Creating Rare Species Artificial Populations of the Genus Hedysarum L. (Fabaceae)

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Abstract. In connection with the intensive mineral resource development, it is now topically relevant to conserve the gene pool of plant rare species, whose populations find themselves within the exclusion zones (quarry mining, disposal of overburden and waste rocks, etc.). However, methods for moving plants from such zones to safe places in order to create artificial populations are still poorly developed. In Bashkortostan, tests are being undertaken to develop the methods for creating rare species artificial populations, including tick trefoils, such as Hedysarum grandiflorum Pall. and H. razoumovianum Fisch. et Helm. These species are included into the Red Data Books of the Republic of Bashkortostan and the Russian Federation. The works are being performed in the Natural Botanical Garden (natural protected area “Gurovskaya Gora” near Ufa). The aim of the research is to develop the methods for evacuating rare species and to create artificial populations and source nurseries for obtaining seeds and planting materials in order to conduct reintroduction works in the crucially endangered populations. In the test with H. grandiflorum we used 15 specimens from different places of Bashkortostan, among them from two sites where mining works are planned to be launched. The number of testing plots created since 2006 is 53. Seedlings are recorded during the first 4-6 years of observation. A massive loss of young plants is observed. Generative plants and self-seeding occur in the majority of the tests. Six specimens of H. razoumovianum are under investigation. Starting in 2005, 17 tests have been undertaken. Seedlings are recorded during 4-6 years. Generative plants emerge in the fourth or fifth year of observation. These plants bloom, produce fruits and self-seed every year. By 2014, two self-reproducing micropopulations have been formed. In 2017, 487 plants were recorded in one of them, with 24 maternal plants. The tests suggest the possibility to create artificial populations of these species and to conserve them under eco-phytocoenotic conditions comparable to those in nature. Even now they can serve as the seed source for the reintroduction works.

1. Introduction
With the intensive development of natural areas and mineral resources in the South Urals, it is now topically relevant to conserve the gene pool of plant rare species, whose populations find themselves within the habitat destruction zones (quarry mining, construction of engineering communications and settling tanks, disposal of overburden and waste rocks, etc.). However, methods for moving plants (alive or by seeds) from such zones to safe places in order to create artificial populations are still poorly developed. The difficulty resides in the fact that these methods should take into account eco-phytocoenotic and biological features of the rare species to be moved, which are very peculiar. Each species...
or a group of closely related species with identical ecology and biomorphology require the development of individual methods and techniques for their transplantation with the selection of eco-phytoocoenotic conditions comparable to natural ones, creation of artificial populations in situ and ex situ and reproduction in the source nurseries [1]. There are the examples of successful pursuance of the works aimed at saving (transplanting) and conserving rare species that find themselves in the industrial construction sites. These are the rare species transplantation during the construction of the Olympic Complex in Krasnaya Polyana (Sochi), coal mining in the Kuzbass, construction of the Nizhne-Bureyskaya Hydropower Station, etc.

Since 2002, in the Republic of Bashkortostan tests are being conducted to increase the number of plants in the crucially endangered populations and also the tests to create artificial in situ and ex situ populations of rare and endangered species of the families Alliaceae, Fabaceae, Globulariaceae and Paeoniaceae [1-5]. Positive results have already been achieved in evacuating three species that grow on rocks (Cystopteris dickieana R. Sim., Woodia alpina (Bolt.) S.F. Gray, Potentilla kuznetzowii (Govor.) Juz.) from the flood area of the Yumaguzino water storage reservoir to the places resembling their natural habitats and to the Botanical Garden in Ufa [6-8] and in restoring the Rhodiola irenelica Boriss. populations dwindled in the South Urals because of poaching [9].

The use of the areas of botanical gardens to conduct large-scale tests in species introducing, creating artificial populations and source nurseries in order to obtain seeds and planting materials for reintroduction works, evacuating rare species from the habitat destruction zones, etc., is not always possible primarily because of their limited areas and frequent inconsistency with natural environmental growing conditions for rare and economically valuable species. Therefore, the Natural Botanical Garden (NBG) was established in Bashkortostan near the city of Ufa over an area of more than 200 ha with different habitats (meadow-like and petrophytic steppes, meadows, pine stands, broad-leaved and birch forests, eroded slopes, etc.) [10, 11]. In coordination with the Ministry of Natural Resources of the Republic of Bashkortostan a natural protected area was established, where the introduction and reproduction of rare plant species from other areas were legally prescribed for use. For instance, in this area it is possible to temporarily grow plants evacuated from mining sites and return them back after quarries will be exhausted and recultivated. Artificial populations of different rare and endangered species are currently being created in the NBG, with seeds and planting materials grown in large amounts for reintroduction works in different parts of the republic that allows eco-biological features of the introduced species to be studied. The introduction tests in the “Gurovskaya Gora” site include 56 species. Currently, 37 of them are represented by living plants, with 20 species in the generative phase.

2. Subject of the Study

The subject of our study is concerned with rare species of the family Fabaceae included into the Red Data Books of the Republic of Bashkortostan (2011) [7] and the Russian Federation (2008) [12]. These are the rare species Hedysarum grandiflorum Pall. (group 3) and the rare endemic species H. razoumovianum Fisch. et Helm. of the Cis-Volga and Trans-Volga regions (group 3).

Particular attention in the republic is paid to studying these species. In the Bashkir Cis-Urals, investigations are carried out on the eco-biological features of these species in nature and under cultivation, the current state of the populations is assessed, long-term and short-term monitoring is performed for a number of model populations, technologies are developed to conserve and restore the crucially endangered populations and to create artificial populations using the reintroduction method [7, 13-18].

In Bashkortostan, these species occupy the eastern boundary of their distribution area. Within the entire area the major anthropogenic factor limiting them is the destruction of habitats resulting from the construction of engineering communications and extraction of building materials. For instance, on Mt. Shakhtau in the Ishimbaysky District of Bashkortostan the H. grandiflorum population and those of many other rare species were completely dwindled during the extraction of limestones over the past several decades. There is a real risk of destroying the two largest populations of this species in the
Bashkir Cis-Urals located on the unique residual mountains Tratau and Yuraktau, since the company that used limestones from Mt. Shakhtau in its production process needs urgently new sources of raw material. In 2010, 26 thousand plants of the species *H. grandiflorum* were recorded on Mt. Tratau (plants of all age states) and 3.2 thousand plants on Mt. Yuraktau [16].

Due to their ecological (plants grow on very stony soils) and biomorphological features (plants have long taproots), these species cannot be evacuated by moving the living plants. Even the attempts to transfer to the Botanical Garden some juvenile and immature plants with only slightly injured roots dug out of lime or gypsum marl did not succeed. In most cases the plants died in a year or two. That is to say, the gene pool of these species can be conserved only through creating artificial populations *ex situ* using seeds.

The aim of our research is to develop the methods for evacuating rare species of the genus *Hedysarum* L., create artificial populations and source nurseries *ex situ* in order to obtain seeds for possible reintroduction works in the crucially endangered populations or mined-out quarries.

3. Research Materials and Methods

Reintroduction works were performed according to recommendations developed and approved by foreign researchers [19-21] and Russian scientists [1 and others]. Materials for conserving the species *ex situ* and for the purpose of reintroduction were collected according to the Rare Species Collection Regulations [1, 19 and others].

Seed sowing was done in spring (the second decade of May) and mainly in autumn (the second and third decades of September and October). Seeds were sowed in the ground in sets of 25 to 50 per hole at a depth of 1.0-2.0 cm. The pattern for sowing was 10-20 holes in a row at 40-50 cm intervals, and the distance between the rows was 80-100 cm. No care for seeds was taken.

4. Results and Discussion

The test with *H. grandiflorum* includes 11 specimens from different parts of the Bashkir Cis-Urals, among them two sites (Mts. Tratau and Yuraktau), where the danger of the species extinction exists. Starting in 2006, 32 testing plots have been established under different environmental conditions. Seedlings are observed every year during the first 4-6 years of observation. A massive loss of young plants (seedlings, juveniles and immature plants) is observed. Natural populations are characterized by similar elimination of plants in these age states. The major limiting factor for young plants is the soil moisture deficit caused by the absence of rains in spring and early summer as well as hot dry winds. Droughts are responsible for plant slow development and massive loss. Less dangerous effects are exerted by seed washout or overcrust with soil when raining and also by the work of burrowing animals. Because of seed hardness typical for the Fabaceae species, the soil seed bank is formed, with germination occurring over several years. The rate of seed germination from the soil seed bank depends heavily on weather conditions. It tends to increase during humid years.

Generative plants emerge in the majority of the tests. They start flowering in 3-5 years of their life. Judging by biometric indices, test plants do not differ from natural ones. The plants maintain their natural rhythm of seasonal development. Self-seeding can be observed in some tests. Tests over the whole period of observation showed a single, sparse and thick types of self-seeding depending on weather conditions during the vegetative period, location of the plot, source of original seeds and seed productivity values.

Starting in 2005, 17 tests have been undertaken in the NBG under different environmental conditions aimed at creating artificial *H. razoumovianum* populations. All the tests show seedlings, yet with a massive loss of young plants. Seedlings are recorded every year during the first 4-6 years of observation. The pregenerative period lasts from 1 to 3 years. During the generative phase the plants pass to the fourth and fifth years of their life (in three tests). These are self-seeding plants; abundant self-seeding was observed in 2017. Self-seeders are resistant, achieve a generative state, produce flowers and fruits and have vigorous seeds. By 2014, two self-reproducing micropopulations have been formed in two plots. Shown below are the results of the tests undertaken in autumn 2005 (250
seeds collected on Mt. Mayaktau in the Kugarchinsky Districts of Bashkortostan were sown in testing plot 1) and in autumn 2007 (200 seeds collected on Mt. Vysokaya in the Kugarchinsky District of Bashkortostan were sown in testing plot 12 years old, and the rest plants were self-seeders of different age states and calendar age, namely, regenerative plants 1-4 years old, young generative plants 3-4 years old and mid-aged generative plants 4-7 years old. Pregenerative plants comprised 73.5% and generative plants made up 26.5%. Active self-reproduction can be observed (68.0% of juvenile plants). In 2017, 121 plants were recorded in testing plot 2: 14 generative mid-aged maternal plants 12 years old (11) and old generative plants (3), and the rest plants were self-seeders (similar to test 1). Pregenerative plants comprised 62.8% and generative plants made up 37.2%. Self-reproduction was also observed (42.1% of juvenile plants).

Figure 1. Young plants *Hedysarum grandiflorum* (seed sowing in autumn 2016).

Figure 2. Self-seeders young plants *Hedysarum razoumovianum*.

Figure 3. Generative plants *Hedysarum grandiflorum* (in May 2015).

Figure 4. Generative plants *Hedysarum razoumovianum* (in June 2015).
A comparison of plant biometric indices under reintroduction and in natural populations shows a clear-cut difference according to 13 traits (42% of 20 traits analyzed), in which reintroduced plants exceed natural ones by a factor of 1.1-3.1. The reintroduction conditions exert a favourable effect on the state of plants, with an increase in size and more abundant flowering. Even now these “handmade” populations can serve as the seed source for the reintroduction works (on Mts. Mayaktau and Vysokaya).

5. Conclusions
Our tests show the possibility of creating ex situ artificial populations of the rare species *H. grandiflorum* and *H. razoumovianum* as well as source nurseries of these species in order to obtain planting materials for possible reintroduction works in crucially endangered populations and in destroyed populations within the industrial construction and mining sites (after recultivation) as well as for moving these populations by seeds to safe places.

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