The stamens are stuck out. The ovary is elongated downy; the thyroid (style) is hairless. The round stigma is covered with thin-walled glandular cells. The stigma is dry at this stage. The nectarines is funnel-shaped. The inner surface is smooth, the outer surface and the spur are covered with dense, long hair. The thickened, twisted spur covered with dense hair is a specific feature. The phase of colored bud begins on the 17^{th}-26^{th} day. The length of the bud is 1.4-2.4 cm. At this stage the stamen begins to turn yellow. The stamen is as long as the pistil seated on the receptacle, although sometimes stamina is fixed to the inner surface of the funnel-shaped nectar.

The semi-colored bud phase begins on the 19-30^{th} day and lasts for 7-8 days. The stamens are physiologically active at this stage. The special position of a stamen is changed, leaning to the sides, the angle of inclination increases and the pollen grains start falling. At this stage, the pistil is taller than the stamens.

In the open flower phase, the flowers are bicolor. The sepals are blue, ovary. The sepal margins are covered with short white hair. The nectarines of the plant are funnel-shaped white curving. The nectarines are of the same size. At this stage the stamen withers, thin-membrane cells that are arranged in several rows inside the epidermis of the nectarine intensively separate the nectar. The pistil grows larger; the glandular cells of stigma become uneven and excrete liquid. The stigma is physiologically active that is ascertained by the peroxidase test. The flower phase includes pollination, fertilization and seed formation.

The biometric study of the flower parts showed that the shape and size of the sepals are changeable. The size of the petals is equal. The number of stamens is variable and ranges within 45-50

Table 2. Percentage of the seed germination and viability at different sowing time and conditions

Year	Place of Seed collection	Date and number of seed collection	Date and number of seed cutting	Date and number of sowing seeds	Conditions light and darkness (21/14)/ days-nights (12/12)	Duration of experiment (week) Start and end (day)	Result of experiment				G%	V%
							g (germi-nated)	F (fertile ungermi-nated)	M (moul-dy)	E (empty)		
2015	Rocky slope of the southwest exposition on the right bank of the river Jruchula	01.07.15 1500	05.07.15 20	05.07.15 20	Incubator	3 (7-21)	27		3		90	100
					Refrigerator +4C	4 (18-25)	26		3	1	89	100
					Filter paper	3 (7-21)	25	1	4		83	100
2016		18.07.16 1000	20.07.21 20	25.10.16 150	Incubator	4 (15-25)	44	2	4		88	100
					Refrigerator +4C	5 (21-30)	40	3	7		80	100
					Filter paper	4 (18-23)	39	4	7		79	100
2017	Rocky slope	16.08.17 900	18.08.17 20	15.03.18 60	Incubator	3 (14-21)	13	3			85	100
					Refrigerator +4C	4 (20-25)	16	2	2		80	100
					Filter paper	3 (17-19)	16	4			80	100
	Immediate-ly on the river bank	16.08.17 200	18.08.17 21	15.03.18 20	Incubator	3 (14-19)	19	1			95	100
2018	Rocky slope	18.06.18 500	16.06.18 20	20.07.18 20	Incubator	3 (18-20)	18	2			90	100
	Immediate-ly on the river bank	18.06.18 600	16.06.18 21	20.07.18 20	Incubator	2 (10-14)	19			1	95	100
2019	Rocky slope	08.07.19 1000	10.07.19 20	10.07.19 20	Incubator	2 (7-13)	18	2			90	100

depending on the number of staminodia. According to the flower development phases the length of the filaments is changeable both to each other and the other parts of the flower. The number of the pistils in the flower and the color of the flower are also variable. The most frequently variable morphological sign is the number of generative sprouts and flowers. The least variable sign is the degree of hair covering of different parts of the flower.

In laboratory conditions, with the purpose to determine the capacity of seed germination and viability, the optimal conditions and terms sowing was performed at different times (immediately after collecting seeds, after three, six months, after one or two years) and under different conditions: 1% agar under controlled temperature (21/14 light-dark) and lighting (12/12 day-night) conditions in the incubator, in the refrigeration at + 4^0C, under the non-controlled temperature and lighting conditions, on the filter paper. Germination is optimal when seeds are sown in the incubator immediately after they are collected. Seasonal sowing was done in pots to create live collections. During the summer sowing we received a higher percentage of germination in a shorter time but with a lower germination rate. Better results were observed as a result of autumn sowing (the number of germination rate is higher) (Table 2).

The number of individuals and the ontogenetic spectrum of individuals were determined on three

plots of the fixed area of population (15 m^2, 55.5 m^2, 300 m^2). From the 23 plants on the first plot (15 m^2), 18 are progenerative, 5 generative; From the 27 plants on the 2nd plot (55.5 m^2), 19 are progenerative, 6 are generative; 215 progenerative, 65 generative from 280 plants on the third plot (300 m^2). The population is viable, with no abundance of senile individuals. However, in 2015-2019 the total number of the individuals in the area where the species is distributed (2080 m^2) was reduced by 33.5% due to anthropogenic stress. The experimental plot (16.5 m^2) immediately on the bank of the river Jruchula was destroyed due to the artificial alteration of the riverbed. Active technical work is being carried out in the area, and the construction material is taken out from the river and piled at the slope of the rock. This section of the valley is important from the point of biodiversity conservation (along with *Aquilegia colchica*, several endemic species of Georgia like *Campanula kemulariae* Fomin are spread. The ongoing technical work here causes disturbance and destruction of the living conditions of these plants.

Conclusions

The research revealed:

- Normal course of formation and function of the generative sphere.
  The plant is characterized by:
- Complete ontogenesis. the number of generative shoots and flowers increases as the plant grows older;
- 5 phases of morphologically different flowering. The stamen is physiologically the most active in the phase of semi-opened colored bud and the pistil in the flower phase;
- The quality of seed germination and viability is high. The seeds kept in 30% silica gel preserve germination ability for three years;
- Characterized by abundant seed production and rapid development of germination;
- High adaptability;
- Ex-situ conservation measures are optimal for placement in the seed bank as soon as the seed is collected, as the duration of seed storage causes a decrease in emergent energy.

We have estimated the reason for reduction of the population in the Jruchula gorge that is caused due to anthropogenic factors. A living collection was created with the purpose to restore the population in nature. From the live collection database 104 plants were planted to restore the population (In an area of 15 m^2 on the right slope of the river Jruchula planted 67 individuals and the Katskhi monastery complex 37 individuals). The monitoring carried out on 20 August of 2021. 21.4% of transplanted plants are viable. The seeds were placed in the Seed Bank of the National Botanical Garden of Georgia. The duplicates were sent to the Millennium Seed Bank of the Royal Botanical Garden of Kew.

ბოტანიკა

კავკასიის წითელ ნუსხაში შეტანილი სახეობის *Aquilegia colchica* Kem.-Nath. თესლით თვითგანახლების შესაძლებლობის შესწავლა და *ex situ* კონსერვაცია

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**ილიას სახელმწიფო უნივერსიტეტი, თბილისი, საქართველო

(წარმოდგენილია აკადემიის წევრის გ. ნახუცრიშვილის მიერ)

შესწავლილია საკონსერვაციო სტატუსის მქონე საქართველოს ენდემური სახეობის კოლხური წყალიკრეფიას *Aquilegia colchica* kem.-Nath. ბუნებრივი პოპულაციის თანამედროვე მდგომარეობა, რეპროდუქციული ბიოლოგია, ონტოგენეზი. პოპულაციის ფიქსირებული ფართობის რამდენიმე ნაკვეთზე განისაზღვრა ონტოგენეტიკური სპექტრი, თესლით თვითგანახლების შესაძლებლობა. ბუნებრივი გავრცელების ადგილიდან შეგროვილი თესლი დამუშავდა ლაბორატორიულად, განისაზღვრა მისი აღმოცენებისა და სიცოცხლისუნარიანობის ხარისხი, ჩატარდა *ex situ* საკონსერვაციო სამუშაოები, შექმნილია თესლის ბანკი და დოკუმენტირებული ცოცხალი კოლექცია *in situ* რეინტროდუქციისათვის. პოპულაციის აღდგენის მიზნით 2020 წელს, ცოცხალი კოლექციის ბაზიდან 104 მცენარე გადაირგო მდინარე ჯრუჭულას მარჯვენა ნაპირზე და კაცხის სამონასტრო კომპლექსის მიდამოებში. რეინტროდუცირებული მცენარეების დაცვის მიზნით მიმდინარეობს ყოველწლიური მონიტორინგი. გადარგული მცენარეების 21,4% სიცოცხლისუნარიანია.

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