

## RESEARCH ARTICLE

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## Reproductive capacity of four medicinal plants in Nature Park “Rilsky Manastir” – West Bulgaria

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**ABSTRACT**

The territory of “Rila Monastery” Nature Park established 203 vascular medicinal plants. Four of them - *Pulsatilla vernalis* (L.) Miller, *Gentiana lutea subsp. symphyandra* (Murb.) Hayek, *Rhodiola rosea* L. and *Taxus baccata* L. were subject of our study due to their conservation importance and the threats to their development in the park. According to IUSN criteria, used in Red Data Book of Bulgaria. *R. rosea* is critically endangered species (CR), and other 3 species from the survey are included in the category “endangered species” (EN). During the survey on the territory of “Rila Monastery” Nature Park we were working with three target populations of *G. lutea subsp. symphyandra* and by one target population for the other studied species. Some important biological and ecological characteristics were described about all of them. The evaluation of the seed reproductive capacity was investigated about three of the species, because individuals of *T. baccata* were only male. Seed reproductive capacity was established by calculating the ratio between the number of actually existing generative individuals in the investigated population, regardless of the phenological stage, in which they are, and the number of seeds in all identified generative individuals. Best reproductive capacity was established in *P. vernalis*.

**Key words:** : *Pulsatilla vernalis*, *Gentiana lutea subsp. symphyandra*, *Taxus baccata*, *Rhodiola rosea*, reproductive capacity

**Introduction**

The “Rila Monastery” Nature Park (25 253, 2 ha) is situated in the southwestern part of the Rila mountain. On this territory are established 203 vascular medicinal plants. Four of them - *Pulsatilla vernalis* (L.) Miller, *Gentiana lutea subsp. symphyandra* (Murb.) Hayek, *Rhodiola rosea* L. and *Taxus baccata* L. were subject of our study, due to their conservation importance and the threats to their development in the park. These species are included in Appendix 3 of Biodiversity low (2002) like protected species. According to IUSN criteria, used in Red Data Book of Bulgaria (Peev (ed), 2011) *R. rosea* is critically endangered species (CR), and other 3 species from the survey are included in the category “endangered species” (EN). *P. vernalis* is local endemic for Rila Mountain. The recommendations for conservation

measures are to study the state of the localities, reproductive biology and the factors that have impact on the frequency of the populations.

The study is a part of a project of the NP Rila Monastery. It covers the activities of maintenance and protection of the population of the target species.

The calculation of the seed reproductive capacity will enable us to determine the spatial structure of the species. The results from the survey can be the basis for objective management decisions that ensure the existence of the studied species.

**Materials and Methods**

The object of this research are *P. vernalis*, *G. lutea subsp. symphyandra*, *R. rosea* L. and *T. baccata* in target zones - “Ribni ezera” Circus, “Kalin” Reservoir, “Tiha Rila”,

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“Kirilova poliana” and “Mramoretski preslap” localities.

The study was carried out during the vegetation season of 2013. The distribution of the target species was evaluated by oriented transects, which is suitable for subalpine terrains, mountain meadows, forests and mountain streams. Borders of the established populations were marked by their GPS coordinates. The area, altitude, slope inclination, soil type and project cover of the species were described (Table 1). The habitat type where the target species are distributed was determined according to Directive 92/438EEC and Red Data Book of Bulgaria (Biserkov, 2011).

All individuals in the found populations of the target

species were count. Their phase of development and threats from pests and diseases was noted. For *G. lutea*, *subsp. symphyandra*, *R. rosea* and *P. vernalis* were numbered seeds in one fruit and seeds on one individual. The seed reproductive capacity was calculated as a ratio between the number of actually existing generative individuals in the population, regardless of the phenological stage, in which they are, and the number of seeds in all identified generative individuals, using the formula:

$$RC (\%) = \frac{\text{real number generative individuals}}{\text{total number of the seeds from real individuals}} \times 100$$

**Table 1.** Localities of the studied species

Species	Locality	GPS coordinates	Area (ha)	Altitude (m)	Exposure	Slope inclination
<i>Rhodiola rosea</i>	Kalin Reservoir	E 23°14.335 N 42°10.016	0.12	2142	North	35°
<i>Pulsatilla vernalis</i>	Mramoretski preslap	E 23°28.755 N 42°06.258	0.05	2544	North	20°
<i>Gentiana lutea</i>	Tiha Rila	E 23°29.050 N 42°07.911	3.71	2057	Southwest	5°
<i>G. lutea</i>	Iliina River	E 23°26.921 N 42°06.134	0.3	2027	Southwest	10°
<i>G. lutea</i>	Suhoto Lake	E 23.40993 N 42.16190	0.45	1801	West	5°
<i>T. baccata</i>	Kirilova poliana	E 23.40024 N 42.15379	0.5	1483	North	5°

## Results

During studies in the target zones we found three local populations of *Gentiana lutea subsp. symphyandra* and by one population for the other three studied species.

*Gentiana lutea subsp. symphyandra* – Endangered species, with recommendation to study the state of the localities, reproduction biology and the factors that have impact on the area and number of individuals of the populations with the aim to optimize the conservation measures (Evstatieva, 2011).

In the target areas have been identified three isolated from one another local populations of the species - in “Tiha Rila” locality, over “Suhoto” Lake and over “Iliina” River. Populations are diffuse, formed by single individuals or groups of 2-5 individuals. The coverage is between 5 - 6 % .

*G. lutea subsp. symphyandra* has been found in 3 habitat

types - *Pinus peuce forests* [EN – A1, 2 B2 C2 E2 F2 G2 H2 I L3]; *Pinus mugo mountain scrub* [VU – A1, 2 B1 C2 F2 G2 I L3] and *Mountain scrub of Juniperus sibirica* [NT – A1, 2 B1 C1 D1 I L3] (Roussakova, 2011). The localities are with West or South exposure, with semi-humid, mountain meadow soils. Dominant species in the plant community are *Chamaecytisus absinthioides*, *Juniperus sibirica*, *Verbascum sp.*

The plants in juvenile (10-15 cm length of the stem), vegetative (leafy rosettes developed without stems) and generative state (rosette leaves and generative stems with flowers) were observed. Dominates the vegetative state of the plants. Disease or infected individuals have not been observed.

The seed breeding potential of an individual was established (6 875 seeds) by calculating the average number of vertebrae on flowering stem (5 pcs.), fruit capsules in one vertebra (25 pcs.), fruit capsules on generative steam (125

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pcs.) and seeds in a fruit capsules (55 pcs.). The seed reproductive capacity of the three studied populations was calculated (Table 2).

*Rhodiola rosea* – Critically endangered species, with recommendation for cultivation, in order to satisfy the market demands and to protect the natural resources (Evstatieva, 2011).

In the target areas have been identified only one population in the area of Kalin Reservoir. The population has mosaic structure and includes 146 clumps with 10-15 floral stems and different sizes. The coverage is about 10 %.

The habitat type is 8220 - Siliceous rocky slopes with hasmophytic vegetation (HD 92/43), included in Red Data Book of Bulgaria like vulnerable [VU – A1, 2 B2 D2 H2 I J] (Roussakova & Gushev, 2011). The locality is with North exposure, with wet soil. Dominant species in the plant community are *Carex sp*, *Calamagrostis arundinaceae*, *Cirsium appendiculatum* and *Pinus mugo*.

The plants in juvenile and generative state were observed. The lack of vegetative clumps can be explained by the peculiarities of the species, namely that this species blooms immediately after snowmelt. Disease or infected individuals have not been observed.

The fruit of *R. rosea* is follicle, that form a large amount of small (under 1 mm) seeds. The seed breeding potential of 1 clump (32 760 seeds) was established by calculating the average number of generative stem (13 pcs.), average number of fruits in one stem (42 pcs.) and average number of seeds in one fruit (60 pcs.). The seed reproductive capacity of studied population was calculated (Table 2).

*Pulsatilla vernalis* – Endangered species, with recommendation for regular monitoring of the populations (Bancheva, 2011).

In the target areas have been found only one small

population in the area of “Mramoretski preslap”. Population has mosaic structure and included 122 individuals with 15% coverage.

The habitat type according to Red data book of Bulgaria is Alpine acidophilic herbaceous communities near melting snow patches [EN - A1, 2 B1 C2 D3 E2 H3 I J] (Roussakova, 2011), which corresponds with 6150 - Siliceous alpine and boreal grasslands (HD 92/43). The locality is with North exposure, the soils are shallow and poorly developed, including small stones and rocks. Soil moisture is moderate, supported throughout the year by melting snowdrift. Dominant species in the plant community are *Juniperus sybirica*, *Sesleria comosa*, *Nardus stricta*, *Vaccinium myrtillus*, *Phleum alpinum*.

The plants in vegetative and generative state, with fruits were observed. Population has a very good reproductive capacity. The seed breeding potential of an individual was established (946 seeds) by calculating the average number of flowering stem (22 pcs.) and average number of seeds on one generative stem (43 pcs.). The seed reproductive capacity was calculated (Table 2).

*Taxus baccata* – Endangered species with recommendation for studying the localities, breeding biology and the factors that affect the size and number of the populations (Evstatieva, 2011).

In the target areas have been found only six male individuals in the area of “Kirilova poliana” They do not form population. The trees are blooming regularly. The coverage is under 1 %. Habitat type is 9410- Acidophilous *Picea* forests of the montane to alpine levels (HD 92/43), included in Red data Book of Bulgaria as Nearly Threatened [NT - A1, 2 B1 C3 D1 E1 F1 G1 H1 I L3] (Roussakova & Dimitrov, 2011). The locality is with North exposure and brown forest soil, humus-rich, acidic, high moistured.

**Table 2.** Seed reproductive capacity of the studied populations

Species	Number of species	Number of seeds from 1 individual	Number of generative individuals	Number of seeds from all generative individuals	Reproductive capacity, %
<i>Rhodiola rosea</i>	146	32 760	13	425 880	0.03
<i>Pulsatilla vernalis</i>	122	43	22	946	12.9
<i>G. lutea</i> - Tiha Rila	1 452	6 875	82	563750	0.3
<i>G. lutea</i> - liina reka	184	6 875	3	20625	0.9
<i>G. lutea</i> - Suhoto ezero	248	6 875	100	687500	0.04

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The forest is formed by *Fagus sylvatica*, *Picea abies*, *Abies borisi-regis*, *Acer platanoides*, *Acer heldreichii*. Calculation of seed breeding potential cannot be done, because there are no female individuals.

## Discussion

The data obtained for the studied populations of target species showed that the seed reproductive capacity in all of them is very low, and even missing in *T. baccata*. About *G. lutea* subsp. *symphyandra* and *R. rosea* this is a result of several complex factors. First - the established low seed germination (Nikolaeva 1967, 1988, Ким и Днепровский 1973). Second - the presence of competing species such as *Chamaecytisus absinthioides* and *Juniperus* sp., which interrupt the survival of seedlings. Known is the fact, that *G. lutea* subsp. *symphyandra* does not bloom every year, but has a good vegetative propagation (Brouz 1992). The inhibition of the species and the formation of 3 to 5 vegetative individuals around mother plant confirm Brouz (1992) observations. Our previous investigations on *G. lutea* in "Tiha Rila" locality show, that maturity plants bloom on a 3-4 years period. In *R. rosea* also dominates vegetative propagation by division of rhizomes segments in early spring or late summer (Platikanov & Evstatieva, 2008).

*P. vernalis* plants showed the highest seed reproductive capacity, which, together with the vegetative propagation, gives opportunity for normal natural regeneration of the clone-population.

*T. baccata*, like a part of the conifers, does not reproduce vegetatively. The seed breeding of this group of individuals is impossible for the moment. These factors make the studied group of individuals vulnerable, because of the lack of opportunities for natural reproduction.

The results established on the basis of theoretical calculations of seed reproductive capacity suggests, that *G. lutea* subsp. *symphyandra*, *R. rosea* and *P. vernalis* in the observed groups of individuals are with clone-population structure, with domination of clonal structure.

On the basis of this preliminary study we strongly recommend to monitor about illegally collecting of rhizomes from *G. lutea* subsp. *symphyandra* and *R. rosea*, and to be organized planting of female individuals of *T. baccata*, near to the observed male individuals, in order to enable further seed propagation of the species.

## Acknowledgement

The authors are grateful for the financial support of NP "Rila Monastery" by the EOP 2007-2013, Contract № 5113326-C-007.

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