

Botany

Biological Peculiarities of F1 Generation of Hybrids of Two Georgian Endemic Species *Aquilegia colchica* Kem.-Nath. and *Aquilegia gegica* Jabr.-Kolak.

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Morphological features of the F1 generation obtained by crossing two Georgian Endemic species *Aquilegia colchica* Kem.-Nath. and *Aquilegia gegica* Jabr.-Kolak., the process of microsporogenesis and gametogenesis, viability and germination capacity of hybrid seeds have been studied. Research has shown that the parental forms are genetically closely related species that is proved by: 1) The same number of chromosomes ($2n=14$) and high degree of homology; 2) The normal course of the process of microsporogenesis, meiosis; In both native and hybrid forms, the process of microsporogenesis and gametogenesis passes within the norm. 3) High fertility of hybrid seeds and high percentage of germination. A hybrid plant combines the traits of both parents. Besides similarities, a number of different features were revealed (the stamens are strongly stuck out from the perianth; heterophily and a short pre-regenerative period). The studies estimated that in the process of ontogeny, the hybrid plants have rapid pre-regenerative phase and are capable to produce fertile offspring. © 2021 Bull. Georg. Natl. Acad. Sci.

Aquilegia colchica Kem.-Nath., hybrid, morphological signs, spur, staminodia

Aquilegia L. is a relatively young genus, formed in the Pliocene. It is in the process of active development, which is indicated by the degree of differentiation of the genus into systematic groups, the weak morphological isolation of the younger species, and the almost continuous distribution range of the genus [1].

The representatives of the genus are distinguished by high ornamental properties and content of alkaloids. Some of them contain flavonoids, saponins, high quality oils [2].

Two Georgian endemic species of columbine (*Aquilegia colchica* Kem.-Nath. and *Aquilegia gegica* Jabr.-Kolak.) are plants with high ornamental potential and medicinal properties. The studies of the hybrids obtained by their crossbreeding may reveal hitherto unknown connections between these species, some dominant and recessive signs, valuable ornamental and medicinal forms, which was the purpose of our study.

Materials and Methods

Aquilegia colchica Kem.-Nath. the species Endemic to Georgia, currently evaluated as an endangered taxon (EN), was described by L. Kemularia-Natadze in the gorge of the river Kvirila in 1934 [3, 4]. The plant is glandular-sticky, characterized by silvery *pubescence*. The lower leaves are doubly or triply lobed on a long stalk. The upper leaves are trifoliate, triply lobed or entire. The leaf lobes are wedge-shaped at the base. The flowers are two-coloured. The perianth petals are either blue or light blue, ovate. The nectary is funnel-shaped with white curve and blue spur that is strongly twisted at the end and densely pubescent. The stamens are longer than the curve of the nectary. The seeds are black, glossy [3, 4].

Aquilegia gegica Jabr.-Kolak. is described from Abkhazia (valley of the Gega River) in 1953 [5, 6]. Due to its pubescence the plant looks greyish. The stem has glandular *pubescence* at the top. The lower leaves are double trifoliate with long sparsely pubescent stalks. The upper leaves are two-three lobed or entire. The petals are elongated and light blue. The nectary is funnel-shaped, blue in the upper part is and whitish in the lower part. The spur is thin, curved like a hook. The seeds have a cellular surface.

The material of *Aquilegia colchica* Kem.-Nath for the research work was collected during the period of 2016-2020 in the gorge of the River Jruchula, of Zestaponi Municipality of Imereti Region. Pollen of *Aquilegia gegica* Jabr.-Kolak (pollen) for artificial pollination was obtained from the plants, cultivated on the Conservation Plot of the Department of Plant Conservation of the National Botanical Garden of Georgia from the material collected in 2016 on Laenjeri summer pasture, on the Southern slope of Egrisi Ridge, Chkhorotsku Municipality of Samegrelo-Zemo Svaneti Region. Flower castration, isolation and artificial pollination of the plants obtained from the seed-grown plants, cultivated on the collection plot of the Conservation Department of the National

Botanical Garden of Georgia were carried out. The degree of fertility and germination of the obtained hybrid seeds was determined in accordance with the international standards (ISTA 2020) [7]. The material for the embryological examination was fixed in Carnoy's solution (3/1). Fixation of the material and preparation of the temporary specimen were carried out by the accepted cytological methods [8]. The specimens were studied and photographed under a light microscope (Carl Zeiss, Germany).

Results and Discussion

The F1 generation of hybrid plants, obtained by crossing ♀*Aquilegia colchica* and ♂*Aquilegia gegica* is 30 cm tall, with sparse pubescence and slightly sticky glands. The lower leaves with two or three leaflets, rounded at the base. The leaves of the generative shoot differ from each other in shape, size and length of the petiole. Some leaves are wedge-shaped, rounded at the base, while others are wedge-shaped narrowed. The flowers are larger than in the parental forms (10-12 cm), the petals of perianth are uniform, long, lanceolate, light blue, almost whitish. The nectaries are funnel-shaped, spur narrow, slightly curved or straight, densely pubescent. The number of stamens is 45-50, depending on the number of *staminodia*. On each staminodium there are five yellow stamens that differ according to the length of the filaments. Staminodiams are narrow and slightly wavy. The stamens are longer than the curve of the nectaries (stuck out from the perianth more than in the parental forms). The carpels are not fused, each of them produces individual pistil, which number varies from four to seven. Ovary is green, heavily pubescent. The style is naked, the stigma is covered with thin-membraneous mucous cells.

Since the parental forms differ from each other in the shape, size of the flower, the petals of the perianth, the shape and size of the spur, and the degree of staminodium folds, during the

experiment special attention was paid to the study of these signs.

In the plants of F1 generation were manifested traits characteristic to parental as well as intermediate signs. According to the degree of pubescence the individuals of F1 generation are intermediate forms. Their leaves are covered with white soft hair. According to the spur shape and the structure of the staminodia they are similar to *Aquilegia gegica* (the spur is straight, narrow, long, with a slightly curved bottom. The staminodia are narrow and slightly wavy). According to the shape of lower leaves, the hybrid resembles *Aquilegia colchica* (Leaf lobes are wedge-shaped, rounded at the base). The structure of stamens and stigma is similar in both parental and hybrid forms. Comparison of parental forms and the F1 generation is presented in the Table.

Microscopic examination revealed that development of pollen sac in both parental and hybrid plants passes according to the main type and the wall consists of endothecium, two intermediate layers and a binucleate secretory tapetum. Meiosis is accompanied by slight deviations (4-5%). The following anomalies were revealed: disturbance of bivalents' orientation, chromosome lag in the anaphase of the first division of meiosis. Asynchronous division in the metaphase-anaphase of the second division of meiosis, the presence of chromatine globules in the area of the spindle. Formation of triads and pentads rarely occurs. These anomalies do not significantly affect the course of the meiosis process and eventually viable pollen grains are obtained.

As it is known from the literature [1, 9], the species of genus *Aquilegia* L. differ not in the

Table. Parental forms and the F1 generation

#	Morphological features	♀ <i>Aquilegia colchica</i>	♂ <i>Aquilegia gegica</i>	F1
1	Stem	Glandular-sticky	Upper part of the stem - slightly glandular	Moderately glandular-sticky
2	Pubescence of leaves	Densely pubescent, covered with white soft hair	Slightly pubescent	Moderately pubescent
3	Leaf lamina at the base	Rounded wedge-shaped	Narrow wedge-shaped	Rounded wedge-shaped
4	Flower size/color	Small, bluish-sky blue	Medium, blue	Large, light blue
5	Perianth petals	Long ovate	Oblong lanceolate	Long lanceolate
6	Nectary	Funnel-shaped, thickly pubescent on the outside	Funnel-shaped, slightly pubescent	Funnel-shaped, moderately pubescent
7	Spur	Blue, thick, short, strongly twisted at the end and densely pubescent	Bluish –light blue, thin, long hooked, slightly hooked pubescent	Light blue, straight, long, moderately slightly pubescent
8	Stamens	Longer than curved yellow nectary	Long	Long
9	Ovary	Pubescent	Pubescent	Pubescent
10	Style	Naked	Naked	Naked
11	Seed	Black, glossy	Black, with cellular surface	Black, with slightly cellular surface
12	Stamonodia	Wide, with wavy edges	Narrow, slightly wavy edges	Narrow, with less wrinkled edges

number of chromosomes, but according to their morphological signs. Their chromosomes are small, metacentric, rarely subcentric. In various species, the fourth pair of chromosomes has satellites of different shapes and sizes. The chromosomes of the forms, which we have already studied are similar, having a round shape and the same size. In the prophase of the first meiotic division six closed and one open bivalent are formed, indicating the homology of chromosomes. Formation of tetrads are of *simultaneous type*. Tetrads are tetrahedral by shape. The completed pollen grain is binucleate, monosiphonous, though as an exception some pollen grains develop two pollen tubes. The pollen grains are spherical, tricolpate. The surface of the grooves is tubercular. The tubercles are round. The generative cell divides into a pollen tube. Spermias are round by shape and uneven in size. The ovule is antropous, crassinucellate, with two *integuments*. There are 7-10 ovules in the ovary. The seeds are small (1.7-2.8mm long and 0.7-1.4mm wide). The seed has is unequal rib all along the full length. The rib is wider in the comparatively narrow part of the seed where a small embryo is placed. The surface of the seed is slightly cellular.

The seeds were sown in laboratory conditions, on a Petri dish, with the one percent plain agar medium, under controlled temperature (21/14) and illumination (12/12) in the incubator. They germinated in 14-21 days after sowing. First root is developed from the seed, then a thin, short (0.5-0.6 cm), arcuately bent hypocotyl is produced. The oval-shaped, naked seed-lobes of green colour turn yellow in the 4-5 leaf phase, wither and fall off in

the six-leaf phase. The first leaf develops 7-9 days after the germination. The leaves are three lobed, wedge-shape rounded at the base. The stalk is covered with sparse long thin hairs.

The study has demonstrated that the hybrid plant is characterized by a rapid pre-regenerative phase (14 months) of ontogeny. Unlike the parents, 5 generative shoots were developed in the first year. Flowering begins 13-14 months after the plant is sown, while in the native forms a generative shoot is developed after two years. Abundant flowering takes place after three years.

Conclusion

- 1) In both native and hybrid forms, the process of microsporogenesis and gametogenesis passes within the norm.
- 2) F1 hybrid seeds are characterized by a high viability (100%) and good germination capacity (86%).
- 3) A hybrid plant combines the traits of both parents. Besides similarities, a number of different features were revealed (the stamens are strongly stuck out from the perianth; heterophily and a short pre-regenerative period).

The study has shown that the parental forms are genetically closely related species, which is confirmed by: the same number of chromosomes ($2n=14$) and a high degree of their homology, high fertility of hybrid seeds, high germination capacity and ability to produce fertile offspring.

F2 generation is being studied and further observations on the detection of hereditary traits are in progress.

ბოტანიკა

„საქართველოს ენდემური სახეობების *Aquilegia colchica* Kem.-Nath. და *Aquilegia gegica* Jabr.-Kolak. შეჯვარებით მიღებული ჰიბრიდული მცენარეების ბიოლოგიური თავისებურებობის შესწავლა“

ლ. გაბედავა

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(წარმოდგენილია აკადემიის წევრის გ. ნახუცრიშვილის მიერ)

შესწავლილია *Aquilegia colchica* Kem.-Nath. და *Aquilegia gegica* Jabr.-Kolak-ის შეჯვარებით მიღებული F1 თაობის მცენარეების მორფოლოგიური თავისებურებები; მიკროსპოროგენეზის და გამეტოგენეზის პროცესები; ჰიბრიდული თესლის სიცოცხლისუნარიანობის და აღმოცენების ხარისხი. მიღებული შედეგები საფუძველს გვაძლევს დავასკვნათ, რომ მშობლიური ფორმები გენეტიკურად ახლომდგომი მახლობელი სახეობებია, რასაც ადასტურებს: 1. ქრომოსომთა ერთნაირი რიცხვი ($2n=14$) და ჰომოლოგიურობის მაღალი ხარისხი. 2. მიკროსპოროგენეზის, მეიოზის პროცესის ნორმალური მიმდინარეობა. 3. ჰიბრიდული თესლის მაღალი ფერტილობა და აღმოცენების მაღალი პროცენტი. კვლევის პროცესში დადგინდა ჰიბრიდულ მცენარეებში ონტოგენეზის პროცესში პრეგენერაციული ფაზის სწრაფი ტემპი და ნაყოფიერი შთამომავლობის მოცემის უნარი.

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