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Ecologo-Physiological and Caryological Investgations of Some Rare Halophyte Plant Species from Ararat Plain

(Submitted by corresponding member of NAS RA Zh. H. Vardanyan 30/VI 2021)

Keywords: *halophytes, water regime, transpiration, photosynthesis intensity, plastid pigments, adaptation.*

Introduction. Halophyte plant communities in general are of great scientific and environmental interest, primarily due to their rich, original biodiversity. There are numerous plant species known as true halophytes and species that thrive on saline soils. Overwhelming majority of these species are stenotop, with a narrow, often disjunctive area of distribution. At present, halophytic plant communities are best represented in the Ararat plain in Armenia, where relatively small areas of salt marshes, salt bodies (solonchaks) and salt licks (solonets) have been preserved. It has to be noticed that Ararat plain is one of the extremely arid regions of Armenia with about 200 mm per year precipitation (water supply is carried out mainly due to groundwater). By the decree of the Government of Armenia dated 14.08.2008 (N967-N), the area of salt marshes in the vicinity of Ararat town has been identified as a "natural monument". This area has long attracted the attention of botanists as a rare ecosystem, consisting of a prodigious number of very rare plant species. Many publications have been devoted to it, highlighting the uniqueness of these marshes, their floristic richness and the urgent need to preserve the entire ecosystem [1 –3]. The Ararat salt marshes are identified as an Important Plant Area and a Key Biodiversity Territory [4, 5].

In their essence and origin, the salt marshes of the Ararat Plain, as well as similar ecosystems of Anatolia and Iran, represent a kind of refugium of the hygrohalophytic flora of the Ancient Mediterranean, preserved since the drying up of the ancient Tethys Ocean [6 – 9]. According to our classification of habitats in Armenia the ecosystem of salt marshes in the Ararat Plain is classified as EUNIS D6.24, – Salt marshes dominated by *Juncus acutus* [10].

Unfortunately, despite their conservation status, salt marshes on the Ararat plain are subject to intense anthropogenic impact (annual burning of tussocks of *Juncus acutus* in autumn, intensive almost year-round grazing, land use for fodder crops, construction of pools on mineral waters) [3]. This impact is superimposed on the global processes of climate change, intensification of desertification, and a decrease in the level of groundwater taking place on the Ararat Plain. All this requires immediate concrete measures to protect the unique ecosystem. In this regard, for a better understanding of the threats to the existence of the rarest and characteristic plant species of this ecosystem, we accomplished an ecological and physiological study, the purpose of which was to identify some ecological and physiological features (water regime, content of plastid pigments, intensity of photosynthesis and transpiration) of eight rare plant species. The study was aimed at the assessment of the degree of adaptation of the species to natural conditions and the real threat to their existence when these conditions change.

Natural conditions of the Ararat salt marshes. The natural monument "Ararat salt marshes" is located in the eastern part of the Ararat valley at an altitude of about 850 m above sea level, and according to unofficial data it covers an area of about 50 hectares.

The climate is sharply continental, arid, with little snowy winters and dry hot summers. The average annual temperature (period 2007-2014) is 12.8°C with an absolute maximum recorded in July of 42°C and a minimum in January of -31°C. The average annual precipitation for the same period is 237 mm, the largest amount falls in the spring (April-May), and the least in August.

The soils on the territory of the natural monument are of the meadow type, moist, loamy, mineralized with salts of groundwater, sulfate weakly alkaline (pH 7.6-8.0). The salt content in the soil is about 0.2%, and in the groundwater - about 0.3% (Table 1). At the same time, due to the very high evaporation rate, the highest salt content was recorded in the surface soil layer [3].

Table 1

The content of salts in the soil on the territory of the natural monument "Ararat salt marshes"

Index	Sampling depth		
	Soil surface	30 cm	60 cm
Na, mg/kg (water extract)	165.0	2500.0	200.0
Na, mg/kg (exchange)	265.0	2550.0	320.0
K, mg/kg (water extract)	30.63	550.3	41.25
K, mg/kg (exchange)	287.5	906.5	316.26
HCO_3^-, %	0.049	0.045	0.044
SO_4^{2-}, %	1.05	0.21	0.41
Cl^-, %	0.128	0.021	0.023
Ca^{2+}, %	0.055	0.017	0.013
Mg^{2+}, %	0.033	0.002	0.004
pH	7.6	7.8	8.0
Salts' content, %	1.9	0.2	0.2

Features of flora and vegetation. The main edificator of the Ararat salt marshes is *Juncus acutus* L., and one of the most characteristic species is *Iris musulmanica* Fomin. Due to changes in environmental conditions (climate change, a decrease in the level of groundwater, the impact of an anthropogenic factor) in recent years some other species became abundant in the area: (*Aeluropus littoralis* (Gouan) Parl., *Gypsophila perfoliata* L., *Thymelaea passerina* (L.) Coss.et Germ., *Elytrygia repens* (L.) Nevski, *Cynodon dactylon* (L.) Pers., *Alhagi pseudoalhagi* (M.Bieb.) Desf. ex B. Keller et Shap., *Sphaerophysa salsula* (Pall.) DC.). The unique feature of the flora of the studied ecosystem is that 16 highly rare species included in the Red Data Book of Plants of Armenia grow here [2]: *Inula aucheriana* DC., *Cirsium alatum* (S. G Gmel.) Bobrov, *Merendera sobolifera* C. A. Mey., *Juncus acutus* L., *Puccinellia grossheimiana* V. I.Krecz., *Sphaerophysa salsula* (Pall.) DC., *Iris musulmanica* Fomin, *Falcaria falcarioides* (Bornm. et H. Wolff) H. Wolff, *Microcnemum coralloides* (Loscos et Pardo) Font-Quer ssp. *anatolicum* Wagenitz, *Linum barsegianii* Gabrielian et Dittr., *Thesium compressum* Boiss., *Frankenia pulverulenta* L., *Salsola soda* L., *Trigonella capitata* Boiss., *Dianthus cyri* Fisch. et C. A. Mey., *Sonchus araraticus* Nazarova et Barsegyan.

This highly rare ecosystem is classified as EUNIS D6.24, according to our classification of habitats in Armenia – Salt marshes dominated by *Juncus acutus* [10].

Material and methods. For the research, we selected 8 rare species known as true halophytes, or as species that successfully grow on saline soils: Merendera sobolifera, Sphaerophysa salsula, Iris musulmanica, Falcaria falcarioides, Microcnemum coralloides, Linum barsegianii, Frankenia pulverulenta, Aeluropus littoralis. The studies were carried out in 2016-2019 in 4-6-fold repetition, during the period of intensive plant growth. The parameters of the water regime of plants (free and bound water, water deficit), the intensity of transpiration and photosynthesis, the quantitative content of chlorophylls and the amount of carotenoids were determined. Physiological studies were carried out according to generally accepted methods [11,12], the content of pigments was assessed using an SF-26 spectrophotometer, and their concentration was calculated according to generally accepted equations [13].

The caryological investigations were based on specimens, collected during expeditions in 2016-2017 to Ararat district of RA. The seeds were germinated on wet filter paper in Petri dishes in the laboratory (19-21°C). The caryological investigations were made on the mitotic metaphases of the meristematic cells from root tips. The root tips were pretreated in 0.4% colchicines solution for 2 hours; fixed in fluid 3:1 alcohol and glacial acetic acid for at least 2 hours at room temperature. After hydrolysis in HCl 1 N for 10-15 minutes at 60˚C the root tips were stained in Schiff reagent at 1.5 hours. Then the root tips were squashed on a glass slide with 45% acetic acid. For all chromosome counts, a minimum of 10 plates were examined for each taxon.

Results and discussion. It is obvious that the study of water regime is the most important at studying halophyte plants, since water is the environment

where all the main biochemical reactions that determine the vital activity of plants take place [14]. Most of the plants absorb highly mineralized water with difficulty because of the insufficient osmotic pressure in plant cells. Moreover, the degree of accumulation of salts by plants depends on both: the peculiarities of species and the amount of salts in the soil. Under the natural conditions of high salt content in soil and groundwater the water content in plant organs will be much lower than under conditions of normal moisture [15]. The indicators of the water regime, the intensity of transpiration and photosynthesis of the studied plants are shown in Table 2.

Table 2
Indicators of the water regime, the intensity of transpiration and photosynthesis of the investigated plant species

Species	Indicators of water regime (raw weight)				Intensity	
	Total water,%	Free water,%	Bound water, %	Water deficit, %	Photosynthesis, mg/dm^2, per hour	Transpiration, mg/graw weight,per hour
Linum barsegianii	63.02	22.81	40.21	27.07	3.47	257.86
Microcnemum coralloides	62.30	23.83	38.47	1.53	2.59	315.93
Falcaria falcaroides	61.97	23.51	38.46	25.26	2.21	162.92
Merendera sobolifera	57.0	22.88	34.12	37.3	1.82	186.1
Iris musulmanica	56.51	21.69	34.82	11.68	2.63	122.05
Sphaerophysa salsula	52.33	23.71	28.62	16.85	2.15	200.0
Frankenia pulverulenta	39.84	18.12	21.72	20.64	2.16	283.6
Aeluropus littoralis	29.94	10.62	19.32	38.40	1.29	102.93

Before analyzing eco-physiological peculiarities of investigated species it has to be noticed that these species are characterized by different ploidy [16]. Accordingly, there are diploid species (*Microcnemum coralloides* x = 9, 2n = 18; *Falcaria falcarioides* x = 11, 2n = 22; *Frankenia pulverulenta* x = 5, 2n = 10), tetraploid (*Iris musulmanica* x = 11, 2n = 44), hexaploid (*Merendera*

sobolifera x = 7, 2n = 42), and for one species (*Aeluropus littoralis*) 3 cytotypes were found – x = 10, 2n = 20, 40, 60. It was revealed that the hexaploid race (2n=60) differs from the diploid (2n=20) by significantly higher energy of vegetative reproduction and viability.

As one can see, among the studied plants, *Linum barsegianii*, *Microcnemum coralloides* and *Falcaria falcaroides* have the highest water content (total water content), and this indicator is the lowest in *Aeluropus littoralis*. The rest of the species on this indicator occupy an intermediate position. The same can be said about the content of free and bound water. As it is known [17 – 19], that one of the internal factors determining the degree of salt tolerance of is the ratio of bound and free water, which correlates with the degree of soil salinity. Best of all, the ratio of the content of different forms of water is expressed in the indicator of water deficit, which among the studied plants turned out to be the lowest in *Microcnemum coralloides* (typical succulent), and the highest in *Aeluropus littoralis* (turf grass with a shallow root system, the least salt tolerant among the studied species, abundantly represented in the most humid areas with the lowest soil salinity). Under the conditions of saline soil, the intensity of transpiration depends not only on the water content in the soil and the ability of plants to assimilate it by the root system, but also on the content of water-soluble salts in the leaves, which create an increased osmotic pressure, on the hydrophilicity of plasma, etc. As it is indicated in A. A. Shakhov [17] research, salts, in particular sodium, reduce the intensity of some biochemical reactions in plants, and their accumulation in vegetative organs increases the osmotic pressure, which is a limiting factor for water evaporation. High osmotic pressure leads to high water retention capacity, which allows more economical water use. It is relevant to notice, that the intensity of transpiration in *Microcnemum coralloides* is much higher than in other species, and in *Aeluropus littoralis* this indicator is the lowest (probably, this is due precisely to the difference in the salt content in the cells of the vegetative organs of the studied plants).

As for the change in the intensity of photosynthesis in the studied plants, this indicator is the lowest in *Aeluropus littoralis*, and the highest in *Linum barsegianii*. *Linum barsegianii* is an elegant annual plant, which, obviously, determines the intensity of its physiological processes aimed at the fastest implementation of the reproductive cycle. Most likely, the difference in this physiological indicator is due to heredity, life form, and structural features of plants [20]. The absorption and transformation of solar energy in the process of photosynthesis is carried out by photosynthetic plant pigments, in particular, chlorophyll "a" and "b" and carotenoids. To assess the state of the photosynthetic apparatus of the studied plants, we investigated the content of these pigments in them, which is a very important internal factor of plant adaptation to unfavorable environmental conditions (Table 3).

Table 3

Chlorophyll and carotenoids content in the studied plants

Species	Chlorophyll "a", mg/g	Chlorophyll "b", mg/g	Chlorophyll "a+b", mg/g	Chlorophyll "a/b", mg/g	Carotenoids, mg/g
Microcnemum coralloides	6.07±0.15	42.77±2.37	48.84	0.14	3.49±1.19
Iris musulmanica	10.97±1.44	1.35±0.07	12.32	8.1	10.90±0.89
Linum barsegianii	19.44±0.61	12.90±0.35	32.34	1.50	15.94±0.94
Falcaria falcaroides	21.96±0.48	11.10±0.56	33.06	1.90	11.40±2.31
Frankenia pulverulenta	24.82±0.48	14.44±0.56	39.26	1.70	16.90±2.95
Aeluropus littoralis	35.44±1.02	18.03±0.53	53.47	1.96	18.10±1.25

As one can see, the highest content of chlorophyll (a + b) is observed in *Aeluropus littoralis* and *Microcnemum coralloides*. It indicates their sufficient internal supply for the processes of photosynthesis, but at the same time *Microcnemum coralloides* has the highest content of chlorophyll "b", which is responsible for the adaptation of plants to extreme growing conditions. This is not surprising, since this species is an obligate halophyte, a stenotopic species with a disjunctive area in the Ancient Mediterranean, found in Spain and South-West Asia [9]. At the same time, on the territory of the natural monument *Microcnemum coralloides* grows in various microconditions (dry areas with deep groundwater, excessively humid relief depressions, *Juncus acutus* tussocks), although all these areas are characterized by a high salt content in the soil.

The rare Armenian endemic annual *Linum barsegianii* (Ararat salt marshes in Armenia are the only known habitat of this species, the closest related species *Linum seljukorum* is endemic to Turkey; it also grows in salt marshes in the Konya province [21] and is characterized by similar ecological plasticity, but at the same time the content of two forms of chlorophyll in it, their total amount and the content of carotenoids is rather high, but not extreme, is at the average level among the studied plants. This is most likely evidence of the intensity of physiological processes associated with the peculiarities of the vital activity of this plant.

The lowest total chlorophyll content as a result of a very small amount of chlorophyll "b" was noted in the rare species *Iris musulmanica*, which grows in the Ararat plain of Armenia and in similar waterlogged habitats of Turkey, Nakhichevan, and northern and northwestern Iran [22]. This is most likely due to the life form of the plant (a relatively large perennial with a powerful rhizome and large leaves), grows mainly in areas with excessive moisture, this does not require special adaptation to arid conditions, a decrease in transpiration intensity and an increase in chlorophyll "b" content.

Conclusion. The results of our studies show that all studied species, despite different adaptation strategies, are well adapted to conditions with a high content of mineral salts in soil and water, arid climate with high summer temperatures and high water evaporation from the soil surface, with changes in the groundwater level during the growing season. If we exclude the direct impact of the anthropogenic factor, then at present their populations can remain unchanged, and in some cases even increase their density on the territory of the natural monument "Ararat salt marshes". Consequently, it is absolutely necessary to take urgent measures to exclude grazing, arson of vegetation, plowing of drier areas in this area, most likely this can be achieved by changing the status of the protected area and organizing an appropriate protection regime. With regard to the possible impact of the projected climate change [23] the expected temperature increase is unlikely to have a direct negative impact on the studied plant species. On the other hand, a decrease in precipitation can have a significant effect on a decrease in the groundwater level, which, together with an increase in temperature can both increase the evaporation from the surface of the soil and, accordingly, increase the degree of its mineralization, and adversely affect the water supply of plants. Worst of all, this can affect plants with a superficial root system and currently growing in more humid areas of the natural monument. In this regard, it is necessary now to intensify work on assessing the possibility of preserving these species in ex-situ conditions, in particular in the Yerevan Botanical Garden, and in case of successful experiments to organize a living collection here.

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Ecologo-Physiological and Caryological Iinvestgations of Some Rare Halophyte Plant Species from Ararat Plain

A study of several physiological features of eight plant species has been conducted in order to assess the vulnerability and adaptability to the conditions of salt marshes of the arid region of Armenia. Water regime, intensity of transpiration and photosynthesis, content of plastid pigments have been investigated for the following species: (*Merendera sobolifera* Fisch. et C. A. Mey., *Sphaerophysa salsula* DC., *Iris musulmanica* Fomin, *Falcaria falcarioides* (Bornm. et H. Wolff) H. Wolff, *Microcnemum coralloides* (Loscos et Pardo) Font-Quer, *Linum barsegianii* Gabrielian et Dittr., *Frankenia pulverulenta* L., *Aeluropus littoralis* (Gouan) Parl.). The results of the study have indicated that all eight species are well adapted to the highly mineralized soil and groundwater conditions. In this regard, it is revealed that the greatest real threat to the existence of the species is the direct impact of the anthropogenic factor (grazing, arson of vegetation, plowing for fodder plants). As a result of karyological studies, it was revealed that these species are characterized by different ploidy.

Ժ. Հ. Հովակիմյան, Ա. Գ. Ղուկասյան, Գ. Մ. Ֆայվուշ

Արարատյան հարթավայրի որոշ հազվագյուտ հալոֆիլ բուսատեսակների էկոլոգո-ֆիզիոլոգիական և կարիոլոգիական առանձնահատկությունները

Ուսումնասիրության համար ընտրվել են ՀՀ Կարմիր գրքում ընդգրկված հազվագյուտ հալոֆիլային 8 բուսատեսակներ՝ *Merendera sobolifera* Fisch: et CA Mey., *Sphaerophysa salsula* DC., *Iris musulmanica* Fomin, *Falcaria falcarioides* (Bornm. et H. Wolff) H. Wolff, *Microcnemum coralloides* (Loscos et Pardo) Font-Quer, *Linum barsegianii* Gabrielian et Dittr., *Frankenia pulveruuslent* L., *Aeluropus littoralis* (Gouan) Parl., որոնք աճում են Արարատ քաղաքի շրջակայքի աղակալած ճահիճներում: Որոշվել են բույսերի ջրային ռեժիմի ցուցանիշները (ազատ և կապված ջուր, ջրային անբավարարություն), պլաստիդային գունանյութերը, և ֆոտոսինթեզի ինտենսիվությունը: Ուսումնասիրության արդյունքները ցույց են տվել, որ բոլոր ութ տեսակներն էլ լավ են հարմարված բարձր հանքայնացված հողի և գրունտային ջրերի պայմաններին: Միաժամանակ պարզվել է, որ տեսակների գոյության ամենամեծ իրական սպառնալիքը անթրոպոգեն գործոնի (արածեցում, բուսականության հրկիզում, կերահանդակների հերկում) ուղղակի ազդեցությունն է: Կարիոլոգիական ուսումնասիրությամբ էլ պարզվել է հալոֆիլ բուսատեսակների պլոիդության առանձնահատկությունները:

Ж. О. Овакимян, А. Г. Гукасян, Г. М. Файвуш

Эколого-физиологические и кариологические особенности некоторых редких галофильных видов растений Араратской равнины

Для эколого-физиологических исследований выбрано восемь редких галофильных видов: *Merendera sobolifera* Fisch. et C. A. Mey., *Sphaerophysa salsula* DC., *Iris musulmanica* Fomin, *Falcaria falcarioides* (Bornm. et H. Wolff) H. Wolff, *Microcnemum coralloides* (Loscos et Pardo) Font-Quer, *Linum barsegianii* Gabrielian et Dittr., *Frankenia pulverulenta* L., *Aeluropus littoralis* (Gouan) Parl., включенных в Красную книгу РА, которые растут на засоленных болотах в окрестностях города Арарат. Определены параметры водного режима растений (свободная и связанная вода, водный дефицит), пластидные пигменты, интенсивность транспирации и фотосинтеза. Результаты исследования показали, что все восемь видов хорошо адаптированы к условиям высокоминерализованной почвы и грунтовых вод. Выявлено, что наибольшую реальную угрозу существованию вида представляет прямое воздействие антропогенного фактора (выпас, поджог растительности, вспашка кормовых угодий). Кариологическими исследованиями выявлены особенности разноплоидности галофильных видов растений.

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