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RESEARCH ARTICLE

Biological features of high altitude rare medicinal plant species *Hedysarum theinum* Krasnob. in Western Siberia cultivation

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Abstract

The paper summarizes the results of a long-term introduction study of a rare alpine plant *Hedysarum theinum* Krasnob., which is widely used in medicine. We found that the species demonstrates sufficiently high seed productivity with adequate agrotechnical care under introduction conditions. Nevertheless, there were cases of a single lack of fruiting, which cannot be considered a reaction to different ecological conditions since these phenomena can also be observed in natural habitats; most likely, this is a feature of the reproductive biology of the species. The phenological characteristics, seed productivity, and seed germination of the species are given, and the range of variation in signs of the vegetative and generative spheres is established. The introduction assessment indicates high plasticity and a high degree of the species adaptation.

Keywords

Hedysarum theinum, medicinal plants, Western Siberia, reproductive biology, cultivated medicinal plants

Introduction

Hedysarum theinum Krasnob. is a perennial herb of the family Fabaceae. It is a valuable medicinal plant, a rare alpine species, an Altai-Dzhungarian endemic, low-abundance, and ubiquitously endangered species (Krasnoborov et al. 1985; Kotukhov 2006).

The unique composition of *Hedysarum theinum* Krasnob. is determined by its medicinal properties: anti-inflammatory, bactericidal, antispasmodic, immunoprotective, and antioxidant (Dong et al. 2013). The following compounds were found in the aerial part of *Hedysarum theinum* Krasnob: monosaccharides, disaccharides, vitamin C, tannins, carotene, mangiferin, and isomangiferin (Kukushkina et al. 2011; Dong et al. 2013). Natural plant populations are limited due to uncontrolled collection of the plants. In this regard, assessment of the population stability and productive potential, development of methods for reproducing species, and production of valuable metabolites using alternative techniques are becoming an urgent problem.

Nowadays, the global science pays much attention to the study of microclonal reproduction of plants, including species of the genus *Hedysarum*, and assessment of the genetic stability of regenerated plants. The researchers have developed a protocol for reproducing *Hedysarum theinum* Krasnob. in culture from axillary buds in vitro, and performed regenerant identification using ISSR markers. It was found that the optimal medium at the stage of reproduction is the GamborgEveleg medium supplemented with 5 μM 6-benzyl aminopurine, and at the stage of rooting it is 1/2 of the mineral base of MurashigeSkoog medium, containing 7 μM α-naphthylacetic acid (Erst et al. 2015).

The researchers evaluated the activity of trypsin inhibitors in the leaves of *H. theinum*. In 2010, the studies of *H. theinum* were performed in the open environment (Altai Republic) and in culture (forest zone of Western Siberia). The studies of *H. theinum* and *H. flavescens* in culture in Western Siberian forest-steppe were performed in 2017 (Zmud et al. 2020). The study of the Hedysarum species showed statistically significant retention of trypsin inhibiting activity in the dry matter of the laboratory-dried material (Zhmud 2018).

The study of chromosomal polymorphism in species of the genus *Hedysarum* growing in southern Siberia showed a high morphological similarity of chromosomes and patterns of distribution of the main clusters of 45S and 5S ribosomal genes in the karyotypes of *H. alpinum* L., *H. austrosibiricum* B. Fedtsch., *H. theinum* Krasnob., which confirms their close relationship. Significant intraspecific polymorphism in the localization of 45S rDNA was found in *H. theinum* (Yurkevich et al. 2020).

Currently, the taxonomic and phylogenetic issues of the genus *Hedysarum* are being actively solved through the comparison of data obtained by molecular genetic analysis (Duan et al. 2015).

The species of the genus *Hedysarum* are distinguished by a rich set of compounds of the phenolic complex. The phenolic complex of the investigated *Hedysarum* consists of various groups xanthones, flavonoids, and coumarins. As previously established, under introduction conditions (in the Tomsk region) *H. theinum* synthesizes a high amount of xanthone glycoside of mangiferin and a number of xanthones in its aerial part, as well as phenolic and terpenoid substances (Zinner 2010, 2011). A sufficiently high protein content was found in the leaves of the introduced species of the genus *Hedysarum*; however, high trypsin inhibitory activity should be considered when using plants for forage purposes. The studied species can be recommended as a feed additive with a high content of biologically active substances and can be used as a source of plant materials with high trypsin inhibitory activity for medical purposes (Zinner 2011).

Material and methods

The introduction studies were carried out in the conditions of Tomsk region located on the West Siberian Plain in the Ob River's middle reaches. The climate of Tomsk region is continental; it is determined by its geographical position (temperate latitudes of 55–61°N) and is characterized by significant seasonal variability of solar radiation. *H. theinum* has been in culture for over 20 years. For the first time, seeds were obtained from individuals in natural habitats (Western Altai, Prokhodnoy ridge, Ivanovsky ridge).

Germination and germination energy of seeds were determined by generally accepted methods; the seasonal development's rhythm was studied by the method reported in Medicinal plant growing ... (1984). Sowing in the open ground was performed using a wide-row planting technique, with a row spacing of 60 cm; the planting depth was 1 to 1.5 cm, depending on the soil type. Individual seed productivity was determined on 20–25 individuals according to generally accepted methods (Methodological guidelines 1980).

The biology of flowering and the pollination features were investigated using the methods developed by A.N. Ponomarev (1960); the fertility of pollen grains was determined by differential staining techniques (Pausheva 1988). The viability of pollen grains was evaluated by method of artificial germination of pollen on the nutrient medium according to D.A. Trankovsky (Barykin et al. 2004). A circadian rhythm of flowering was determined according to A.N. Ponomarev (1960).

A comprehensive assessment of the introduction capability and success rate of the species in culture was performed according to scales developed by N.A. Bazilevskaya (1964), V.N. Florea, (1987), and V.P. Amelchenko (2010).

Statistical data processing was performed using MS Excel and Statistica 6.0 software packages. We calculated the coefficient of variation (CV,%) and determined the variability of features during the morphometric analysis using the empirical scale of variability by S.A. Mamayev (1975). Statistical hypotheses were tested using the nonparametric Mann-Whitney test (U test).

Results

Under the conditions of the forest subtaiga zone of Western Siberia, individuals of *H. theinum* retained natural biorhythms in development, renewal buds of two types: vegetative buds that give rise to vegetative orthotropic shoots, and mixed buds that form not only well-developed rudiments of growth organs but also rudiments of reproductive organs.

The spring regrowth phase in the conditions of Tomsk region begins when the snow cover melts and the average daily temperatures pass through 0–5 °C. Plants are not damaged by either spring or autumn frosts.

The spring regrowth of *H. theinum* under introduction conditions was observed when the snow cover disappeared in late April – early May; in years with a protracted spring, regrowth was found in mid-May. Under introduction conditions, spring regrowth and appearance of the first buds occur within 14–30 days; budding was found to start in late May – early June, the phenophase of budding lasts from 13 to 28 days. *Hedysarum theinum* exhibits a phenophase of budding, flowering, and fruiting. The first flowers appear in the third decade of June, whereas in years with early spring, the first flowers appear in early June. Flowering lasts 2–4 weeks on average (Fig. 1A). The first fruits were set at the end of June; mass fruiting occurs at the beginning of July and lasts about 14–29 days (Fig. 1B).

In some years of observation, we noted the absence of the fruit setting process. The lack of fruiting can be attributed to an increased temperature during the phenophases of flowering – fruiting. In natural habitats of *H. theinum* (Altai Mountains, Mount Krasnaya, Seminsky pass), in some years, fruits are also absent on both the entire individual and on individual peduncles (Fig. 2).

In mid-August, the leaves changed to autumn color, partially faded, and gradually fell off. The stem, devoid of leaves, dried up. Complete wilting of the plant was found in late September – early October. Depending on the observation year, the duration of the growing season was 130–140 days. In general, the rhythm of seasonal development remains periodic.

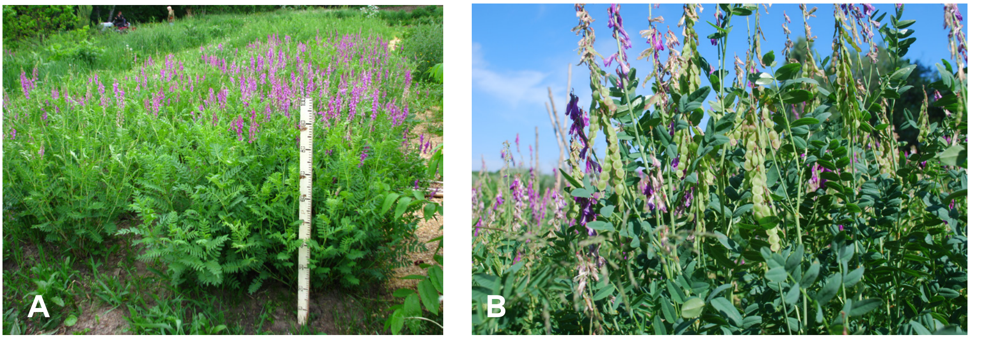

Figure 1. A – Mass flowering of *Hedysarum theinum*; **B** – The beginning of *Hedysarum theinum* fruiting during the introduction.

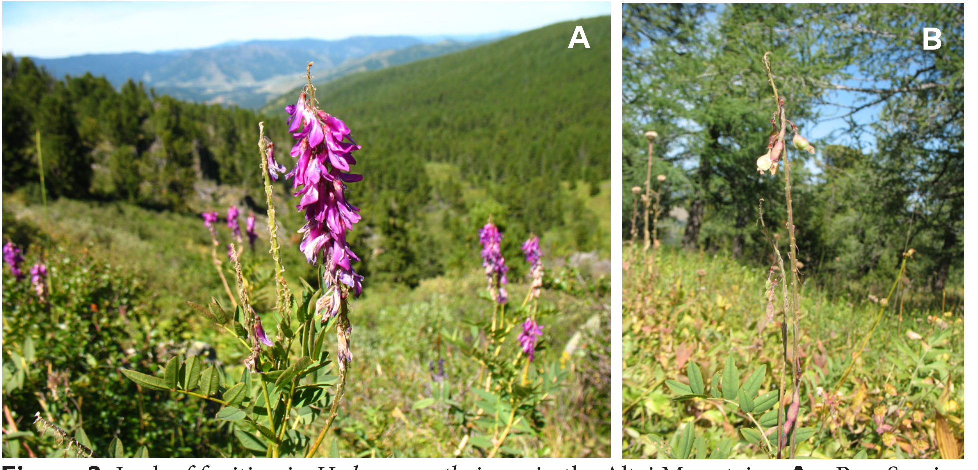

Figure 2. Lack of fruiting in *Hedysarum theinum* in the Altai Mountains; **A** – Pass Seminsky 1800 m a.s.l.; **B** – Mount Krasnaya 1880 m a.s.l.(2010).

Under introduction conditions, the studied individuals were distinguished into groups of early flowering, medium flowering, and late flowering individuals. The overwhelming number of individuals of *H. theinum* reproduced in Siberian Botanical Garden belongs to the group of medium flowering individuals and accounts for about 70%. About 30% of the introduced individuals belong to the groups of early and late flowering plants. No groups of early flowering individuals were found in *H. theinum* grown from seeds collected in natural habitats that had been involved in the introduction experiment for more than 20 years. The majority of individuals belong to the group of medium and late flowering plants. The difference between the phenophases of the three groups is 6–12 days during budding, 5–10 days during flowering, and 4–6 days during fruiting.

The development proceeds with the regular alternation of phases; the onset of phases occurs at intervals that remain relatively stable over several years of observation. In terms of the growing season duration, *H. theinum* belongs to species with a long growing season; the duration of the active growing season is 119–140 days.

The presence of the early, medium, and late flowering individuals in the phenostructure of the introduction population indicates the ecological plasticity of the population and results from the ongoing process of adaptogenesis.

We have studied the patterns of development and functioning of reproductive structures for five years; the seed productivity during the introduction has been studied experimentally for ten years. The analysis of the flowering rhythm features revealed that the investigated *Hedysarum* species belong to the diurnal plant group by the circadian rhythm of flowering. Flowers open once during the day and close at night. Flowers begin to bloom early in the morning (at 7–8 a.m.), and close in the evening (at 8–9 p.m.). On clear and sunny days, mass blooming of flowers can be observed at 12–3 p.m., when the air temperature reaches 20–25 °C. On cloudy and rainy days, the number of blossoming flowers significantly reduces. The blooming period of one flower ranges from 2 to 5 days; that of the whole inflorescence is 10 to 20 days.

The sizes of pollen grains vary insignificantly. The mature pollen grains of the studied *Hedysarum* are single, elliptical, isopolar since the grain polarity can be easily detected after the disintegration of tetrads, radially symmetric with three apertures. The pollen grains of *H. theinum* are three-furrowed. The apertures are meridional grooves. The ores are not visible at the magnification used. The texture of mesocolpiums (areas of the pollen surface between furrows in the equatorial projection) is finely reticulated. The shape of pollen grains is almost spheroid, according to the classification by G. Erdtman. The pollen grains do not narrow towards the poles but form blunt rounded ends. The average equatorial diameter of pollen grains varies from 1.3 to 9.42 µm; the polar axis ranges from 17.6 to 23.5 µm. Some of the *H. theinum* individuals displayed single large pollen grains with an equatorial diameter of 19 µm and a polar axis of 24 µm.

The fertility of pollen grains under introduction conditions is 75–98%, depending on the observation year. Temporal fixation of germinating pollen showed that all potentially viable pollen grains' germination time is 10–15 minutes. The analysis of the viability of pollen grains revealed low percentage of viability (44–49%) of *H. theinum* individuals in a young generative age state; middle-aged individuals have 61–65% of viable pollen grains, and the number of these in individuals in an old generative age state attains 51–56%.

Middle-aged *H. theinum* individuals exhibited the maximum seed productivity. Young generative plants probably have not formed sufficiently adequate reproductive mechanisms. Young generative individuals of H. theinum show indicators of potential seed productivity (PSP) of 46.9–241.0 ovules per shoot, and real seed productivity (RSP) of 8.9–23.0 full-fledged seeds (the coefficient of seed production is 6.7–21.5%). Middle-aged individuals exhibit RSP indices of 210.8–323.7 ovules per shoot and 27.0–163.5 full-fledged seeds (the coefficient of seed production is 9.9–61.2%). Old generative *H. theinum* individuals show RSP indices of 113.7–165.0 ovules per shoot and 22.6–40.2 full-fledged seeds (the coefficient of seed production is 18.5–35.3). Under the conditions of natural phytocenosis (Altai Mountains, Mount Krasnaya), the average number of ovules (RSP) is 196.0 per shoot and 1886.9 per individual; that of full-fledged seeds is 57.0 per shoot and 547.0 per individual (the coefficient of seed production is 30%).

Seeds of *H. theinum* sown into the soil, including scarified ones, exhibited delayed germination; seedlings appeared in the third year after sowing, which indicates the probability of endogenous mechanisms of seed dormancy. The freshly harvested seeds of *H. theinum* show a low germination rate of 3.5%. In the first two years of storage, seeds of *H. theinum* are characterized by a reasonably high germination capacity (48–51%), and then it decreases to 13.3–28%. Germination energy fluctuates during six years of storage from 4–10%; most likely, it depends on climatic conditions of the period of flowering – fruiting – ripening of seeds. The germination of *H. theinum* seeds is characterized by low germination energy of 4–10% on day 8–10. The germination period of *H. theinum* is long, and the germination is often observed in the third year after sowing. Delayed germination of seeds is one of the manifestations of their biological heterogeneity and seed hardness.

Laboratory germination of scarified seeds of *H. theinum* showed germination energy increased two-fold, reduced germination time, and seed germination increased from 30 to 60%. The laboratory germination of seeds of 2010 from populations reproduced in Siberian Botanical Garden and seeds collected in natural habitats (southeastern slope of Krasnaya Mount) revealed a similar type of germination tactics with germination capacity of 51–53% and low germination energy of 4–5% on day 9. The germination rate of seeds in spring sowing is 6–8%. Mechanical scarification of seeds significantly increases germination, which averages 43.8%. We also found that the survival rate of seedlings grown from scarified seeds increases to 30–40% compared to the control that attains 4–7%.

The study of the variability of features of the vegetative sphere in individuals of *H. theinum* in natural habitats (Krasnaya Mount) showed variation in features at low and medium levels of significance (CV of 11.3–18.6%). The same features in individuals from the introduction population varied at the average variability level (CV of 17.6–19.2% on average). Individuals of *H. theinum* in natural habitats showed a high level of variability (CV of 30–38%) of the features of the generative sphere, individuals from the introduction population were characterized by a high and very high level of variability of features of the generative sphere (CV of 39.7–54% on average).

The most stable features in the studied species were the lamina length and width (leaf blade), CV of 13–14%. The number of leaves on the shoot and shoot height vary, CV of 18.3–19.8%. In *H. theinum* individuals from the introduction population, all features of the generative sphere showed a stable level of very high variability; the feature of the number of flowers per inflorescence exhibited the highest coefficients of variation, CV of 46–52.3%. In individuals of *H. theinum* in natural habitats, the most stable features were the lamina length and width (leaf blade) and the shoot height, CV of 11.3–14.3%.

The comparison of the morphological characters of individuals from the introduction population and individuals from natural populations (Altai Mountains, Krasnaya Mount) showed that individuals from the introduction population significantly differ in a higher shoot height of 118.9 cm compared to 76.1 cm, a smaller width of stem leaves, and higher RSP indices (1673.5 and 57.0, respectively). The features of leaf length, number of ovules, and number of inflorescences did not differ significantly.

The study of the intrapopulation variability of features in the species from the introduction population showed their significant variability, especially features of the generative sphere that reflect the heterogeneity of the introduction population.

Conclusion

Thus, the analysis showed that *Hedysarum theinum* growing in the subtaiga forest zone of Western Siberia is resistant to diseases and shows sufficiently high seed productivity. A single lack of fruiting cannot be considered the cause of a weak adaptation process since these phenomena are also observed in natural habitats; most likely, this is a feature of the reproductive biology of the species. The established introduction assessment indicates high plasticity and high degree of adaptation of the species.

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