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Studies on the perennial pea *Vavilovia formosa* (Fabaceae) of mountain Aknasar population (Gegham Highland) and under *ex situ* conditions in the Botanical Gardens of Armenia

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Summary: Wild perennial pea *Vavilovia formosa* is a relic and an endangered species of the upper alpine flora of Armenia, specialized to scree habitats. The populations of *V. formosa* in Armenia, as well as in other sites of the world, are in danger of extinction and need both *in situ* and *ex situ* complementary investigation and conservation. Present article contains information on *V. formosa* Aknasar mountain

population studies. Data on natural conditions, flora composition, ecological and coenotic characteristic of the habitat and bio-ecological features of *V. formosa* are given. The present state of *Vavilovia* population of mountain Aknasar is satisfactory. The activation of seed reproduction along with vegetative propagation is observed. One of the natural threats leading to the change and reduction of *Vavilovia* population was estimated to be the overgrowing of mobile scree habitats with turf-forming grasses, which creates conditions for the mountain-meadow vegetation development. *V. formosa ex situ* conservation strategy is one of the possible ways of its adaptive capacity estimation to environment changes as well as for its saving and using in basic and applied researches. An introductory experiment on the cultivation of *Vavilovia* in the Yerevan and Sevan Botanical Gardens has started, and some data on bio-morphological and eco-physiological peculiarities under *ex situ* conditions were obtained.
Key words: Aknasar mountain, Armenia, bio-ecological studies, *ex situ* cultivation, *Vavilovia formosa*

Introduction

Wild perennial pea *Vavilovia formosa* (Steven) Fed. (Fabaceae) is a relic and endangered species of the alpine flora of Armenia, highly specialized to small areas of moving detritus and scree. *Vavilovia* Fed. (Fedorov, 1939) is a separate branch in the tribe Fabeae (Fabaceae). Its origin is associated with upper alpine, high mountain zones of Great and Small Caucasus, North and North-Western Iran, Northern Iraq, Anatolia, Syria and Lebanon.

In Armenia *Vavilovia* occurs on Gegham highland, Kapudjugh mountain (Zangezur alpine zone) and on Syunik upland. Gegham highland is a part of the volcanic zone of Armenia, it arose as a result of volcanic effusions of upper tertiary and quaternary time, and was partially altered by glacial and erosion processes (Aslanyan, 1958). Highland is dominated by about 30 large crater cones with maximum height 3598 m asl (Azhdahak peak). In some of craters small lakes have been formed. The climate is temperate continental and in top areas – cold mountain. By the Yeratmber weather station database, the average annual temperature is –2.5°C (from –6.4°C to +1.7°C), precipitation – 871 mm. Snow lasts about 218 days a year. Wind speed reaches 25-30 m/s. Slopes with moving detritus and screes of volcanic light porous slag are found on almost all the cones. As a result of the continuous process of weathering of rocks, a series of transitions from large rocks to fine sandy crushed stone are formed. Primary and secondary by the origin, as well as fixed and mobile screes of large-gravel, fine-grained and sand-like slag are observed on Gegham mountains (Kazaryan, 1939).

The populations of *V. formosa* in Armenia, as well as in other sites of the world, due to the narrow specificity of habitat requirements, are considered to be regressing (Red Book of Plants of RA, 2010; Akopian et al., 2010; Mikic et al. 2013, 2014; Vishnyakova et al., 2016). Narrow ecological amplitude, isolation and fragmentation of *Vavilovia* populations are among expansion limiting factors, the main human-induced threat is grazing. Another possible threat to *V. formosa* is forecasted warming, conditioned by the influence of Global climate change, which can lead to redistribution of territories of different ecosystems. By the niche modeling results, *V. formosa* is expected to have further range reduction in various scenarios for climate change (Smýkal et al., 2017).

Taking into account such predictions, a better knowledge of the evolutionary potential of the rare species respond to environmental change is needed. Targeted bio-ecological studies to identify the adaptive potential of *Vavilovia* to forecast warming on the territory of Armenia has not been carried out yet. That is why a special monitoring for *Vavilovia* populations status estimation is demanded.

V. formosa ex situ conservation strategy is one of the possible ways of its adaptive capacity estimation to environment changes, as well as for its saving and using in basic and applied researches. The most important goal in *Vavilovia ex situ* cultivation is to grow the plants that are capable of developing reproductive organs and produce seeds. *V. formosa* has a high potential for breeding, due to its adaptive features: perennial life cycle, tolerance to frost, drought, pests and diseases. In case of *V. formosa* flowering in culture, interspecific and intergeneric hybridisation can to be done (Mikich et al.,

2014). Nowadays, according to the definition of Botanic Gardens Conservation International (BGCI) in the International Agenda for Botanic Gardens in Conservation (IABGC), an important role of the rare and endangered plants *ex situ* conservation is given to botanical gardens.

This studies was carried out with the aim of *V. formosa* bio-ecological peculiarities evaluation in natural habitats of Gegham mountains and under *ex situ* conditions in Botanical Gardens of Armenia.

Materials and methods

Studies of *Vavilovia* were carried out in the upper alpine zone of Mt. Aknasar, in Yerevan and Sevan Botanical Gardens and in the Institute of Botany NAS RA. Field works were implemented during the expeditions to Kotayk and Gegharkunik districts of RA in June-October 2018. The following methods used in research: a unified system of environmental monitoring (Hill et al., 2005); bio-ecological (Serebryakov, 1962); phaenological (Beydeman, 1974); *ex situ* cultivation of wild plants (Akhverdov, Mirzoeva, 1949); water regime, transpiration, chlorophyll and photosynthesis intensity determination (Osipova, 1947; Practicum on plant physiology, 1990; Mezhunts, Navasardyan, 2010), measurements were taken from 11am to 1pm, the determined results were subjected to statistical processing. Site monitoring involves: visual observation of population and individual plant species, photographic records, preparing permanent squares (quadrats) 1x1 meter for field records, measuring of site factors (using GPS device, pH and Moisture Meter, Thermometer with Hydrometer TA-138). Analysis of soil samples was done in the Center for Ecological-Noosphere Studies of the NAS RA.

Results and discussion

The Gegham highland population of *Vavilovia* is the largest one in Armenia. During the field investigations of past years from 1929 to recent time *Vavilovia* was recorded on south-eastern and south-western mobile scree slopes of mountains Aknasar (3258 m), Vishapasar (3157 m), Spitakasar (3555 m), Sevkatar (Sevsar) (3225 m), and in large numbers – in the neighborhood of lake Aknalich (3032 m) and on volcanic cones surrounding the lake (Kazaryan, 1939; Fjodorov, 1939; Makasheva et al., 1973; Baloyan, 2004; Akopian et al., 2010, 2018; data of ERE and EREU Herbarium materials).

With the aim of bio-ecological studies and botanical inventory of *Vavilovia* population, scientific expeditions to mountain Aknasar were realized on July 11 and on September 13, 2018. Population is located on south-eastern moving scree slope of mountain Aknasar, it has an area of 0.12 ha at an altitude of 3135-3150 m asl. The slope is sunlit and sun-warmed, covered by a layer (up to 4-6 cm) of red and grey volcanic fine-grained slag of 0.5 cm to 2-3 cm in size and by larger tuff stones of up to 20-30 cm. The base of the slope is large rocky. The water that is formed during the melting of the snow from the top of mountain is absorbed by the slag under which the aquifer layer is formed. The habitat is characterized by precipitation during the spring and autumn-winter months, strong heating of the soil in the summer dry period, and winds that dry up the surface layer of slag and the adjacent layer of soil. These conditions lead to the xerophytization of the plants growing here. The following parameters were obtained for climatic and soil conditions of Aknasar: daytime air temperature is 26.5°C, the air temperature in the sun is 32.1°C, relative air humidity is 37%-40%. The results of soil chemical and mechanical analysis are represented in Table 1. Sandy loam soil moisture under the gravel on the depth about 40-50 cm content is 1-1.2%. The high percentage of sand (72.2 %) in the soil promotes water infiltration to deeper layers, which creates dryness both on the scree slag and under it.

Table 1. Soil chemical and mechanical composition of *Vavilovia formosa* habitat on Mt. Aknasar

Determined indicators	Outcomes, %
K	0.00085

K_2O	0.00102
Na	0.00075
Cu	$2.66 \bullet 10^{-5}$
Pb	$3.2 \bullet 10^{-4}$
P_2O_5	0.95
SO_4	0.0082
N total	0.52
Ca	0.008
Mg	0.004
Cl^-	0.0084
HCO_3^-	0.0183
CaO	1.68
Hummus	37.0
pH	6.38
Soil type	Sandy loam
Sand	72.2
Clay	5
Silt	22.2

Vegetation on scree habitat is low with surface coverage about 20-25%. The density of *Vavilovia* population is from 40 to 150 plant samples per 1 sq. meter. Samples of *Vavilovia* represent 30 – 85 % of the total number of plants per 1 sq. meter. Plants are represented by three life forms: annual, biennial and perennial herbs. The leading role in the vegetation cover of Aknasar slopes belongs to low-growing perennial herbs. The floristic composition of studied vegetation is represented by the following plant species, which were recorded in July-September aspects: Alliaceae – *Allium schoenoprasum* L.; Apiaceae – *Chamaesciadium acaule* (M. Bieb.) Boiss.; Asteraceae – *Centaurea fischeri* Willd., *Cirsium rhizochephalum* C. A. Mey., *Doronicum oblongifolium* DC., *Leontodon hispidus* L., *Senecio vernalis subsp. sosnovskyi* (Sof.) V.E. Avet., *Tripleurospermum caucasicum* (Willd.) Hayek; Brassicaceae – *Sibbaldia procumbens* L., *Draba araratica* Rupr., *D. siliquosa* M. Bieb., *Erysimum gelidum* Bunge, *Coluteocarpus vesicaria* (L.) Holmboe; Campanulaceae – *Campanula glomerata* L.*, C. saxifraga* subsp. *aucheri* (A. DC.) Ogan., *C. tridentata* Schreb.; Caryophyllaceae – *Arenaria dianthoides* Sm.; *Cerastium araraticum* Rupr.*, C. szowitsii* Boiss., *Dianthus raddeanus* Vierh., *Minuartia oreina* Schischk., *Silene ruprechtii* Schischk., *S. saxatilis* Sims; Fabaceae – *Astragalus incertus* Ledeb., *Trifolium ambiguum* M. Bieb.*, T. canescens* Willd., *T. trichocephalum* M. Bieb.; Gentianaceae – *Gentiana pontica* Solt.; Lamiaceae – *Nepeta lamiifolia* Benth.*, Thymus kotschyanus* Boiss. & Hohen., *Ziziphora raddei* Juz., *Z. clinopodioides* Lam.; Juncaceae – *Luzula pseudosudetica* V. I. Krecz.; Papaveraceae – *Papaver fugax* Poir., *P. caucasicum* M. Bieb.; Poaceae – *Alopecurus tuscheticus* Trautv., *A. laguroides* Balansa, *A. textilis* Boiss. subsp. *textilis*, *Bromopsis variegata* (M. Bieb.) Holub *, Festuca woronowii* Hack. subsp. *caucasica* (St.-Yves) E.B. Alexeev*;* Polygonaceae – *Rumex acetosa* L. subsp. *acetosa*; Ranunculaceae – *Ranunculus oreophilus* M. Bieb.; Rosaceae – *Alchemilla sericea* Willd., *A. grossheimii* Juz., *A. retinervis* Buser; Rubiaceae – *Asperula glomerata* (M. Bieb.) Griseb., *Galium sosnovskyi* Manden.; Saxifragaceae – *Saxifraga sibirica* Pall. ex Ledeb.; Scrophulariaceae – *Pedicularis sibthorpii* Boiss., *Scrophularia olympica* Boiss., *Veronica orientalis* Mill., and some others.

On many stones of mountain Aknasar, saxicolous lichens develop, which belong to the first settlers of stones, involved in the formation of scree. They give the stones a bright orange or grayish color. The following lichens species were discovered on Mt. Aknasar: *Aspicilia desertorum* (Kremp.) Mereschk.*, Candelariella vitellina* (Ehrh.) Müll. Arg.*, Lecanora muralis* (Schreb.) Rabenh., *Rusavskia elegans* (Link) S.Y. Kondr. & Kärnefelt*, Xanthoparmelia stenophylla* (Ach.) Ahti & D. Hawksw.

Scree plants specialization is aimed to fixing the underground organs in a moving substrate and to adapting the above-ground parts to the conditions of continuous slag coating. In *Vavilovia* this is achieved by the rapid formation of numerous thin, long, branched roots and above-ground sprouts, which, when filled with a substrate, continue to grow under its surface, take root and give a new leafy above-ground shoot. Roots and rooting above-ground shoots, spreading in all directions, consolidate scree and, thus, reach the state of their relative immobility.

Figure 1. Fragment of *Vavilovia formosa* Aknasar population, 11.07.2018, stage of the mass flowering

Some fragments of the slope, free of vegetation, are firstly occupied by *V. formosa*, as well as by accompanying endemic species *Gallium sosnovskyi* described from Gegham highland (fig. 1). *G. sosnovskyi* is fine perennial herb, which also firmly fixes to the scree thanks to very dense and thin branching roots. *Vavilovia* plants are often found in small groups together with other species, such as *Astragalus insertus, Campanula saxifraga* subsp. *aucheri*, *Cerastium araraticum, Thymus kotschyanus*, *Trifolium ambiguum*, *Tripleurospermum caucasicum*. Intertwined by roots, these plants fix the scree in the place where they grow.

Mass flowering of *Vavilovia* was observed in the first decade of July (fig. 1), on average ten days earlier than usual. Flowers fallen from night co
ld were not noted. During the autumn expedition in early September a large number of seedlings and juvenile *Vavilovia* plants were observed, which indicates the activation of seed reproduction along with vegetative propagation. The present state of *Vavilovia* population of mountain Aknasar can be estimated as satisfactory. No facts of grazing were noted on the altitude of investigated site.

Over the past decades, an increase in air temperature has been observed in Armenia (an average of 5.5°C) (Melkonyan, Gevorgyan, 2017). With increasing altitude above sea level, a recession in the temperature increase is observed: temperature gradient up to 800 m asl is 1.7°C, and at an altitude of 3000 m asl is 0.7°C. Precipitation for 1935-2016 decreased in Armenia by 9%. Above 2500 m asl there is a significant decrease in precipitation. As environmental niche modeling predicts, species may respond to temperature increase by moving to higher altitudes, which is hard for the high mountain *V. formosa*, as it already occurs at the elevations of 3000–3500 m in Armenia. The observations we carried out in natural habitats showed that the discussed scenario of *Vavilovia* range boundary rise is not feasible: this is prevented by the constant movement from above and the slag shedding from the top of the mountain. At the bottom the plant is limited by large stones, which cover a base of the slope. As a result, *Vavilovia* populations always occupy the middle part of the slope, which was also observed on the scree slopes near the Mt. Ukhtasar in Syunik district (Akopian et al., 2010). Being a highly specialized species, *Vavilovia* belongs to the biological group of high-altitude xerophytes. In this regard, it can be assumed that the observed increase in temperature and a decrease in precipitation in the high-mountainous zone of Armenia cannot have appreciable threatening impact on *Vavilovia.*

The possible threat factor leading to the reduction of *Vavilovia* habitat can be the overgrowing of mobile scree with turf forming species of grasses *Alopecurus tuscheticus*, *A. laguroides*, *A. textilis* ssp. *textilis*, *Bromopsis variegatus, Festuca woronowii* ssp. *caucasica*, which creates conditions for the development of mountain-meadow vegetation. The overgrowing of Gegham ridge mobile screes was observed earlier by E. Kazaryan (1939) and it continues to the present. The expansion results of grass vegetation can be seen not far from Aknasar on the gentle slopes around Lake Aknalich (3090 м asl). It led to vanishing of *Vavilovia* near Lake Aknalich, which occurs there in abundance in the first half of the past century. The grazing factor and using this area as pastures is a secondary negative factor accompanying the natural degradation of scree.

Attempts of *V. formosa* cultivation and *ex situ* conservation have been undertaken in Armenia from the 40s to 80s of the past century in the "Flora and vegetation of Armenia" Plot of the Yerevan Botanical Garden (Akhverdov, Mirzoeva, 1964). We have obtained some results of *V. formosa ex situ* cultivation in 2008-2010 (Akopian et al., 2010), and in laboratory conditions (Akopian et al., 2014).

Many years' experiences on the Armenian flora wild species cultivation by A. A. Akhverdov and N. V. Mirzoeva (1961, 1964) have revealed that alpine mesophyte species despite good care, grow not so well in the dry continental conditions of the Yerevan Botanical Garden. But in the same conditions, the upper alpine xerophytes, including *Vavilovia*, which in wild grow on well warmed lighted southern slopes, are more successfully cultivated. The southern position of mountain habitats and the sharply continental climate of the Yerevan Botanical Garden (severe winters and sharp fluctuations in day and night temperatures in summer) contribute to acclimatization of upper alpine xerophytes in the Garden.

In the frame of the present research *V. formosa* and some other alpine plant samples were transplanted (in July 12 and in September 14, 2018) from Mt. Aknasar in the Yerevan (stony semi-desert zone) and Sevan (high mountain steppe zone) Botanical Gardens. Climate condition average characteristics in July-September in Mt. Aknasar and in Botanical Gardens are presented in Table 2.

Table 2. Climate conditions in Mt. Aknasar, Yerevan and Sevan Botanical Gardens in July-September

Altitude (m)	Air Temp (°C)	Relative air humidity (%)	Soil moisture (%)	Soil Ph (%)	Light (Klux)
In situ: Mt. Aknasar, 3135 -3150 m	32	29	1.1	6.8-7	40
Ex situ: Sevan, 1950 m	20	56	8.5	6.9	33

(under partial shadow)					
Ex situ: Yerevan, 1250 m (under partial shadow)	30	47	7.5	6.8-7	22

Alpine hillocks were prepared in Botanical Gardens, using slag and earth from scree habitats, as well as tuff stones and mixture (with pH 6,8-7) of black soil, peat, red sand. *Vavilovia* samples were planted on the slopes of various exposures. Planting was carried out according to the method described in Akopian et al. (2010). After 7-12 days there were noticeable signs of rooting. Plant survival in the Sevan Botanical Garden was at first about 80-85% and in the Yerevan Botanical Garden – about 60%. Further, in the summer-autumn period, the growth and vegetative propagation of plants in the Yerevan Botanical Garden became more active, especially on the south-eastern and south-western slopes of hillocks. The shoots reached 11-15 cm length with 14-17 leaves and 2-3 lateral branches with 5-6 leaves. The vegetation of the plants in Yerevan continued almost until the middle of November, they were green, new sprouts appeared permanently. The plants in the Sevan Botanical Garden developed more slowly, the shoots remained short, up to 5-8 cm in length, as in plants in natural places.

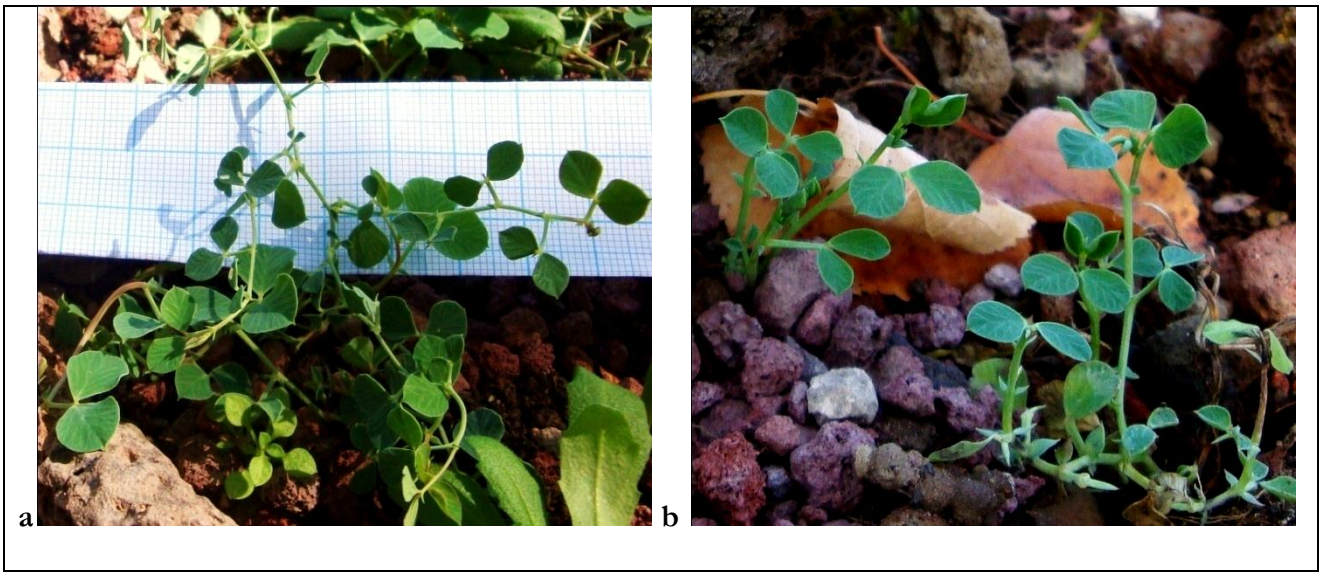

Figure 2. *Vavilovia formosa* growing at the "Flora and Vegatation of Armenia" Plot of the Yerevan Botanical Garden on September 25 (a) and on October 10, 2018

To identify the adaptive capacity of *Vavilovia* in nature and in the Yerevan Botanical Garden, measurements of such eco-physiological parameters as plastid pigments (chlorophyll, carotenoids), the intensity of photosynthesis, water regime (free and bound water, water deficiency) and transpiration, were made. The comparative study of some parameters in *Vavilovia* fresh leaves was carried out two months after transplantation from nature, when plants under *ex situ* conditions multiplied and the possibility of taking material appeared.

Table 3. The content of plastid pigments in *Vavilovia formosa* fresh leaves, depending on the height of growth in different climatic conditions, mg / 100 g

Altitude (m)	Chl *a*	Chl *b*	Chl *a* + *b*	Chl *a* / *b*	Carotenoids
Mt. Aknasar 3150 m	174	77	251,7	2,25	94

The Yerevan Botanical Garden 1250 m	198	85	283	2,32	142

The quantitative decrease in the chlorophyll content in *Vavilovia* leaves (Table 3) in mountains is explained by the biological feature considered as a plant response to high light intensity, while more chlorophyll accumulates at a lower light intensity in the Botanical Garden. An increase in the content of carotenoids indicates their adaptive and protective effect for the photosynthetic apparatus in altered *ex situ* growing conditions.

Table 4. Intensity of water regime, photosynthesis and transpiration in fresh leaves of *Vavilovia formosa* under *in situ* and *ex situ* conditions

Altitude (m)	Total water % wet weight (M, m)	Free water % wet weight (M, m)	Bound water % wet weight (M, m)	Free/ Bound	Water deficit % wet weight (M, m)	Transpiration intensity mg/g wet weight, hr	Photosynthesis intensity mg CO_2/dm^2-h
Mt. Aknasar 3150 m 11.07.2018	79	47.4	31.6	1.5	22.1	245.4	2.13
Mt. Aknasar 3150 m 13.09.2018	81.7	56.1	25.6	2.2	23.3	363.6	2.8
Yerevan 1250 m 18.09.2018	69.89	45.1	24.8	1.8	20.6	240.5	2.1

As a result of comparison of water regime indicators (Table 4), it was identified that in the arid conditions of the Yerevan Botanical Garden despite regular watering of the rocky hillocks and soil moisture of 7.5%, the water deficit in *Vavilovia* was 20.6%. It is not almost different from that of Aknasar (23.3%), where soil moisture was 1.1%. Some studies on the transpiration of mountain plants reveal the dependence of changes in plant transpiration on the height of their habitat. According to Stanko et al. (1958), an increase in the intensity of transpiration in plants with elevation to greater height is due to an increase in light intensity. It is known that the intensification of transpiration leads to an increase in water deficiency. In *Vavilovia*, water deficit is compensated by well-developed root system, which increase absorbtion of water. Some other parameters obtained under *in situ* and *ex situ* conditions changed not so significantly and did not exceed statistical deviations.

Conclusions

The populations of *Vavilovia formosa* in Armenia, as well as in other sites of the world, are in danger of extinction and need both *in situ* and *ex situ* complementary investigation and conservation. The present investigation contribute to the bio-ecological knowledge on *V. formosa* of mountain Aknasar population (Gegham highland) and under *ex situ* conditions. As a result of population monitoring data on natural conditions, flora composition, ecological and coenotic characteristic of the habitat and bio-ecological features of *V. formosa* are given. One of the natural threats leading to the change and reduction of *Vavilovia* population were estimated the overgrowing of mobile scree habitat with turf-forming grasses, which creates conditions for the development of mountain-meadow vegetation. A slight increase in temperature and a decrease in air humidity in the alpine zone currently not adversely affect the vital activity of *Vavilovia*, which belongs to the eco-biological group of high mountain xerophytes. The present state of *Vavilovia* population of mountain Aknasar can be estimated

as satisfactory. A large number of seedlings and juvenile *Vavilovia* plants were observed in population, which indicates the activation of seed reproduction along with vegetative propagation. *V. formosa ex situ* conservation strategy is one of the possible ways of its adaptive capacity estimation to environment changes. An introductory experiment on the cultivation of *Vavilovia* in the Yerevan and Sevan Botanical Gardens has been started, some data on bio-morphological and eco-physiological peculiarities under *ex situ* conditions were obtained.

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Studies on the perennial pea *Vavilovia formosa* (Fabaceae) of mountain Aknasar population (Gegham Highland) and under *ex situ* conditions in the Botanical Gardens of Armenia

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Sažetak: Wild perennial pea *Vavilovia formosa* is a relic and an endangered species of the upper alpine flora of Armenia, specialized to scree habitats. The populations of *V. formosa* in Armenia, as well as in other sites of the world, are in danger of extinction and need both *in situ* and *ex situ* complementary investigation and conservation. Present article contains information on *V. formosa* Aknasar mountain population studies. Data on natural conditions, flora composition, ecological and coenotic characteristic of the habitat and bio-ecological features of *V. formosa* are given. The present state of *Vavilovia* population of mountain Aknasar is satisfactory. The activation of seed reproduction along with vegetative propagation is observed. One of the natural threats leading to the change and reduction of *Vavilovia* population was estimated to be the overgrowing of mobile scree habitats with turf-forming grasses, which creates conditions for the mountain-meadow vegetation development. *V. formosa ex situ* conservation strategy is one of the possible ways of its adaptive capacity estimation to environment changes as well as for its saving and using in basic and applied researches. An introductory experiment on the cultivation of *Vavilovia* in the Yerevan and Sevan Botanical Gardens has started, and some data on bio-morphological and eco-physiological peculiarities under *ex situ* conditions were obtained.
Ključne reči: Aknasar planina, Jermenija, bio-ekološka istraživanja, *ex situ* gajenje, *Vavilovia formosa*