RESEARCH ARTICLE

Study of *Aconitum barbatum* in the southern Tomsk region in wild and horticultural conditions

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Abstract

Background: The recent finding that the diterpene alkaloid songorine, isolated from *Aconitum barbatum* Pers., has anxiolytic properties highlights the need for reliable sources of this plant.**Objective:** This study seeks to produce a highly productive introductory population of *A. barbatum* Pers. **Materials and Methods:** Monitoring of this species in natural coenopopulations allowed selection of two *A. barbatum* coenopopulations in southern Tomsk, Russia, for initial experiments. Individual *A. barbatum* plants having favorable morphobiological factors were identified to form the basis for an introductive population production. **Results:** The seasonal development rhythm of *A. barbatum* is long vegetative, with a green cycle from spring-to-autumn. Under horticultural conditions, *A. barbatum* regularly blooms and bears fruit. The number of chromosomes in plants is retained at the diploid number ($2n = 16$). Comparative estimation of *A. barbatum* pollen grain morphology showed slight differences in grain size among the studied coenopopulations. **Conclusion:** The high fertility of pollen grains and low level of meiotic anomalies suggested that plants from these coenopopulations could be used successfully as an introductory population. Based on the predictable developmental rhythm of *A. barbatum* in southern Tomsk, this region could be a foundation to create a raw material base and preserve plants growing in the subzone of the southern taiga of Western Siberia.

Key words: *Aconitum Barbatum*, coenopopulation, culture, cytogenetic characteristics, Morphobiological, Tomsk Region

INTRODUCTION

In Russia, over 6–7% of the population is estimated to suffer from anxiety or depression. Of these, more than 30–40% of patients are diagnosed with pathological anxiety symptoms. At least 25% of all individuals are predicted to experience anxiety at some point in their lives. Excessive anxiety can increase the likelihood of myocardial infarction by 2.3-fold and sudden death by 4.5-fold. Although many drugs (anxiolytics) have pronounced anti-anxiety effects that reduce anxiety, restlessness, and increase resistance to stressors, several of these drugs have negative side effects, including daytime drowsiness, cognitive dysfunction, distraction, and loss of memory and muscle tone. These drugs also have the risk of addiction.[1] As such, many individuals will be unable to use these drugs to treat anxiety. Therefore, new drugs that have high anxiolytic activity but lack serious side effects are needed. Many alternative drugs involve compounds isolated from medicinal plants. Research conducted at the E.D. Goldberg Institute of Pharmacology and Regenerative Medicine (Tomsk) identified various physiological activities of diterpene alkaloids, including songorine, which is extracted from *Aconitum barbatum* Pers. In animal studies, 0.25 mg/kg songorine had anxiolytic activity. Moreover, songorine was more effective than phenazepam and did not induce sedation.[1] Given the promise of diterpene alkaloids as novel anxiolytics, raw material sources for *A. barbatum* that can be used to produce these compounds are needed.

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The possibilities for culturing *A. barbatum* as valuable medicinal and decorative plant have been studied earlier under the Eastern Siberia[2,3] and Central Asia[4] conditions.

In the Western Siberia (Tomsk Region), the studies of *A. barbatum* as valuable medicinal plant have been carried out for the 1st time. The aim of these studies was to study the natural populations of *A. barbatum* in the South of the Tomsk Region and possibilities of culturing it in connection with necessity of providing an agro-population for pharmacological industry of the region.

MATERIALS AND METHODS

The plant *A. barbatum* Pers. (Ranunculaceae family) is a Central Asian meadow-steppe species[5] found in western, central, and eastern Siberia, as well as the Far East. *A. barbatum* can also be found in northern Mongolia and northern China.[6] The range of the species covers steppe regions and adjacent parts of the forest zone. In mountainous regions, *A. barbatum* can grow up to the upper border of the forest belt. *A. barbatum* grows on the steppe - rarely upland – of meadows, rocky or gravelly slopes, forest edges, in scrubs, and sometimes in rare deciduous and mixed forests.[7] The common habitat of the species is near southern slopes, upland meadows, and sparse tall grass birch forests. *A. barbatum* does not have a dominant position in plant communities, occurs sporadically, and does not form undergrowth.

In the Tomsk region of Russia, *A. barbatum* is relatively rare[8] but can be found in the southern and southeastern areas of the region in Tomsk, Shegarsky, and Zyryansky [Figure 1].[9] Based on long-term observations, the species was included in the seed bank of plants recommended for protection in the Tomsk region.[10] To maintain studied species in natural habitats, since 1984, stocks of *A. barbatum* have been grown in the Siberian botanical garden as part of a collection of rare plants.[11]

Figure 1: *Aconitum barbatum* in wild population near Kolarovo village (Tomsk region)

This study focused on *A. barbatum* growing either in culture or in natural vegetation areas in the southern Tomsk region. To create a raw material source for *A. barbatum*, we examined the morphobiological features of the species growing in herb-rich areas of gramineous upland meadows in southern Tomsk. The study in nature was carried out for 2 years (2015, 2016) in two coenopopulations. Coenopopulation No 1 was located near Kolarovo village and coenopopulation No 2 was located near Yarskoe village. The herbarium samples from the studied populations were kept in the P.N. Krylov Herbarium (TK) (Tomsk State University, Tomsk): Voucher numbers of specimens were TK-004015 for Kolarovo village and TK-004014 for Yarskoe village). We also examined individual plants from the Altai Republic (Coenopopulation 3; Kosh-Agach District, surrounding the village of Kurai) that would enhance the genetic potential of cultured plants. The study of seasonal rhythm of development was carried out on specimen growing in the collection of Siberian Botanical Garden of Tomsk State University.

Population-ontogenetic Method

This study used a population-ontogenetic approach.[12] Methods described by Bejdeman, Golubev, and Zajcev were used to study seasonal development rhythms of individual plants.[13-15] In the studied coenopopulations, 25 plants had been sampled, in which shoot height, inflorescence length, number of rosellate, and cauline leaves, length and width of leaves were measured, then, the aerial part of the plant was dried under room conditions and weighed to determine its weight. According to the obtained data, the following main parameters had been calculated: Average feature value (M) and standard deviation (SD). The significance of the feature differences was determined by Student's t-test. Seed production was studied using methods of Vajnagij and Levina.[16,17] Morphobiological features of seed germination were determined using the method of Majsuradze *et al*.[18] Cytological and cytogenetic research was conducted as described by Puhalskij *et al*.[19] The diameter of pollen grains, plant sterility and fertility, number of chromosomes, and meiotic stage characterization was determined using an Axiostar Plus microscope (Carl Zeiss) with magnification of ×10 and ×100 and AxioVision LE software.

RESULTS

In the coenopopulation 1 (Kolarovo village), the plant biomass was higher – dry weight of the aerial part of the specimen was 25–30% higher compared to the specimen from coenopopulation 2 (Yarskoe village) [Table 1]. The biomass accumulation depends on the amount of atmospheric precipitation in spring and summer periods. Thus, in the spring and summer period (March to August) of 2016, 360.2 mm precipitated in the territory of studied coenopopulations which was 20% higher compared to the analogous period of 2015 when 291.1 mm precipitated.[20] Increase in the precipitation

Table 1: Morphometric characteristics of *Aconitum barbatum* grown in southern Tomsk

Features	Coenopopulation No. 1 (Kolarovo village)		Coenopopulation No. 2 (Yarskoe village)	
	2015 year	2016 year	2015 year	2016 year
Shoot height, cm	90.5±16.0[a*]	132.4±12.9[b]	99.9±13.5[a]	136.2±18.7[b]
Inflorescence length, cm	12.9±3.5[a]	29.8±6.4[b]	21.4±3.6[c]	27.4±6.3[b]
Number of rosellate leaves, pcs.	3.6±0.9[a]	4.1±1.1[b]	3.1±0.7[c]	3.5±0.7[a]
Number of cauline leaves, pcs.	3.3±1.1[a]	3.2±0.6[a]	3.1±0.4[ab]	2.8±0.7[b]
Rosellate leaves length, cm	11.6±2.2[ab]	12.1±1.9[a]	10.2±2.1[b]	11.3±2.3[ab]
Rosellate leaves width, cm	22.3±4.0[ab]	24.2±4.2[a]	20.4±4.3[b]	21.7±3.5[ab]
Cauline leaves length, cm	9.0±2.8[a]	8.5±2.1[a]	8.6±2.1[a]	8.2±1.6[a]
Cauline leaves width, cm	16.3±4.4[a]	16.7±2.2[a]	17.0±2.9[a]	16.4±2.4[a]
Dry weight of aerial part of the plants, g	22.8±5.2[a]	28.2±6.1[b]	18.2±4.2[c]	21.6±4.1[ac]

*Data having different letter indices significant differ at P<0.05

amount leads to increase in some of the morphological features of the specimen affecting the biomass accumulation: Shoot height, inflorescence length, and number of rosette leaves. Some of morphological features of the specimen (number of cauline leaves and leave sizes) do not change significantly at increase in precipitation and do not significantly differ between coenopopulations. However, both coenopopulations had morphometric parameters that were suitable for culture.

A. barbatum is a long vegetative, spring-to-autumn green plant that has active vegetation of between 135 and 158 days under typical conditions. Spring regrowth in some years begins at the end of April but can begin as late as the first half of May. The period from the beginning of the vegetation until the bloom in mid-summer to early autumn averages from 64 to 80 days. The duration of blooming can range from 40 to 58 days.

In addition to blooming, *A. barbatum* also bears fruit. The period from fruit inception to ripening ranges from 50 to 70 days and this species falls into the category of late-ripening plants. The end of the vegetation occurs between the end of September and the first 10 days of October. By this time, the seeds fall, and the generative shoot and radical rosette of the leaves shrink. The triangular seeds are relatively small (2.9 [2.7–3.0] mm long, 1.9 [1.8–2.0] mm wide), have transverse brown scales, and weigh approximately 2 mg. *A. barbatum* seeds have high (93%) laboratory germinating capacity after stratification.

To identify the ecological valence of the species, its genetic potential must first be estimated by cytological and cytogenetic analysis. The chromosomal number of *A. barbatum* is maintained at a constant diploid number of $2n = 16$.[21] Estimates of chromosomal numbers in all three coenopopulations of this study confirmed the stability of the diploid number of chromosomes [Figure 2]. The degree of irregularities in gamete formation can be estimated by examining meiosis and evaluating the fertility of pollen grains and seed production prospects. Here, microspore gametogenesis proceeded appropriately in the evaluated samples as evidenced by the low level of meiosis anomalies. Those anomalies that were

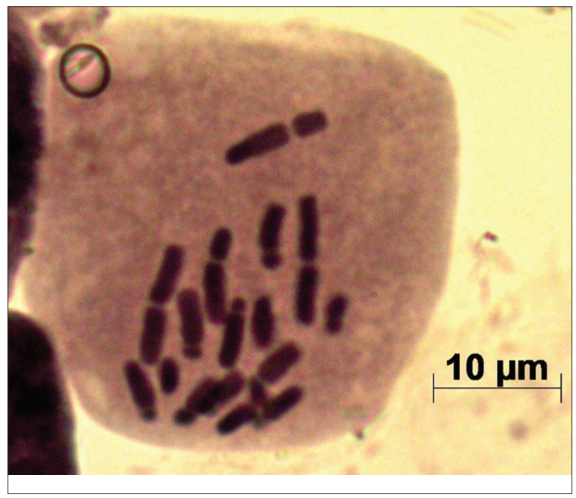

Figure 2: *Aconitum barbatum* metaphase plate ($2n = 16$)

observed included chromosome aberrations and disrupted cell division and cytokinesis [Table 2].

Although evaluation of cytogenetic characteristics of medicinal plants can provide a basis for the successful introduction of a species to horticultural conditions, for many plants, there is a lack of information concerning chromosome morphology, microspore gametogenesis, and cytological estimation of pollen grain quality. Notably, a comparative estimation of pollen grain morphology allows the characterization of slight differences in pollen grain size in various coenopopulations.

A high pollen grain fertility and low level of meiotic anomalies suggest that plants from these coenopopulations could be used successfully to produce an introductive population of the studied species.

CONCLUSION

A. barbatum has a steady developmental rhythm and produces full and sound seeds in the south of the Tomsk region. The

Table 2: Cytogenetic characterization of ***A. barbatum***

Features	Coenopopulation No 1 (Kolarovo village)	Coenopopulation No 2 (Yarskoe village)	Coenopopulation No 3 (Kurai village)
Pollen grain diameter, μm	19.90±0.24	20.30±1.82	19.86±0.33
Pollen grain fertility, %	98.30	92.06	92.95
Frequency of meiosis anomalies, %	0.6	-	1.8
Number of chromosomes 2n	16	16	16

A. barbatum: Aconitum barbatum

morphological heterogeneity and cytogenetic species stability in the studied areas also confirm the ecologic flexibility of this plant. This study provides the foundation for developing a material base for *A. barbatum* in the subzone of southern taiga of western Siberia over the long term. The studies will help to save the species in its natural vegetation area while still providing sufficient quantity and quality of raw material to produce alternative compounds for anxiolytics.

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