Original Article

The First Anatomical, Morphological, and Ecological study of the Endemic Iranian *Moltkia gypsacea* from the Boraginaceae family

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ABSTRACT

The climate and soil characteristics of a given region play an important role in the endemism of plants. There are few studies on gypsophytes species vegetation in gypseous desert areas of Iran. Ecological adaptation in ecosystems such as gypsophytes species plant communities in Semnan province leads to the biodiversity of endemic plants. The genus *Moltkia Lehmann* consists of 7 species, belonging to the Boraginoideae family and the Lithospermeae tribe. Three species named *M. caerulea*, *M. gypsaceae*, and *M. longiflora* are listed in the flora Iranica. *M. gypsaceae* was first collected and named by Rishinger from the gypseous areas of Semnan. Studies have shown that *M. gypsaceae* is an independent species. In this paper, western parts of Semnan with an area of 28000 ha were studied as the main habitat of *M. gypsace*. Gypsum soils are widely distributed in certain areas in the western and northwestern parts of Semnan. Spearman's rank correlation coefficient between descriptive variables was studied by ordination analysis on this species. *M. gypsaceae* was found in gypsum areas of Semnan with a frequency of 63% and high frequency at altitudes of 1230 to 1980 m above sea level. *M. gypsaceae* was found only in gypsum soils with endemic and gypsophytes species of Semnan, especially *Astragalus fridae*, but it does not exist in calcareous and salty communities. Studies on the adaptation of this species showed that this species like other gypsophytes species prefers gypsum and elevation and avoids magnesium and sodium. In the anatomical slices of different organs of *M. gypsaceae*, calcium sulfate crystals are well observed. *M. gypsaceae* has obvious and long trichomes with gypsum crystals. These features somehow show the plaster outflow pathway from calcium sulfate crystals called Cystolith, which is stored in its Lithocyte cells.

Keywords: Gypsophytes species, Moltkia, Endemic, Semnan, Ecology.

Introduction

One of the limiting factors of gypsophytes species in different habitats is the characteristics of gypsum soils that distinguish these plants from other plants. Gypsum soils often form in arid and semi-arid regions with an annual rainfall of less than 400 mm and are usually found in places where gypsum bedrock is abundant [1]. The biosystematics of gypsophytes plants to identify plants with limited distribution based on ecological factors such as soil and elevation determines the range of distribution of endemic plants.

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Gypseous species are those that grow exclusively in gypsum soils. There is a great diversity of plants in gypsum habitats [2], but some of them due to climatic and soil characteristics are endemic. Meyer (1986) studied the occurrence of endemic plant species concerning soil type and identified gypsophytes as plants growing on gypsum soils [3]. In the past few decades, sustainable ecological and evolutionary studies have been conducted on the flora of gypsum plants [4]. Pueyo et al. (2007) investigated the relationship of vegetation patterns with a wide range of soil properties related to the topographic position [5]. Except for the Spanish gypsum flora, gypsophytes have been less studied in most parts of the world, especially compared to serpentine and halophyte vegetation. Even in gypsum areas of Spain and the United States with well-documented botanical studies, many new gypsophytes plants in the past decade have been identified. The endemism of plants can be related to genetic changes and manifests themselves in morphological, anatomical, and ecological traits of plants. Special features of climate and soil of the region also play an important role in the endemism of plants. Despite the mentioned points, in gypseous desert areas of Iran, few studies have been conducted on gypsophytes vegetation [6, 7]. Ecological adaptation in ecosystems such as gypsophytes plant communities in Semnan province leads to the biodiversity of endemic plants [8].

The Boraginaceae family, with 1600 to 1700 species and about 90 genera, is one of the largest and most valuable families in the world in terms of anatomical, morphological, ecological, and medicinal characteristics [9]. Common systematic features of this family species include scorpioid inflorescences [10], gynobasic style, and an ovary with two carpels, which are divided into four nutlets [11-14]. The Boraginaceae family is divided into four subfamilies of Boraginoideae, Cordioideae, Ehretioideae, and Heliotropioideae [15-18].

The *Moltkia Lehmann* (1817: 6) belongs to the Boraginoideae subfamily and the Lithospermeae tribe [9, 19-21]. The *Moltkia Lehmann* has 7 species worldwide [22]. Different data have been reported about Moltkia species [19, 23, 24]. In 1817, Lehmann introduced the genus called M. caerulea (Willdenow). In 1844, Boissier mentioned the genus Moltkia with 4 species in the flora Orientalis and divided the species based on the surface of their achenes. Some species have a smooth surface and some have a rough surface. In another study, De Candolle (1846) classified Moltkia into five species in two distinct groups according to the color of their corolla: The first group included species with blue wreath such as *M. punctata* and *M. angustifolia* and the second group included species with yellow wreath such as *M. libanotica* and *M. aurea*. Also, Cecchi and Selvi's (2009) reported that Moltkia is a monophyletic genus [25]. The study of matK chloroplast genes reveals a fundamental gap between this genus and other species. Subsequent studies also created a division between European and Asian species and showed that there are special differences in the flower, fruit, and pollen components of these two areas. Five species comprise two sister clades, the first of which includes European species *M. petraea* and *M. Suffruticosa,* and the second includes three West Asian species *M. aurea*, *M. angustifolia* and *M. caerulea* [25, 26]. The Moltkia section is in Anatolia and the Irano-Turanian highlands and the Echianthus section in the eastern central Mediterranean. The Moltkia section is characterized by an urceolate corolla with curved anthers and ovate achenes. In the Echianthus section, the corolla is not separated from the tube, the anthers are straight, and the achenes are hood-shaped with smooth and shiny surfaces. Three species named *M. caerulea*, *M. gypsaceae* Rech. F. and *M. longiflora* are listed in Flora Iranica [22].

Khatamsaz (2002) believed that *M. gypsaceae* and *M. longiflora* are both called *M. coerulea*, but Ghaffari et al. (2005) conducted a cytological study and proved that *M. gypsaceae* is a species independent of *M. coerulea*. Notably, the only study of the chromosomal content of *M. gypsacea* (n = 20) was conducted by Ghaffari et al. (2005) [27, 28].

Moltkia gypsacea Rechinger and Aellen species were first collected by Rechinger (1950) from the western gypsum area of Semnan and were named Gypsacea (Gypsophyte) [29]. *M. gypsacea* is an endemic species of Iran [30] that is found in its central and southern parts such as Alborz, Kerman, and Semnan. Ranjbar (2019) reported the distribution of *M. gypsacea* in the west of the country and showed that it is an Irano-Turanian species in terms of photographic and grows in dry clay slopes. It grows ecologically in arid and semi-arid regions.

In nature, *M. gypsacea* and *M. caerulea* are not easily recognizable. However, the most important diagnostic features are slight differences in leaf size and type of inflorescence that are significantly more compact in *M. gypsacea* than in *M. caerulea*. This species has been reported from Semnan and Kurdestan [28]. In this regard, morphological, anatomical, and ecological studies of *M. gypsacea* were performed for the first time in gypsum areas of western Semnan. This paper seeks to answer the question of what adaptations have been made to the anatomical, morphological, and micro-morphological characteristics of *M. gypsacea* based on specific ecological conditions and gypsum soils.

Materials and Methods

The western part of Semnan was studied as the main habitat of *M. gypsace*. Semnan is located in the south of the central Alborz Mountains. It has a severe semi-desert and dry and temperate climate, respectively. Geologically, the study area is in the foothill section. Geological structures mainly consist of Eocene sandstones and tuffs of the Miocene marl, gypsum, and salinity formations and Miocene and Pliocene conglomerate formations. Due to the unfavorable climatic conditions in a large area of the province, the process of soil development is very slow. Generally, in the study area, the soil has no evolution and no definite horizon is observed except the gypsum accumulation horizon. Gypsum soils cover most of the area and most of the gypsum mines in the province are found in this area. Topographically, the study area is in the hilly land type and piedmont plain. Gypsum is present in almost all the studied habitats from the ground surface and the highest amount of gypsum is usually observed below an average depth of 5 cm (Figure 1).

Gypseous soils are widely distributed in certain areas in the western and northwestern parts of Semnan, including many gypsophyte plants. The study area is located in the western gypseous habitats of Semnan and in the northern part of the famous desert of Iran (called Kavir plain) between three regions of the west of Momenabad with geographical coordinates of 35° 32′ 38.96″N and 53° 17′ 36.64″E, north, and east of Lasjerd with coordinates of 35° 26′ 27 27.33″N and 53° 05′ 2. 2.8″Eand the south of Aftar with coordinates of 35° 35′ 54.63″N and 53° 7′ 18.63″E. Also, the northern parts of Sorkheh city, located between Lasjerd and Momenabad, were studied. The area has a warm and dry climate and the dominant vegetation cover is gypsophytes. The average annual precipitation of this region is 112.3 mm/year for 10 years and the monthly precipitation range is 0-20 mm (even without summer precipitation). The average annual temperature of this region is 11.3° (ranging from -5 ° to + 37 °) with high evaporation capacity (102 mm on average). These data were obtained from the Meteorological Organization of the Islamic Republic of Iran (IRIMO). The drought period (about 7 months) with the highest drought rate extends from June to September in the study area (Figure 2).

Soil and plant sampling as well as collecting ecological data were carried out at different stations in the gypseous areas in the west of Semnan with an area of 28,000 ha and 100 plots (15 m × 15 m) with a distance of 300-500 m. In addition to studying the species of *M. gypsacea*, its associated species were examined in each plot, soil samples were collected from 30 to 50 cm above the rhizosphere in each plot, and characterization analyses (pH and electrical conductivity (EC)) were performed using standard operating methods by the validated soil laboratory of Semnan Natural Resources Department. Gypsum contents were determined using acetone precipitation method and calcium (Ca), potassium (K), sodium (Na), magnesium (Mg), and calcium carbonate (CaCO3) were measured by standard methods. The correlation between the descriptive variables was measured using Spearman's correlation coefficient and Canoco for Windows software 4.5 was used for ordination analysis (ter Braak and Smilauer, 1998). The ordination charts were drawn in CanoDraw 4.5. RDA is a linear ordination method that is applied to investigate factors on a given species. The importance of each axis of RDA analysis is expressed by eigenvalues, which explain the extent of variations in species data with a combination of environmental variables for each axis [31, 32]. For each group of plants, mean and standard deviation were calculated for environmental parameters. In the anatomy section, transverse slices of the root, shoot, and leaf of *M. gypsaceae* were manually prepared from specimens fixed in ethanol and glycerol (1: 1). Samples were stained with Bismarck brown and methyl green. At least, 5 replicates were carried out for each plant species. Photos were taken with a Leitz optical microscope (Wetzlar, Nikon camera model Coolpix). The tissues of these plants were scaled and measured by Digimizer.4.1.1.0 software. Also, photographs of leaf surface Trichomes of M. gypsacea were taken by scanning electron microscopy (SEM). RDA analysis revealed gypsophytes species position with 9 environmental variables including the content of gypsum, Ca (Ca), potassium (K), Na (Na), Mg (Mg), calcium carbonate (CaCO3), pH, EC, and height. The elevation and content of gypsum and soil calcium are determining factors in the growth of gypsophytes species, especially Moltkia gypsaceae.

Results

Moltkia gypsaceae Rech from the Boraginaceae family, known as the *Moltkia gypsacea*, is a species endemic to Iran. It was first collected and named by Rechinger (1950) from the gypseous areas of Semnan. It is abundant in gypseous areas of Semnan, which is called gypsaceae due to its abundance and distribution in gypseous areas of western Semnan. This species, along with *Amygdalus leciodes*, are among the first plants to start flowering in late winter. Its fruit ripens in April and in June, and its seeds begin to germinate when the weather warms up (Figure 3).

Other species of the Boraginaceae family in the area include *Onosma longiloba* Bunge, *Echium ammonium*, *Heliotropium aucheri* DC ssp. Carmanicum (Bunge) Akhani & Forther., known as desert Chameleon, are endemic to Iran and with frequencies ranging from 10% to 20% in the study area (Figure 4).

Morphological and Anatomical Characteristics of *Moltkia gypsaceae*

Moltkia gypsacea Rech. f. & Aellen has rhizomes of about 10 cm tall. Leaves are lanceolate, without a petiole. Like other species of this family, they have clear Trichomes and basifixed (Figure 5).

The calyx is 5 to 7 mm in height. Fruits are capsule-shaped with 8-9 mm long, inflorescences are scorpioid, and the corolla is tubular. The flowers of this species, which are taller than the corolla, are pollinated by butterflies (Figure 3b). *M. gypsaceae* characteristics include curved corolla, sharp tip, blue to purple flowers, up to 15 cm height, broad and round corolla, and dense inflorescences. The perennial plant, with an average plant elevation of 10 cm, sometimes, reaches 30 cm at the fruiting stage. This species has a rhizome. The leaves are cauline-shaped, without a petiole. Leaves are lanceolate with10-30 mm long and 7-10 mm wide. Inflorescence type is dense cyme, Calyx is 5-7 mm long, and the corolla is cup-shaped and 12-15 mm long. The stamens are projected from the calyx. The corolla lobes are round. The styles are 20 mm long, 5 mm taller than the stamens. The number of stamens in the corolla is four. They are shorter than the styles and the anthers are twisted. The achenes have a rough surface, about 4 mm long and 3 mm wide (Figure 6).

In the spongy parenchyma of the leaves, calcium sulfate crystals are well visible. The plant exudes extra gypsum that has been crystallized with a special function. The plethora of these gypsum crystals on the trichomes is well visible. These gypsum crystals are also observed on the stem trichomes, but in the anatomical cuts of the root and stem, the presence of these gypsum crystals is very limited compared to the leaves (Figures. 7 and 8). The average size of trichomes was estimated to be 674.33 μm (Figures 5, 7, and 8).

Studying the ecological characteristics of *Moltkia gypsaceae*

M. gypsaceae with a 63% frequency was observed in the study area. This is one of the most abundant species in gypseous areas. The mean elevation of this species is 1537.6 ± 223.9 meters above sea level. Table 1 presents the mean soil properties including pH, EC as well as gypsum, calcium, potassium, sodium, magnesium, and calcium carbonated in this study.

RDA analysis revealed the effect of environmental factors on *M. gypsaceae*. The height and gypsum and calcium contents had a positive effect on *M. gypsacea*. EC and magnesium and sodium contents hurt *M. gypsacea*. Elevation and content of gypsum had a significant negative relationship with sodium content, EC, and

PH (Figure 9). Calcium had a significant negative relationship with magnesium and pH. Sodium had a significant positive relationship with EC and consequently, EC had a significant positive relationship with pH. Finally, potassium had no significant relationship with any of the factors (Tables 2, 3, and 4).

Discussion

This article reports on the growth of *M. gypsaceae* in Semnan gypsum soils. The species *M. gypsaceae* were studied for the first time. *M. gypsaceae* was found in 63% of plots at altitudes of 1230 to 1975 meters above sea level. A mixture of gypsum and carbonate was present in all soil samples. The increase in height is related to the content of gypsum and carbonate in the soil (carbonate is indicated by CaCO3 index). *Moltkia gypsaceae* in most of the plots with other gypsum species including *Astragalus fridae*, *Dendrostellera lessertii*, *Dorema ammoniacum*, Euphorbia gypsicola, *Euphorbia bungei*, *Cousinia deserti*, Allium bunge, *Matthiola ovatifolia*, *Gypsophila semypiolia, Acantholimon cymosum, Ajuga chamaecistus, Amygdalus glycosides, Anabasis pelliotii, Calligonum junceum, Centaurea lachnopus, Eremurus loteus, Echinops nizvanus, Heliotropium aucheri, Jurinea radians*, and *Nepeta eremokosmos* form beautiful communities in this area. Rabizadeh et al. (2018) identified six endemic gypsophytes species from western Semnan: *Astragalus fridae*, *Gypsophila mucronifolia, Astragalus semnanensis, Centaurea lachnopus, Euphorbia gypsicola*, and *Nepeta eremokosmos*. The groups of gypsophytes and non-gypsophytes plants were identified by the biosystematics study of gypsophytes plants in the west of Semnan. *M. Gypsaceae* was observed only in gypsum soils. This species was not found in calcareous and salty communities. Ecological factors such as soil and climate factors play an important role in this zonation. Also, by examining the adaptability of *M. gypsaceae* and investigating the morphological, micro-morphological, and anatomical changes of the species, it was found that although the conditions of the substrate are influenced by climate, they affect the adaptation of the plant species. The genetic variability of species over time provides exclusive conditions in specific substrates such as gypsum substrates [33]. In general, soil permeability has a direct relationship with the exchange rate of calcium and sodium. If there is enough moisture at the soil depth, the velocity of salts differs and occurs in different layers [34]. Usually, these dynamic layers are carbonates (on the surface), sulfates (in the middle), and chlorides (in the depth). In these layers, the soil permeability increased by increasing calcium and decreasing sodium. The amount of this permeability affects the electrical conductivity of the soil and vegetation. Results of soil pH and EC analysis indicated that gypsum soils, unlike our expectation, improve soil conditions for growing some plants [35, 36]. *Moltkia gypsaceae* is more abundant at higher altitudes such as the Aftar area (northwest of Semnan), which has higher attitudes and humidity than the other studied places. Compared to species such as *Dorema ammoniacum* and Stachy inflate, which grow in areas with high CaCO3, these two plants are less frequently observed. Interestingly, EC and pH values in gypsum soils are much better than those of saline soils and indicate the suitability of these soils for agricultural work due to optimal ion exchange capacity in these soils. *M. gypsaceae* has a high abundance in gypseous areas and is often seen with *Astragalus fridae*. A study of the adaptation of this species showed that it, like other gypsophila species, prefers gypsum and elevation and avoids magnesium and sodium [33, 35]. In most soils of the desert, sodium is an impediment to plant growth [37], because it is dispersed in the soil and thus prevents other particles from approaching each other and forming the soil structure. Gypsum removes this problem because calcium has twice positive charges and easily replaces sodium, brings the clay particles closer together, and forms a structure [38]. An increase in the soil gradation results in easy air, water, and root penetration. Gypsum also modifies the ratio of calcium to magnesium, which is in favor of calcium. Magnesium cation has a negative (antagonistic) interaction with calcium and potassium cations. So, if the amount of one of these elements in the soil solution increases, the absorption of the other two elements decreases. High magnesium has also directly negative effects on plant growth and development. The presence of gypsum as an available source of calcium increases and modifies the calcium to magnesium ratio. Calcium in soil solution reduces the root absorption of sodium. Therefore, in saline and sodium soils, which the dominant salt is sodium chloride, the use of gypsum increases the salinity and sodium resistance. The adsorption sites for calcium and sodium on the root are the same. By increasing the content of calcium in the soil solution, a greater number of adsorption sites are dedicated to calcium, leading to a decrease in the absorption of the dangerous sodium element [39]. All these have been proven in RDA analysis. The presence of gypsum in the soil has provided ecological conditions for the species in the area. The anatomy study of Iranian species *M. gypsaceae*, despite its endemic and exclusive species, has not been considered in previous studies. This paper addresses the adaptability of *M. gypsaceae* species, which has special conditions in gypsum soils. It also points to the relationship of their anatomy and micromorphology with their ecology or pattern of distribution in the region. Important results were obtained by comparing the adaptability of *M. gypsaceae*. Many plants of specific ecosystems such as gypsum, calcareous, and saline soils have some morphological adaptations that limit transpiration and preserve water in the plant. In gypsum soils, it shows the adaptive abilities of these species and also broadens our understanding of specific endemic gypsum agents [40]. Anatomical cuts of root and stem and leaf of this species indicate a kind of adaptation to the warm and dry habitat of Semnan in this plant, including the presence of gypsum crystals in the leaf and stem gypsophila of M. gypsaceae. Also, Escudero (2014) pointed out that plants that grow under special conditions of soil and climates find special adaptations in their morphological, anatomical, and ecological characteristics [41]. One of the adaptations found in the leaves of gypsophytes plants is the presence of calcium sulfate crystals, sometimes known as cystoliths (elongated cells containing calcium carbonate crystals). Calcium sulfate is accumulated calcium salts [42]. Crystals are found in different organs of *M. gypsaceae*. *M. gypsaceae* has clear long Trichomes with gypsum crystals. They somehow show the gypsum outflow pathway from calcium sulfate crystals called Cystolitte, which are stored in its Lithocyte cells. Anatomical and micro-morphological studies of the species *Moltkia Lehman* were carried out by Dogui et al. (2012) in Turkey. They reported that no calcium sulfate crystals were found in the parenchyma and leaf surface [43]. Also, they confirmed that the anatomical characteristics of *M. gypsaceae* were due to the specific ecological conditions and adaptations that have been created in the gypsum substrate. Grigore et al. (2011) investigated two species of *Ononis tridentata* L. and *Gypsophila struthium* L [44]. The presence of calcium sulfate crystals in the leaf parenchyma, the presence of secretory trichomes in the leaf and stem, and the unique features of the vascular cylinder were the features reported in these two species. These results were confirmed by the characteristics of *M. gypsaceae*. Specific features of *M. gypsaceae*, which were established based on ecological conditions, have also been introduced into the plant genome [28].

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Figure 1. Images of gypseous areas in the west of Semnan (Photo by Fatemeh Rabizadeh, 2017)

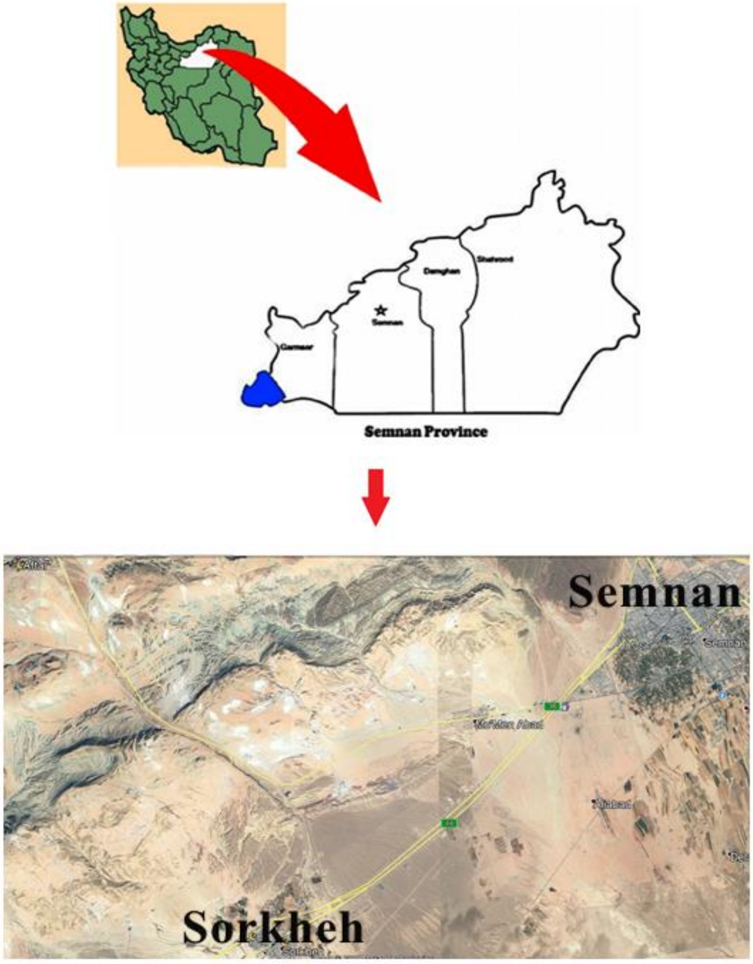

Figure 2. Map of Iran, Semnan province, and gypseous areas studied in western Semnan

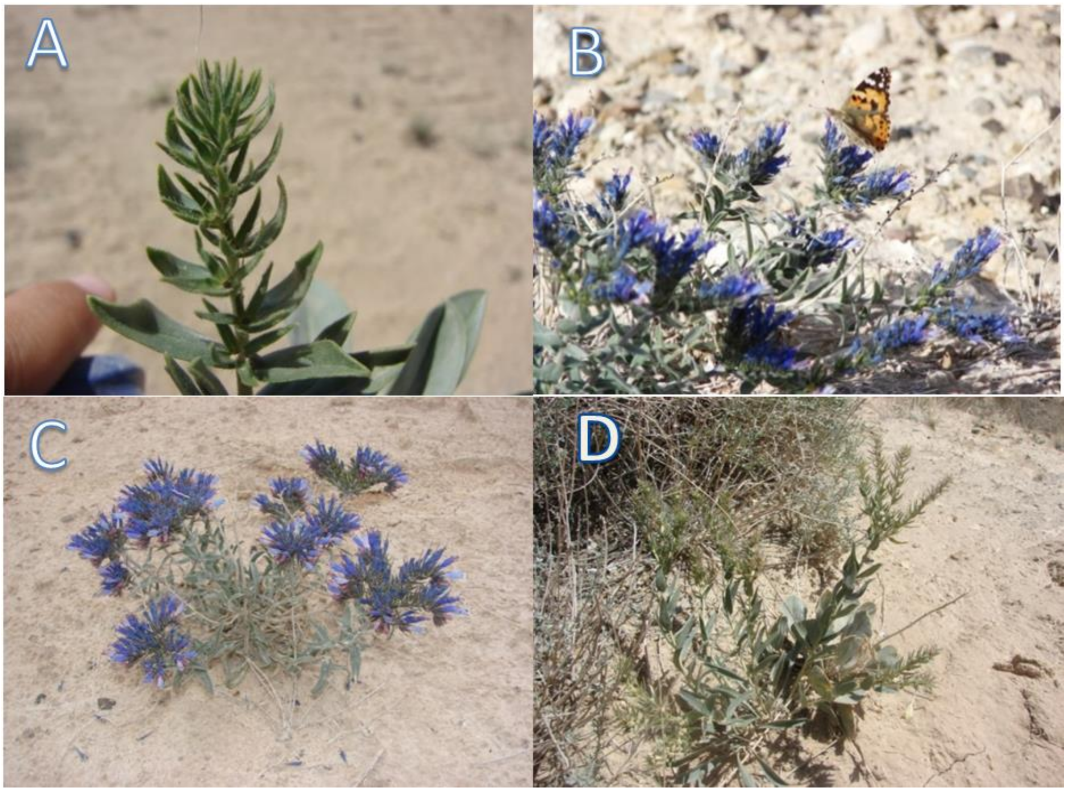

Figure 3. Different vegetative and flowering stages of M. gypsaceae. A) The beginning of plant growth and before flowering, B) and C) Flowering stages, D) End stages of plant growth and fruit and seed formation (Rabizadeh 2017)

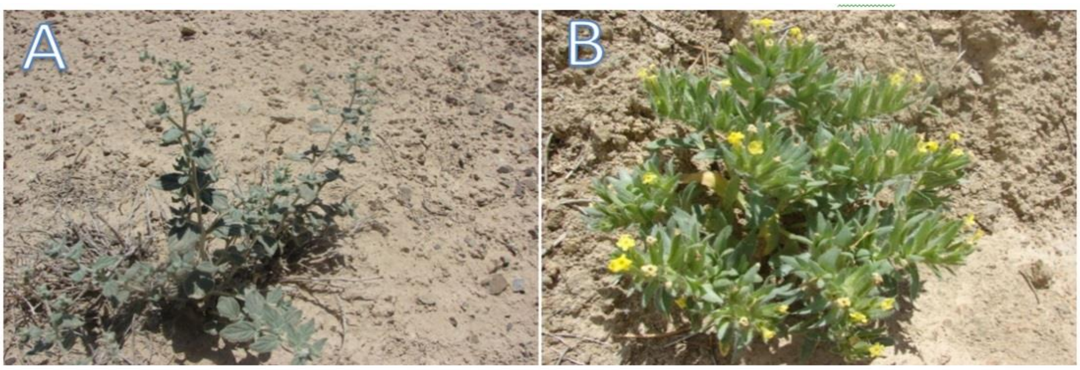

Figure 4. A. .Heliotropium aucheri; B. Onosma longiloba

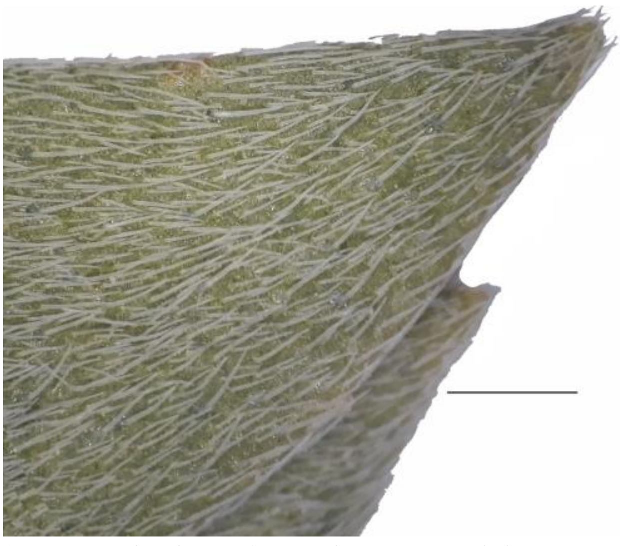

Figure 5. Leaf surface trichomes of *M. gypsace* (scale line = 1 mm)

Figure 6. Upper and the lower surfaces of *M. gypsacea* species (scale line = 1 mm)

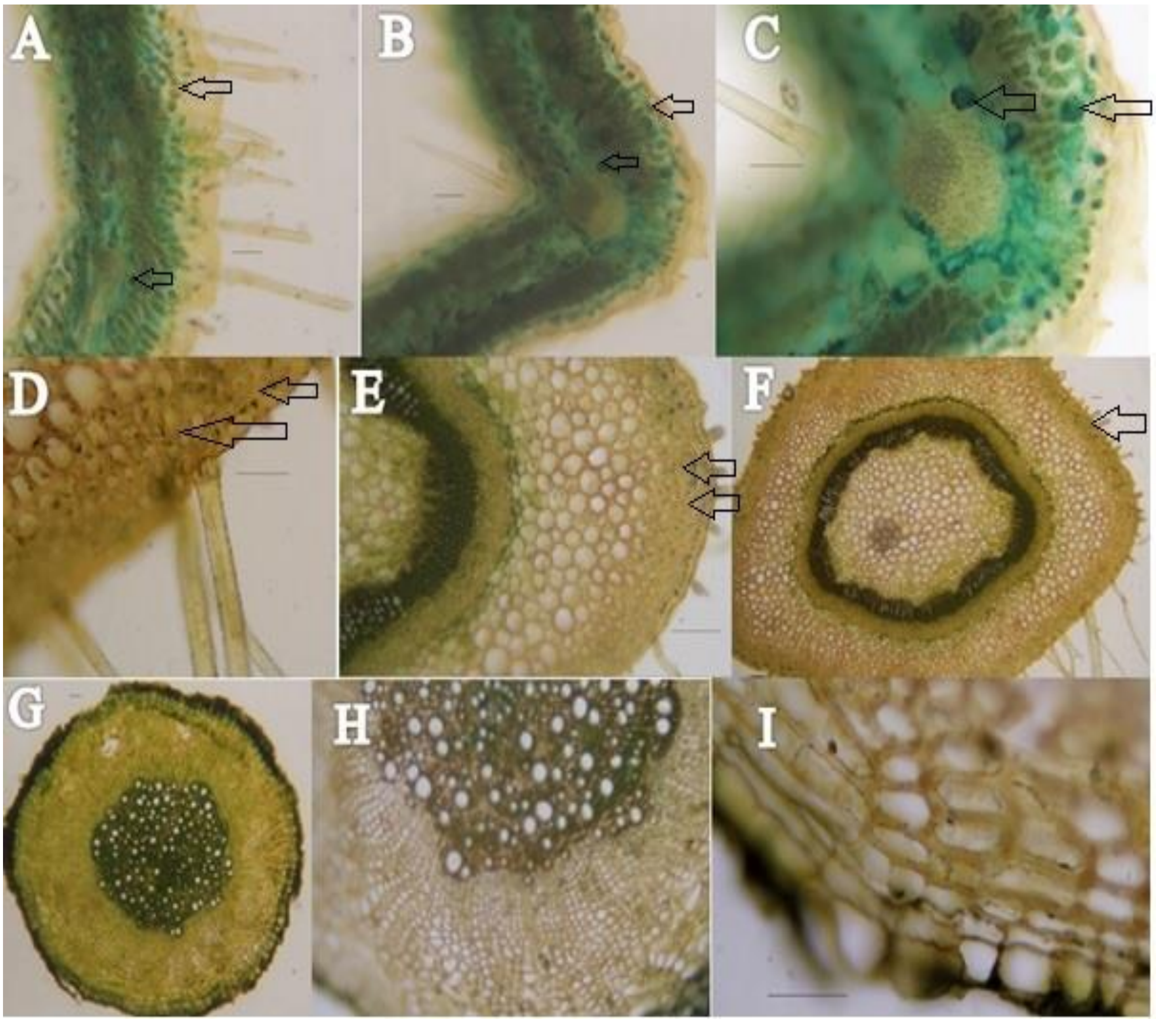

Figure 7. Anatomical cuts of leaves, stem, and root of *Moltkia gypsaceae*: A), B) and C) leaves of the plant; D), E) and F: stem of a plant; and G), H) and I) the root of the plant (scale lines = 200 μm). Signs indicate the location of gypsum crystals in stem and root parenchymal tissues.

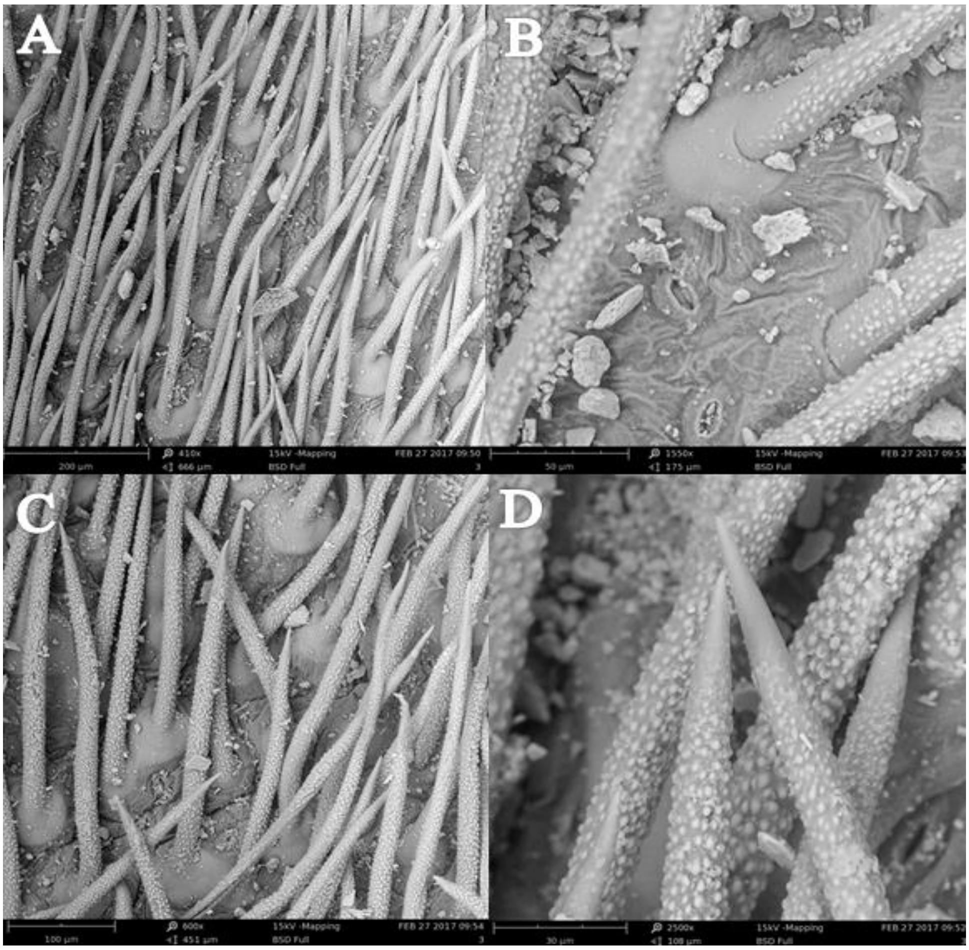

Figure 8. SEM images of the leaf surface of Moltkia gypsaceae

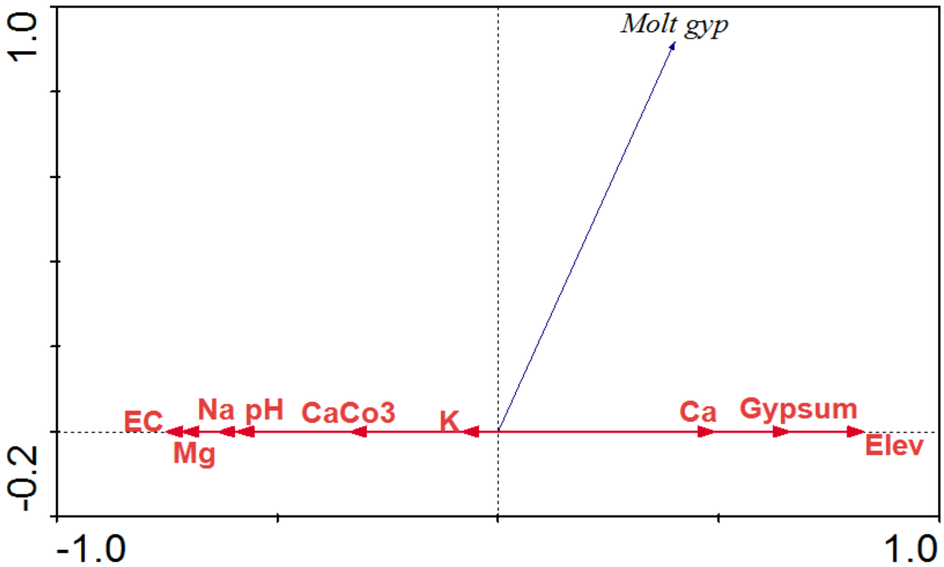

Figure 9. RDA analysis shows 9 environmental factors affecting M. gypsacea including gypsum, calcium (Ca), potassium (K), sodium (Na), magnesium (Mg), calcium carbonate (CaCO3), PH, EC, and height.

Table 1. The mean of ecological factors, soil types, and elements present in the areas where Moltkia gypsaceae grows

Name	(weighted) mean	stand. dev.	inflation factor
Elevation (m)	1537.634	223.9174	2.7164
EC (d.s/m)	2.7683	0.6074	7.3343
pH	7.6244	0.1832	2.8554
Ca (mg/kg)	25.361	6.2517	4.7967
$CaCO_3$ (%)	8.3902	7.0568	1.6424
Gypsum (%)	17.0976	6.4309	2.4705
K (mg/kg)	80	36.8252	1.5853
Mg (Meq/l)	11.9902	5.7008	4.3852
Na(Meq/l)	8.488	10.317	10.3506

Table 2. Correlation Matrix in First Axis of RDA Analysis (* = Correlation >0.5)

Environment variable	Ax1
Elevation	0.83*
Ca	0.49
Mg	-0.63*
$CaCO_3$	-0.33
Gypsum	0.66*
K	-0.08
Na	-0.71*
EC	-0.75*
pH	-0.59*

Table 3. Eigenvalues, species correlation, and environmental factor of the first two axes of RDA analysis

Axes	1	2
Eigenvalues	0.159	0.841
Species-environment correlations	0.399	0
Cumulative percentage variance of species data	15.9	100
Cumulative percentage variance of species-environment relation	100	0