

The Study of the Relationship between Distribution and Anatomical of *Astragalus semnanensis*

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Abstract

Astragalus L. is from Fabaceae. *Astragalus* is the largest genus among the flowering plants. *Astragalus semnanensis* Bornm. & Rech. is from sec. *Leucocercis*. A. sect. *Leucocercis* is one of the few sections of thorny *Astragalus* that has basifixed hair. *Astragalus semnanensis* is the only species of this section that is a narrowly endemic one known from a small area in north-central Iran near Semnan. This study focuses on the distribution and anatomy of this plant. The present study was done on gypsum area west of Semnan that is 30,000 hectares in Aftar, Momen Abad, and Lasjerd. Ecological factors such as altitude and physical and chemical characters of soil are studied in this area. For observing the impacts of ecological factors on *A. semnanensis* used from RDA and CCA of Canoco for windows 4.5. companion plants with *A. semnanensis* in this area are *Acantholimon cymosum*, *Amygdalus lycioides*, etc. *Astragalus fridae* is another endemic gypsophyte in this area but there is in higher altitude than *A. semnanensis*. In this area there are many miner and factories for extracting gypsum that damage growing these gypsophytes such as *A. semnanensis* in this area.

Keywords: Gypsophyte, Ecological factors, Semnan, RDA, CCA

INTRODUCTION

Astragalus is the largest genus among flowering plants with about 2500-3000 species belonging to Fabaceae family and classified in 245 sections. The center of diversity of this genus is Eurasia, especially in the southeast and the center of Asia and the Himalayas. This genus has more than 800 species in Iran, among which 500 are endemic to Iran. Spiny *Astragalus*, which makes up a relatively large group of this genus, is distinguished from other groups by features such as cushion-like growth, spiky, presence of tragacanth production ducts, small fruit, and inflorescence size, and having single-seeded fruits, usually single or double-seeded. These characteristics are observed in some of the sections of older *Astragalus* subgenera such as *Tragacantha*, *Calycophysa*, *Astragalus*, and *Cercidothrix* [1-5]. *Astragalus semnanensis* Bornm. & Rech is one of the Spiny *Astragalus* located in the *Leucocercis* Bunge section [6]. *Leucocercis* is the only section spiny species that also has bifurcated hair. All members of sections are endemic to Iran. The characteristics of the members of this section are short plants, with exclusively white bifurcated hair, paripennee with hard and spiny axes, leaflets with very long Gallinagome dia, short, cup-shaped or tubular-cup-shaped, and non-swollen inflorescences, a sheath protruding from a calyx. These plants are distributed in the desert areas of Iran, especially in the central, southern, and southwestern regions of Iran due to their drought resistance [7-9]. This section has modified hair that are densely located on the leaf, while most other spiny species have basifixed hair [10]. *Leucocercis* section is distributed in the central regions of Iran and the central desert

of Iran. In some cases, it penetrates saline soils and adapts to other halophyte species. This section has 7 species and is distributed in the central regions of Iran (around Isfahan, Yazd, Fars, Kerman, Balochistan, and south of Semnan). Low altitude, high temperature, and low rainfall are the ideal ecological conditions of the species in this section and their strategy for this continuity in this area is flowering in spring. *A. semnanensis* is distinguished from other species in this section due to its dense hair covered with villous hair [7]. Ecologically, *A. semnanensis*, like other *Astragalus* species of *Leucocercis* section are distributed in arid climates, but *A. semnanensis* is the only species in this section that is known as a low endemic from a small area in the center of northern Iran and gypsum areas in the northern strips of the famous desert of Iran (Kavir) near Semnan. Various reports of *A. semnanensis* species have been presented in:

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- In gypsum deserts, Iran, 2-3 km north of Sorkheh at 1300-1400 meters altitude [6].
- 88 km east of Garmsar, 10 km west of Sorkheh gypsum areas at 1350 meters altitude
- 31 km west of Semnan in Sorkheh and Momenabad gypsum areas at an altitude of 1300-1200 meters [10].

Despite the few reports of *semnanensis* species distribution, less study has been done on its anatomical adaptability to soil and altitude factors. In this study, the distribution of *semnanensis* species was studied for distribution in a large area, and all anatomical, morphological, and ecological factors of this species were studied.

MATERIALS AND METHODS

The study area with an area of about 30,000 hectares is located in the gypsum habitats of the west of Semnan and in the northern strips of the famous Iranian desert called Kavir desert between three regions west of Momenabad with geographical coordinates of 35° 32' 38.96"N and 53°17' 36.64" E and north and east of Lasjerd with coordinates of 35° 26' 27.33"N and 53°05'2.2.8" E and south of Aftar with coordinates of 35° 35' 54 54.63" N and 53° 7' 18.63" E. The northern parts of Sorkheh, located between Lasjerd and Momenabad, were also studied. This region has a hot and dry climate and the dominant vegetation cover is *Gypsophila* plants. The average rainfall is 210 mm per year and the average temperature in the region is 11.5°C. The altitude of the study area is from 1100 to 2000 meters above sea level.

For more detailed studies in this area, 40 stations with a distance of 2 km on transects at a distance of 200 m above sea level were sampled. From each station, soil and plant samples and ecological data were collected in at least 3 plots (25x 25 meters). All the information of the region in terms of latitude and longitude, height and direction of slope, vegetation including the frequency of *Gypsophila* species and companion plants, height, the vegetative form of plants, canopy, flowering time, and fruiting time were collected in each plot. In addition, the soil of each plot was collected for physical and chemical analysis, including analysis of organic carbon, lime, pH, Ec, and various soil elements such as calcium, magnesium, sodium, potassium, phosphorus, nitrogen, and soil texture were determined. Soil analysis was performed in the soil laboratory of Semnan Natural Resources Department. Anatomical sections of the leaves of *A. semnanensis* were manually prepared from samples fixed in alcohol and glycerol (1:1) and stained with Bismarck methyl green and brown, and photographs were taken with a Leitz light microscope (Wetzlar, Nikon camera model Coolpix).

Leaf tissues were scaled and measured by Digimizer.4.1.1.0 software. Besides, photographs of the hair on the leaf surface and the surface of the calyx of this plant were taken with an electron microscope (SEM). To study the ecological relationships and soil factors and topography of *Astragalus*

semnanensis species, Canoco for windows 4.5 software and CCA and RDA analyses were used.

RESULTS AND DISCUSSION

A. semnanensis is one of the spiny *Astragalus* that is seen in the cushion-shaped. It blooms in early May. Its flowers have a very beautiful aroma and pollinate with bees and bear fruit in early June before the onset of high temperatures in this region (**Figure 1**).



Figure 1. *Astragalus Semnanensis* Bornm. & Rech. in Semnan (Camera from Fatemeh Rabizadeh, 2020)

Morphology

This is a semi-shrub plant with a height of 20-30 cm with basifixed, white, often flat hair that surrounds the vegetative and reproductive organs. Leaves are very thick, hard with dense hair, 4-8 pairs of hard, green, flat, ovate or round, round at the tip leaflets. The inflorescence is shorter or the size of a leaf, consisting of 8 to 15 florets. The inflorescence is covered with dense silky hair, the calyx is yellowish-white, a vein of a leaf is purple or red, the shape of the calyx is tubular or cup-shaped covered with long hair (**Figure 2**).



Figure 2. a: *Astragalus Semnanensis*, b: Rhachis Segment with Leaflets, c: Calyx, d: Bracteoles, e: Standard, f: Wing, g: Keel, h: Staminal Tube, i: Ovary [10].

Leaf Anatomy

The plant has a thick ladder epidermis and parenchyma cells, and sclerenchyma tissue is also observed above the large-diameter woody tissue, which indicates the presence of rhachis in the leaves of this plant. The mean thickness of the leaf epidermis in *A. semnanensis* is $94.96 \pm 3.4 \mu\text{m}$. The mean thickness of the leaf ladder parenchyma is $230.42 \pm 22.15 \mu\text{m}$. The mean thickness of rhachis is from 190.97 to 298.272 μm (Figure 3).

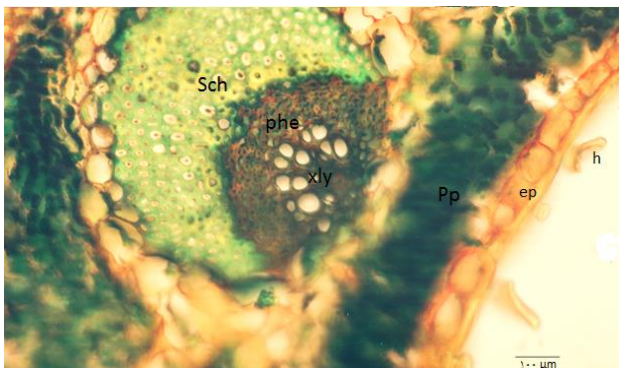


Figure 3. Anatomy of Leaf *Astragalus Semnanensis* X20. h: Hair, ep: Epidermis Cell, P.p: Palisade Parenchyma, Ph: Phloem, Xyl: Xylem, Sch: Sclerenchyma

The hair of *A. semnanensis* is dense, basifixed, flat, and papillose. The presence of gypsum crystals on the hair of *A. semnanensis* indicates the high amount of gypsum in the region. The average distance of stomatal cells from each other is $68.6 \pm 16.1 \mu\text{m}$ and it has long hair with different sizes from 257.3 to 800.7 μm , which is $510.4 \pm 220.1 \mu\text{m}$ on average. The hair is close to each other ($80.6 \pm 12.1 \mu\text{m}$ from each other). It is well shown in electron microscope images (Figure 4).

There is also the hair on the calyx that is basifixed like leaf hair but are taller smoother and without protrusions than the leaf hair and no gypsum deposits are seen (Figure 5).

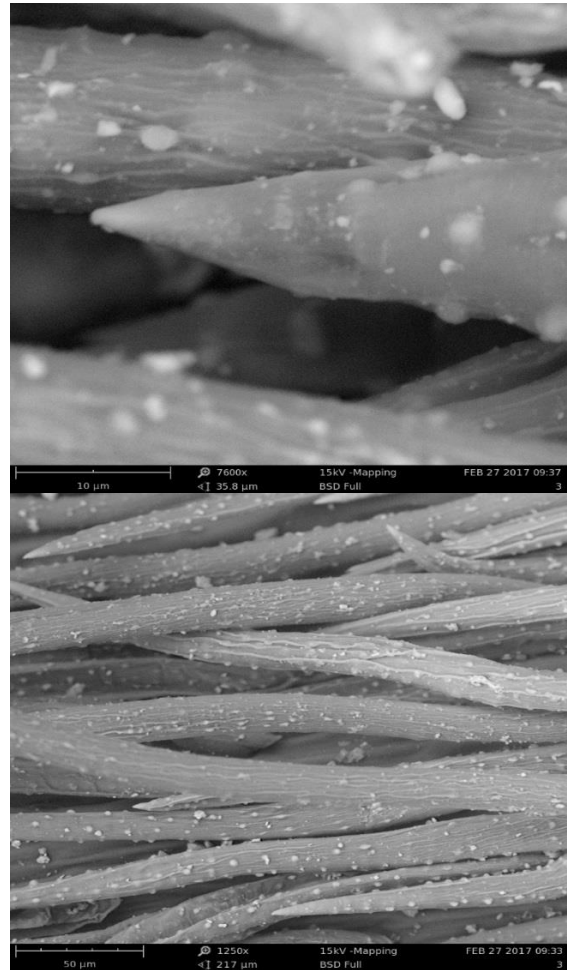


Figure 4. Scanning Electron Micrograph (SEM) of Leaf Surface

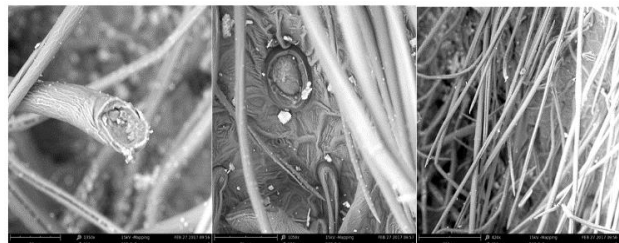


Figure 5. Scanning Electron Micrograph (SEM) of Calyx Surface with Simple Hair and Basifixed in *Astragalus Semnanensis*

Ecology

This plant was found in 68% of the plots and at an altitude of 1500 to 2000 meters (rarely at altitudes of 2245 meters) in most northern and eastern slopes. All the places where *A. semnanensis* is found are shown in Figure 6 and Table 1.

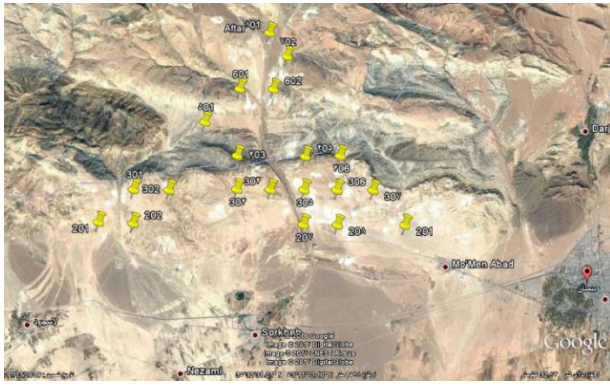


Figure 6. Shows Stations of *Astragalus Semnanensis* in Map

Table 1. Shows Geographical Condition of Stations of *Astragalus Semnanensis*

Station	Slope	Elevation (m)	N	E
2011	S	1608	35 28.758	53 5.209
2021	0	1462	35 29.131	53 6.876
2071	N	1423	35 31.6	53 12.625
2072	0	1400	35 31.76	53 12.452
2073	0	1400	35 31.75	53 12.440
2081	E	1361	35 31.955	53 13.406
2082	0	1423	35 32.088	53 12.739
2083	0	1474	35 32.474	53 12.682
2101	SW	1248	35 33.715	53 16.609
3011	0	1492	35 29.413	53 5.269
3012	S	1455	35 29.44	53 5.744
3021	0	1600	35 29.968	53 7.193
3023	E	1650	35 30.313	53 6.107
3041	SE	1555	35 30.32	53 8.86
3042	0	1479	35 30.809	53 9.466
3051	0	1493	35 32.307	53 11.553
3061	0	1567	35 32.688	53 12.514
3062	S	1457	35 33.386	53 14.254
4031	EN	1647	35 31.637	53 9.445
5011	N	1663	35 32.575	53 7.8
6011	E	1776	35 33.778	53 7.865
6012	0	1680	35 33.667	53 8.013
6021	0	1678	35 33.954	53 9.144
7021	0	1796	35 35.869	53 8.504
8013	N	1840	35 35.893	53 6.336

The average height of the areas where *A.semnanensis* grows was 1537.6 m. The soils of gypsum areas are often flocculate due to the high amount of gypsum and the amount of sand and clay that could not be analyzed. Mean of other soil factors including EC, pH, TNV, gypsum, calcium, magnesium, potassium, and sodium were calculated 2.8 (d.s/m), 7.6, 8.4%, 17%, 25.4 (mg/kg), 12 (Meq/l), 80 (mg/ kg), and 8.5 (Meq/l), respectively (Table 2).

Table 2. Average of Ecological Factors in each of Plots

No.	Name	(Weighted) Mean	Stand. dev.	Inflation Factor
1.	Elevation (m)	1537.6	223.9	2.7

2.	EC (d.s/m)	2.8	0.6	7.3
3.	pH	7.6	0.2	2.8
4.	TNV (%)	8.4	7.1	1.6
5.	Gypsum (%)	17.1	6.4	2.5
6.	Ca (mg/kg)	25.4	6.2	4.8
7.	Mg (Meq/l)	12.0	5.7	4.4
8.	K (mg/kg)	80.0	36.8	1.6
9.	Na (Meq/l)	8.5	10.3	10.4

The effect of ecological factors was investigated in two ways, once only on *A. semnanensis* by RDA analysis in Canoco for windows 4.5 software and for the second time on a set of gypsum plants in this area by CCA analysis.

RDA analysis showed that gypsum and soil calcium factors have the greatest effect on the abundance of *A. semnanensis* species and increasing factors such as magnesium, potassium, EC, sodium, and TNV reduces the abundance of this species. So, they harm the emergence of this species (Figure 7). The eigenvalue and the correlation between the species and the environment in the two axes using the RDA analysis are 0.3, 0.7, 0.5, and 0, respectively (Table 3). Gypsum, magnesium, and sodium are the factors that have the highest value on the axes (Table 4).

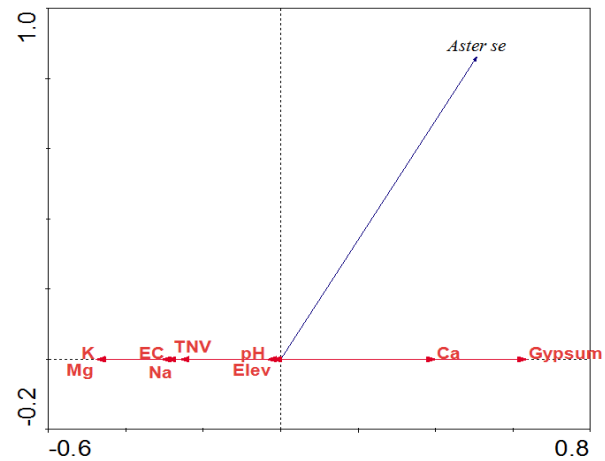


Figure 7. RDA graph of Canoco for Windows 4.5. This Graph Shows Impactions Factors on *Astragalus Semnanensis*.

Table 3. Eigenvalues and Species-environment Correlations of the First Two RDA Axes

Axes	1	2	Total Variance
Eigenvalues	0.3	0.7	1
Species-environment Correlation	0.5	0	
Cumulative Percentage Variance of Species Data	25.7	100	
Cumulative Percentage Variance of Species-environment Relation	100	0	
Sum of all Eigenvalues			1
Sum of all Canonical Eigenvalues			0.3

Table 4. Intra-set Correlations of Environmental Factors with the First Two RDA Axes

	Ax1	Ax2
Elev	-0.0	-0.0
EC	-0.2	-0.3
pH	-0.0	-0.0
TNV	-0.1	-0.3
Gypsum	0.3	0.6*
Ca	0.2	0.4
Mg	-0.2	-0.5*
K	-0.2	-0.5*
Na	-0.2	-0.3

*correlations > ±0.50

Correlation analysis between environmental factors of RDA analysis shows that gypsum factor has a positive and negative correlation with altitude, sodium, EC, and pH factors, respectively. Also, the highest negative correlation was observed between calcium and magnesium. Magnesium, sodium, EC, and pH were positively correlated and all were negatively correlated with altitude. There is a positive correlation between potassium and lime (Table 5).

Table 5. Correlations of Environmental Factors

	Elev	Ca	Mg	TNV	Gypsum	K	Na	EC
Elev	1							
Ca	0.3	1						
Mg	-0.3	-0.8*	1					
TNV	-0.2	0.1	0.1	1				
Gypsum	0.5*	0.5*	-0.5*	-0.2	1			
K	0.2	0.2	0.0	0.5*	-0.1	1		
Na	-0.8*	-0.4	0.5*	0.1	-0.7*	-0.1	1	
EC	-0.5*	-0.2	0.5*	0.2	-0.6*	0.2	0.8*	1
pH	-0.4	-0.6*	0.7*	0.0	-0.5*	-0.1	0.6*	0.6*

*correlations > ±0.50

CCA analysis was used to investigate the effect of ecological factors on a set of gypsophytic species that are also associated with *Astragalus semnanensis* (Figure 8).

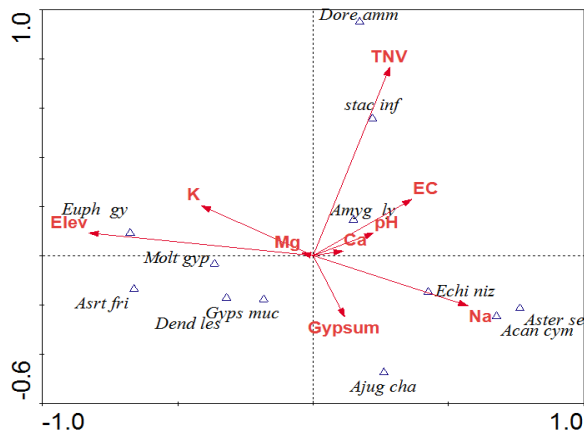


Figure 8. CCA Analysis of Canoco for Windows 4.5

Astragalus fridae, Moltkia gypsaceae, Euphorbia gypsicola, Dendrostellera lessertii, Dorema ammoniacum, Astragalus semnanensis, Acantholimon cymosum, Gypsophila mucronifolia, Amygdalus lycioides, Stachy inflata, Echinops nizvanus

The eigenvalue obtained from CCA analysis in the first two axes is 0.37 and 0.18, respectively; the correlation between species and environmental factors in the first axis is 0.83 and in the second axis is 0.73 (Table 6).

Table 6. Eigenvalues and Species-environment Correlations of the First Two CCA Axes

Axes	1	2	Total Inertia
Eigenvalues	0.37	0.18	2.038
Species-environment Correlations	0.83	0.73	
Cumulative Percentage Variance of Species Data	18.1	26.9	
Cumulative Percentage Variance of Species-environment Relation	47.7	70.8	
Sum of all Eigenvalues			2.03
Sum of all Canonical Eigenvalues			0.774

The factors of height and sodium amount are the factors that have the highest amount in the first axis, the height factor has a negative role and the amount of sodium have a positive role. TNV or lime factor analysis has the most impact on the second axis (Table 7).

Table 7. Intra-set Correlations of Environmental Factors with the First Two CCA Axes

	Ax1	Ax2
Elevation	-0.8*	0.1
EC	0.4	0.2
pH	0.2	0.1
TNV	0.3	0.8*
Gypsum	0.1	-0.3
Ca	0.1	0.0
Mg	-0.0	0.0
K	-0.4	0.2
Na	0.6*	-0.2

*correlations > ±0.50

Gypsum formula is in two forms of dehydrate calcium sulfate and anhydrate calcium sulfate in nature. However, the predominant form of gypsum in the soil is dehydrate calcium sulfate (CaSO4.2H2O) and the anhydrate form is rarely seen [11]. If the gypsum content in the soil is more than 2%, it will be seen as white particles. Contrary to what is expected, gypsum plays an important role in improving the physical and chemical properties of soil [12]. In most soils of the desert, sodium is an obstacle to plant growth because sodium soils are dispersed soil particles, and the presence of sodium prevents these particles from approaching each other and prevents the formation of soil structure. Gypsum solves this problem because C²⁺ easily replaces sodium, bringing clay particles closer together and forming structures. With increasing soil granulation, air, water, and roots penetrate

more easily. Gypsum also improves the ratio of calcium to magnesium, which is in favor of calcium. Magnesium cation is antagonistic to calcium and potassium cations. If the amount of one of these elements in the soil solution increases, the absorption of the other two elements decreases. Too much magnesium also directly affects plant growth. The presence of gypsum as an available source of calcium increases and improves the ratio of calcium to magnesium. Calcium in soil solution reduces the absorption of sodium by the roots. So, in saline and sodium soils, where sodium chloride is the predominant salt, the use of gypsum increases salinity and sodium resistance. The site of calcium and sodium absorption is the same on the root. As the amount of calcium in the soil solution increases, more adsorption sites are allocated to calcium, thus the absorption of the dangerous element sodium is reduced. All of the above were proven in the RDA and CCA analyzes of this article. The presence of gypsum in the soil has provided ecological conditions for *A.semnanensis*. *A. semnanensis* Bornm. & Rech. is a native species of gypsum areas of western Semnan and is an exclusive gypsophila species that grows only in areas with high gypsum. The abundance of this species in the areas around Lasjerd is more than Momenabad and Aftar. *A. semnanensis* has a good association with *Acantholimon cymosum*, especially in the areas around Lasjerd. These two species were predominant in most plots. *Echinops nizvanus* and *Ajuga chamaecistus* are associated with *A.semnanensis* in most areas. Species of almond called *Amygdalus lycioides* was also found in some plots next to these two plants. *A.semnanensis* is less common in the Aftar region, which has a higher altitude and humidity than Lasjerd, unlike other gypsophila species such as *Astragalus fridae*, *Moltkia gypsace*, and *Euphorbia gypsicola*, which tend to higher altitudes. *A.semnanensis* has a higher frequency at lower altitudes and an altitude of 1600 meters towards Lasjerd (near Garmsar). In addition, this plant is observed less than species such as *Dorema ammoniacum* and *Stachy inflata*, which grow in areas with high TNV or lime. The amount of EC and pH in the soils of gypsum area is much more suitable than saline soils and shows the suitability of this type of soil for agricultural work. The vegetative form of the plant, its spiny nature, and its morphological characteristics have greatly contributed to the survival of this plant in the dry climatic conditions of this region. Its anatomical characteristics such as having a high amount of ladder parenchyma tissue and consequently high collenchyma and the presence of Rhachis in its leaves indicate the adaptation of this plant to dehydration and high evaporation in this area. Although this plant grows in conditions with high gypsum soils, the examination of the anatomy of the roots, stems, and leaves of this plant and following the gypsum in this plant showed that a large accumulation of gypsum occurs around the root shell and in the root parenchyma tissue. The plant has adapted to living in gypsum areas and keeps a large part of gypsum in its skin. The thickness of the root skin indicates this, but part of the gypsum still enters the plant. There are these crystals in the leaf parenchyma, but the plant has pushed these crystals out

of the leaf with a special skill, and the presence of dense hair and gypsum sediments on the leaf hair also shows the extraordinary effect of ecological conditions on *A.semnanensis*. There is also dense covering hair on the calyx, but there are no gypsum crystals in it.

CONCLUSION

The study of *Astragalus semnanensis* was carried out in Semnan and specific areas with a dry ecosystem and rich in gypsum and carbonates that are under massive gypsum exploitation. The results showed that there is a limitation of a diverse group of plant species, including many native species, in a potentially endangered environment and possible changes in the range of species distribution soon. Among these variables, soil characteristics and type, as well as height, are the determining factors in *Astragalus semnanensis*. Other gypsum species such as *Astragalus fridae*, *Moltkia gypsaceae*, etc. were also seen with *A.semnanensis*. CCA and RDA analysis confirmed the field observations and showed that Na and K ions hurt the occurrence of gypsum plant species and gypsum factor and in some cases altitude. *A. fridae* grows at high altitudes and low gypsum content while *A.semnanensis* occurs abundantly at low altitudes and higher gypsum content. The anatomical results of leaves of *A.semnanensis* show the plant's high adaptation to drought and gypsum stresses in this area.

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