

Biological features of the rarest narrow-range plant species *Potentilla porphyrantha* Juz. (Rosaceae)

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Abstract

Potentilla porphyrantha Juz. (*Rosaceae*) is a very rare stenotopic species, which area of distribution includes small subpopulations in Armenia, Nakhichevan and North Iran. It was assessed and listed in the Red Data Book of Armenia in the category Critically Endangered Species. The main threats for this species could be anthropogenic factors (mainly mining industry) and forecasted climate change. The purpose of this work was to identify the adaptive potential of the studied species and the possibility of its conservation in ex situ conditions as well reintroduction into nature, and to assess the threat to natural populations from predicted climate change. It was shown that in Armenia there is only one species from the section *Persicae* with pink flowers. In the first time karyology and palynology of this species were investigated, number of chromosomes and karyotype were found, as well in the first time pollen morphology was described and their fertility was estimated. Eco-physiological investigations allowed to estimate potential of species adaptation to new environmental conditions. Special attention was paid to possible adaptation of this species to forecasted climate change. All these investigations allowed us to elaborate methods for conservation, reproduction and reintroduction of *Potentilla porphyrantha* back to the nature.

Introduction

Potentilla porphyrantha Juz. – is a very rare, taxonomically interesting plant species. Most representatives of the genus *Potentilla* have yellow or white flowers, and this and 2–3 other closely related species from the section *Persicae* have pink flowers (Fig. 1). The distribution range of the species is limited only to Armenia (until recently 5 small populations were known), Nakhichevan Republic (1 population) and Northern Iran (3 populations) (Fig. 2) [1] [2]. Due to its rarity, the species was included in the Red Book of Plants of Armenia [2] in the Critically Endangered category.

Mining activities are extremely important for modern day societies. The natural resources that are being mined are crucial to modern everyday life and the backbone of the economies of many countries, including Armenia. The global mining industry is increasingly showing a commitment towards greater respect for environment but is accused of wanting to improve its reputation without seeking real progress. While industry initiatives on certain minerals and metals are helpful, companies are still responsible for undertaking comprehensive environmental diligence and have to work to ensure that fragile ecosystems, critical habitats and endangered species are protected from damage and that responsible mine closure is being planned for. This must become the practice of mining in Armenia. It is important to point out that according to the Law on bowels in Armenia, any economic activity is prohibited, as a result of which plants included in the Red Book of Armenia may be destroyed. However, based on economic importance, a special Decree N 781-N "The order of protection of flora objects of the Republic of Armenia and their use for the purpose of reproduction in natural conditions" was adopted by the Government of the Republic of Armenia, which defines the measures for the protection of plants under threat of extinction as a result of economic activity. The transplantation of plant species under the threat of extinction has been established for favorable climatic conditions (such as protected areas or botanical gardens or in the Red Book as a plant registered area) to ensure the natural reproduction of plants.

The international mining company Lydian International (later Lydian Armenia), with the staff of the Institute of Botany of the National Academy of Sciences of Armenia, conducted an Environment Impact Assessment (EIA) on Mount Amulsar to obtain a license to develop a gold deposit. During the EIA, a population of *Potentilla porphyrantha* was found here. This species is included in the Red Data Book of plants of Armenia [2], and scientific researchers of the Institute of Botany organized multilateral studies of this species with the aim to study the possibilities of reproduction, preservation and subsequent reintroduction of it, the results of which are presented in this article.

Results

In the course of scientific research of the rare stenotopic species *Potentilla porphyrantha* (*Rosaceae*) at the Institute of Botany, the following results were received.

During karyological examination of *Potentilla porphyrantha*, the diploid cytorace (2x), 2n=14 of this species grows on Mount Amulsar was determined (Fig. 3).

After the transplantation of *Potentilla porphyrantha* plants from Mount Amulsar to the Yerevan (1230 m a.s.l.) and Sevan (1940 m a.s.l.) Botanical gardens, the parameters of Water regime and transpiration and photosynthesis intensity of this species in different growing conditions were studied (Table 1).

Table. 1. Water regime and transpiration and photosynthesis intensity of *Potentilla porphyrantha* in different growing conditions

Locality and elevation (m a.s.l.)	Water content, %	Free water, %	Bound water, %	Ration of free and bound water	Water deficit, %	Transpiration rate, mg CO ₂ dm ² / hour	Photosynthesis rate, mg/g wet weight, hour
Amulsar Mountaine, 2900 m, alpine belt	75,1 ± 1,8	51,4 ± 1,4	23,8 ± 1,5	2,1	14,5 ± 1,5	387,1 ± 0,1	3,6 ± 0,2
Sevan Botanical garden, 1900 m, meadow-steppe belt	69,9 ± 4,3	40,9 ± 2,4	28,9 ± 1,9	1,4	21,2 ± 2,1	433,2 ± 1,9	3,1 ± 0,1
Yerevan Botanical garden, 1250 m, semi-desert	67,5 ± 2,8	37,7 ± 1,2	29,8 ± 1,6	1,2	24,3 ± 2,6	419,4 ± 2,3	2,2 ± 0,1

In addition to the direct threat to the studied species from the destruction of its habitat as a result of the mining industry, we assessed the threat to natural populations of *Potentilla porphyrantha* as a result of forecasted climate change.

Using the concept of “bio-temperatures” by L. Holdridge [3], we plotted their changes in connection with climate change forecasts in the implementation of the RCP8.5 scenario (Fig. 4).

Discussion

The Amulsar mountain massif is located in the Vayots Dzor region of Armenia at an altitude of slightly more than 3000 m above sea level. Lydian Armenia has planned and permitted their open pit gold mine here.

Potentilla porphyrantha is a stenotopic species that grows here in cracks in volcanic rocks at an altitude of 2450 to 3200 m above sea level. As studies have shown, the subpopulation on Mount Amulsar is obviously the largest in Armenia - more than 7,500 specimens of different ages were counted. The studies on growth and reproduction of *Potentilla porphyrantha* were carried out on Mount Amulsar, in the Sevan Botanical Garden (in a greenhouse and on a specially created rockery) and in the Yerevan Botanical Garden. It was shown that this species perfectly adapted to the conditions of the Sevan Botanical Garden, grows superbly and reproduces by seeds in rockery conditions. In the greenhouse conditions, the plants also adapted well, bloomed and gave full-fledged seeds with high germination (more than 95%), however, some violations in the growth patterns were noted. In the Yerevan Botanical Garden, the species apparently did not adapt well to climatic conditions, grew and developed for 2–3 years, after which the plants died, and seed reproduction was suppressed. Thus, it was found that in the conditions of the Sevan Botanical Garden it is possible to preserve and even increase the number of cultivated plants in order to return them to nature after mining is completed, as well as for use in the flower decoration of Armenian settlements with similar climatic conditions.

In the polymorphic genus *Potentilla*, a polyploid series was recorded: $2n = 14, 28, 35, 42, 56, 63, 70$ with a base number of chromosomes $x = 7$, moreover, among the species of the genus, di-, tetra- and hexaploid cytoraces are more common, and penta- and octaploid cytoraces are somewhat less common [4] [5] [6] [7]. During karyological examination of *Potentilla porphyrantha* grows on Mount Amulsar (Armenia) we identified a diploid cytorace ($2x$), $2n = 14$, the number of chromosomes was given earlier [8]. The karyotype is symmetrical with very small metacentric chromosomes, $0.76\text{--}1.54\text{ }\mu\text{m}$ in size. Formula of karyotype is $2n = 14M$. Symmetry index $TF\% = 49.67$, total chromosome length is $13.85\text{ }\mu\text{m}$. Unfortunately, we did not have the opportunity to compare the obtained data with the karyological data of other species of the section *Persicae*. On the other hand, the basic chromosome number $x = 7$ and the diploid cytorace ($2n = 14$) are very characteristic of many species of the genus *Potentilla* from other sections [4] [5] [6] [9]. That is, the chromosome number of the studied species, established in the course of our research, undoubtedly confirms its belonging to the genus *Potentilla*, despite the color of the flowers, which is uncharacteristic for the genus.

After the transplantation of *Potentilla porphyrantha* plants from Mount Amulsar to the Yerevan (1230 m a.s.l.) and Sevan (1940 m a.s.l.) Botanical gardens, along with observations of the growth and development of plants in the new conditions, palynological studies to clarify the viability of plants in new conditions were conducted. Namely, morphological changes and pollen fertility were determined. It was shown that pollen grains in-situ conditions (on Mount Amulsar) are slightly larger than in the Sevan Botanical Garden, while in the Yerevan Botanical Garden they are much smaller. It was also shown that in plants from Amulsar and the Sevan Botanical Garden, pollen grains are distinguished by high fertility - more than 96%, and in the Yerevan Botanical Garden, fertility is much lower (78%) [10]. That is, from a palinological point of view, the conditions of the Sevan Botanical Garden had little effect on the pollen of the studied species, while the much hotter and drier conditions of the Yerevan Botanical Garden largely inhibited the processes of pollen formation.

In the most investigations devoted to the study of eco-physiological characteristics of plants, special attention is usually paid to the peculiarities of the water regime, because water availability and the state of water primarily determine the physiological activity of plants and their adaptability in various growing conditions. Not less important for assessing the physiological activity and the success of plant adaptation to new growing conditions are indicators of the intensity of transpiration and photosynthesis. The indicators of the water regime and the intensity of photosynthesis and transpiration of the studied species in the conditions of Mount Amulsar and in the Yerevan and Sevan Botanical Gardens were studied. It is shown that under the conditions of the Yerevan Botanical Garden, plants are in a state of stress, while the indicators of plants in the Sevan Botanical Garden are close to those of plants from *in-situ* conditions. We have conducted similar eco-physiological studies on Geghama mountains in Armenia [11]. The findings from these studies align quite closely with our data regarding *Potentilla porphyrantha*. Despite the artificial irrigation conditions in the Yerevan and Sevan Botanical Gardens, plants from the natural habitat on Amulsar Mountain exhibited higher levels of water regime and photosynthesis intensity.

Furthermore, indicators of water deficit and transpiration intensity displayed an inverse relationship. As the altitude of the area increased, the values of these indicators decreased. This pattern was also observed in many other plant species examined in the alpine zone of Geghama mountains and in the Yerevan Botanical Garden, despite the significant 1740-meter altitude difference between the two locations. The correlation between transpiration intensity and elevation appears to be influenced by a combination of factors, including disparities in soil composition, climatic conditions, and weather patterns (including variations in light intensity, atmospheric humidity, atmospheric pressure, and temperature). Additionally, structural modifications within the plants themselves, such as the quantity and density of stomata on the leaves and the leaves' diffusion permeability, likely play a role in this relationship [12]. In any case, both changes in the structural elements of plants and their functional changes should be considered as a complex of adaptation to climatic changes in the broad sense, aimed at maintaining homeostasis and dynamic equilibrium of plants.

According to the latest data [13] in Armenia, the average air temperature for the period from 1929 to 1996 increased by 0.4°C, in the period 1929–2007 – by 0.85°C, in the period 1929–2012 – by 1.03°C, and in the period 1929–2016 – by 1.23°C. In general, in Armenia in the period 1935–2016 the annual amount of precipitation decreased by 9%, but if the regions of Central Armenia became much more arid, then in the north, southeast and in the southeastern part of the Lake Sevan basin their amount even slightly increased. Based on the data provided by the National Communication [13], forecasts of changes in temperature and precipitation were made for Mount Amulsar and other volcanic highlands of Armenia, where *Potentilla porphyrantha* grows, as well as for the Yerevan and Sevan botanical gardens. If we assume that the current climatic conditions on Mount Amulsar are optimal for the growth of *Potentilla porphyrantha*, and considering that in the conditions of the Sevan Botanical Garden this species has perfectly adapted, grows, is not oppressed and multiplies, then at least until 2100 the conditions on Mount Amulsar and, respectively, in other places of its natural growth will remain. That is, climate change should not be considered a threat to the existence of this species. Also this confirms our opinion that the reintroduction of the studied species to its former habitat on Mount Amulsar after the completion of mining operations will not have natural obstacles.

Conclusion

As a result of a multilateral study of the rare stenotopic species *Potentilla porphyrantha* (Rosaceae), its ecological and biological features were revealed. In particular, the taxonomic independence of the species was clarified, its chromosome number and features of the karyotype were identified, and based on the study of physiology (water regime, intensity of transpiration and photosynthesis), the adaptive capabilities of the species in different environmental conditions were evaluated. All these traits are important indicators of *Potentilla porphyrantha* adaptability to different environmental conditions. In the course of studying the growth and development of plants in ex-situ conditions (Sevan and Yerevan Botanical Gardens), it was shown that in the conditions of Sevan (at an altitude of about 1000 m lower than the natural population), the species adapted perfectly, has good growth, high seed productivity with high similarity of seeds and easily captures new territories. An assessment of current climatic conditions and comparison with those predicted due to climate change made it possible to conclude that global climate change is not a serious threat to the natural populations of this species. In general, it can be concluded that the reintroduction of the species from the conditions of the Sevan Botanical Garden to the place of the natural population, part of which can be destroyed during mining operations, is quite possible and should be crowned with success.

Material and Methods

The material for the research was collected in Armenia on Mount Amulsar (39°44'25"N, 45°42'19"E). Here herbarium specimens, seeds and living plants were collected. The formal identification of the plant material used in our study undertook by Dr. George Fayvush. Herbarium specimens are stored in the herbarium of the Institute of Botany after A. Takhtajan (ERE). **Voucher specimens** are stored and deposited at the Herbarium of the Institute of Botany after A. Takhtajan NAS RA (ERE: 193406, 193408.): Armenia, Vayots Dzor region, Alulsar mnt., Artavazdes peak, cliffs. 39°43'37"N, 45°42'59"E, 2990 m a.s.l. 18 Jul 2016. Leg. G. Fayvush, K. Janjughasyan. Det. George Fayvush.

In addition, pollen was collected there for palynological studies. The collected seeds and live plants were used for cultivation and reproduction in ex-situ conditions in the Sevan and Yerevan botanical gardens, the seeds were also used in carrying out karyological studies and to determine the conditions for their storage while maintaining germination. Live plants have also been used for eco-physiological research. According to the Decree of the Government of Armenia N-781 dated July 31, 2014, species included in the Red Book of Plants of Armenia can be collected only for scientific purposes by employees of specially authorized organizations (including the Institute of Botany after A. Takhtajan NAS RA) for a herbarium or transplanted into the territory of Botanical gardens or specially protected natural areas. The studies were carried out in accordance with this regulation. Institute of Botany after A. Takhtajan has permission of the Ministry of Environment RA for collecting plants on the whole territory of the Republic.

For palynomorphological studies, pollen was collected from the buds of plants growing on Mount Amulsar at an altitude of 2800 m above sea level, and from plants grown in the Sevan (1930 m) and Yerevan (1240 m above sea level) botanical gardens. Pollen was studied using a light microscope (AmScope at x200, x400 and x1000 magnification; measurements were made on 20 pollen grains from each examined sample), for which two classical methods were used: the basic fuchsin staining method and the simplified acetolysis

method [14]. Pollen fertility was assessed using the acetocarmine staining method with the preparation of temporary preparations according to the standard method [15].

Karyological investigations were conducted using mitotic chromosome preparations from metaphase stages, obtained from meristematic cells in root tips. The seeds were germinated on damp filter paper within Petri dishes in a laboratory setting (19°–21°C). The root tips underwent a pretreatment in a 0.4 % colchicine solution for a duration of 2 hours, followed by fixation in a mixture of alcohol and glacial acetic acid at a 3:1 ratio, held at room temperature for at least 2 hours. Following a hydrolysis step in 1N HCl at 60 °C for 10–15 minutes, the root tips were subjected to staining with Schiff reagent for 1.5 hours. Subsequently, the root tips were gently squashed onto a glass slide along with 45 % acetic acid. The prepared slides were sequentially immersed in butyl alcohol for 5 minutes, followed by xylene for another 5 minutes, before being embedded in Canadian balsam. For chromosome counting, a minimum of 10 plates of chromosome preparations were meticulously examined. The determination of chromosome numbers and the characterization of species karyotypes were performed using an Photomicroscope (AmScope 2000xLED Lab Trinocular Compound Microscope SKU: T120B-5M, China, 2020) equipped with an oil immersion objective (×100). Photomicrographs were captured using the same microscope setup. We follow the scheme of chromosome morphology and terminology presented by [16]. The centromere index of chromosomes (I^c) was determined by a formula:

$$I^c = \frac{S}{S+L}$$

where: S – the short arm of the chromosome, L – the long arm of the chromosome.

The investigation of the **eco-physiological** characteristics (water regime, water deficiency, transpiration, and photosynthesis intensity) of *Potentilla porphyrantha* in Armenia has been carried out for the first time. The studies of water regime, the intensity of transpiration and photosynthesis were conducted according to [17]. All the measurements were done in the period between 11⁰⁰ and 13⁰⁰. Each measurement was done with 3 repetitions and in 3 options (7-10 shoots were chosen for each sample). The data presented are the average results of the performed analysis which were subjected to statistical development. Water deficiency was determined based on the principle of water saturation of leaves. The fresh sample was promptly weighed and dried at a thermostat of 105°C for determining the total water content. The intensity of transpiration of the leaf was determined by rapid weighing with a torsion scale. The intensity of photosynthesis was determined by the change in the dry weight of the leaf sample. The amount of dry material synthesized during photosynthesis was determined by the difference between the initial and the final measurements of the dry weight. The intensity of photosynthesis in mg/dm², per hour, was expressed by dividing the amount of synthesized dry material by the time period between the measurements.

To assess the threat to the existence of the *P. porphyrantha* population in Armenia, we used the climate change forecasts given in the National Communications under the UN Framework Convention on Climate Change [18] [19] [13].

Declarations

Contributions

All authors designed the study Zh.H., K.J., L.K., and H.H. performed the experiments. A.G., Zh.H., A.A. and K.J. analyzed the data. G.F. and A.G. wrote the manuscript. G.F. supervised the research. All authors reviewed and edited the manuscript.

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Competing interests

The authors declare no competing interests.

Data availability

All data generated or analyzed during this study are included in this published article

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Figures



Figure 1

Potentilla porphyrantha on Mnt. Amulsar



Figure 2

Map of distribution of *Potentilla porphyrantha*

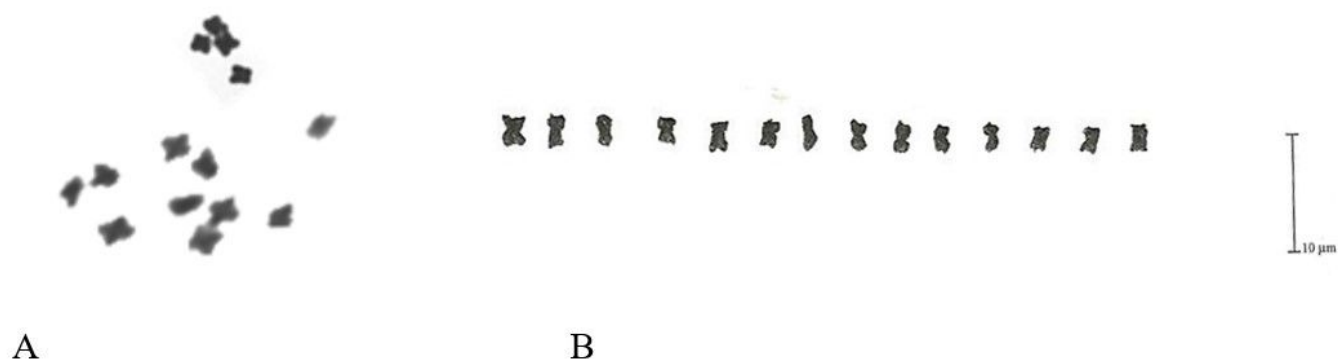


Figure 3

A – Metaphase plate ($2n = 14$), B – Karyotype of *Potentilla porphyrantha*

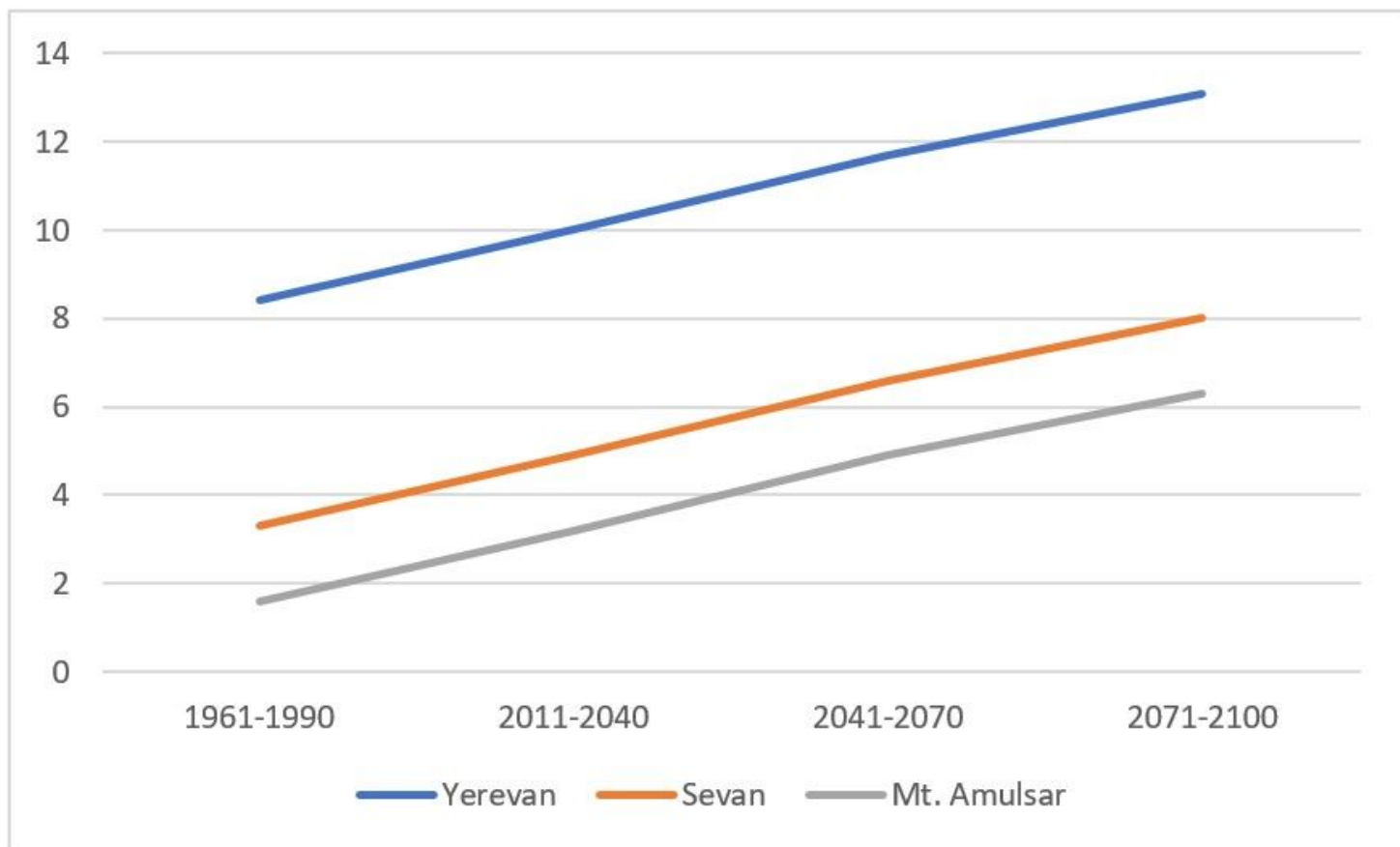


Figure 4

Forecast of changes in bio-temperatures on Mount Amulsar and in the Sevan and Yerevan Botanical Gardens until 2100