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THE ADAPTABILITY OF EUPHORBIA GYPSICOLA AND EUPHORBIA BUNGEI IN GYPSUM SOILS OF WEST SEMNAN, IRAN

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*Euphorbia gypsicola (*Euphorbiacea, *sect. Pithyusa*) is one of the six endemic gypsophyte species in the west of Semnan Province, Iran, and *E. bungei (*sect. *Sclerocyathium)* is native to the gravelly calcareous or gypseous slopes in southwest Asia. In this study, the adaptability of these two species and the effect of ecological factors on their morphological, anatomical, and micro-morphological characters in the gypseous habitats of West Semnan were investigated using CCA and RDA analytical methods, Canoco 4.5 software. The micromorphological investigations using SEM showed the surface of the leaf and stem of *E. gypsicola* has long hairs with an average length of 224.5 µm, and the surface of the leaf and stem of *E. bungei* is smooth and hairless. The size of stomatal guard cells in *E. gypsicola* leaves is 17.5 µm long and 10 µm wide on average, compared to *E. bungei*, with stomata 9 µm long and 3 µm wide, the stomata of *E. gypsicola* species are bigger and deeper. The presence of small and hairy leaves in *E. gypsicola* fleshy leaves in *E. bungei* (13.4 mm in average length and an average 3.8 mm width), as well as gypsum crystals in the leaves of *E. gypsicola* and in stems of *E. bungei,* are the factors, that help these two species to adapt to the hot desert climate condition of Semnan. Results indicated that the altitude had the highest effect on the abundance of *E. gypsicola*. The increase of Na and Mg content in the soil reduced the abundance of both species.

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Keywords. Adaptation; ecological factors; *Euphorbia bungei*; *Euphorbia gypsicola*; gypsum habitat

سازش‌پذیری دو گونه فرفیون (Euphorbia gypsicola و Euphorbia bungei) در خاک‌های گچی غرب سمنان

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گونه Euphorbia gypsicola از بخش Pithyusa یکی از شش گونه گچ‌دوست انحصاری غرب سمنان است و گونه E. bungei از بخش Sclerocyathium بومی جنوب‌غرب آسیا است و در شیب‌های گچی و یا آهکی ماسه‌ای رشد می‌کند. در این تحقیق، سازگاری این دو گونه و تأثیر عوامل اکولوژیکی بر خصوصیات مورفولوژیکی، تشریحی و میکرومورفولوژیکی آنها در رویشگاه‌های گچی غرب سمنان با استفاده از روش‌های تحلیلی CCA و RDA در نرم‌افزار Canoco 4.5 مورد بررسی قرار گرفت. سطح برگ و ساقه E. gypsicola دارای کرک‌های بلند با طول متوسط ۲۲۴/۵ میکرومتر و سطح برگ و ساقه E. bungei صاف و بدون کرک است. اندازه سلول‌های نگهبان روزنه در برگ‌های E. gypsicola به طور متوسط به طول ۱۷/۵ میکرومتر و عرض متوسط ۱۰ میکرومتر، در مقایسه با E. bungei ، با روزنه‌هایی به طول متوسط ۹ میکرومتر و به عرض متوسط ۳ میکرومتر، روزنه‌های گونه E. gypsicola بزرگتر و عمیق‌تر هستند. وجود برگ‌های کوچک و کرکی در گونه E.gypsicola و برگ‌های گوشتی در گونه E. bungee (به طول متوسط ۱۳/۴ میلی‌متر و به عرض متوسط ۳/۸ میلی‌متر) و همچنین حضور کریستال‌های گچی در برگ .E

gypsicola و در ساقه E. bungei باعث سازش‌پذیری این دو گونه به آب‌وهوای گرم و خشک سمنان شده است. فاکتور ارتفاع بیش‌ترین تأثیر مثبت را روی فراوانی گونه E. gypsicola دارد. افزایش غلظت سدیم و منیزیم باعث کاهش فراوانی هر دو گونه می‌شود.

INTRODUCTION

In gypsum soils, plants grow under unique conditions (Eftekhari & Asadi 2001). Gypsum soils often are formed in arid and semi-arid areas with less than 400 mm of annual rainfall and usually exist in places with abundant gypsum bedrock (Boyadgiev 1974). Nutrient levels in gypsum soils are typically low (Eftekhari & Asadi 2001). Because of the high gypsum levels, the soil has a reduced capacity for cation exchange, and nutritional availability is limited (Castillejo & al. 2012; Escudero & al. 2014). Plant species adapted to gypsum soils develop special morphological and physiological compromises to limit transpiration and water absorption in the leaves. Moreover, plants in dry areas produce thick leaves to balance salt toxicity and tissue water content due to the absorption of alkaline ions (Rabizadeh & al. 2019). According to some studies (Jafari & Tavili 2012), gypsum soils positively affect the growth of some plants. As a result of calcium concentrations at high levels, sodium ions can't exchange and soil solutes are easier to wash away. The presence of a high amount of sodium is considered to be an obstacle to the growth of plants in most desert soils. Due to the sodium content of these soils, the particles of soil cannot come close together and the structure of the soil cannot develop. Added gypsum to the soil solves the calcium cation problem and improves the ratio of calcium to magnesium as well as calcium to sodium. Moreover, the exchangeable calcium to sodium ratio in the soil is directly related to its permeability capacity. As this ratio increases (more calcium or less sodium), soil permeability increases. A soil containing calcium sulfate (gypsum) prevents sodium ions from exchanging and facilitates solute washing. Similarly, chloropotassium also exhibits the same pattern (Jafari & Tavili, 2012; Rabizadeh & al. 2018).

Euphorbiaceae s.l. with 322 genera and about 8910 species, is the sixth-most abundant family of flowering plants. These family members are mostly found in humid tropical and subtropical regions both in the northern and southern hemispheres (Bahadur & al. 2022). The genus *Euphorbia* L. is one of the largest genera of the Euphorbiaceae family with more than 2000 species (Barla & al 2006). *Euphorbia* is one of the most extensive and diverse genera with some members adapted to very specific ecological niches and habitats (Govaerts & al. 2000; Frodin 2004; Pahlevani & Akhani 2011; Webster, 2014). Some members of the genus have considerable economic importance and are used in the pharmaceutical, rubber, and nutrition industries. Many of its species contain toxic milky latex, which has a protective and defensive role against herbivores. Some species are also carcinogenic, invasive, and weeds in many regions (Pahlevani 2007; Nasseh & al. 2018).

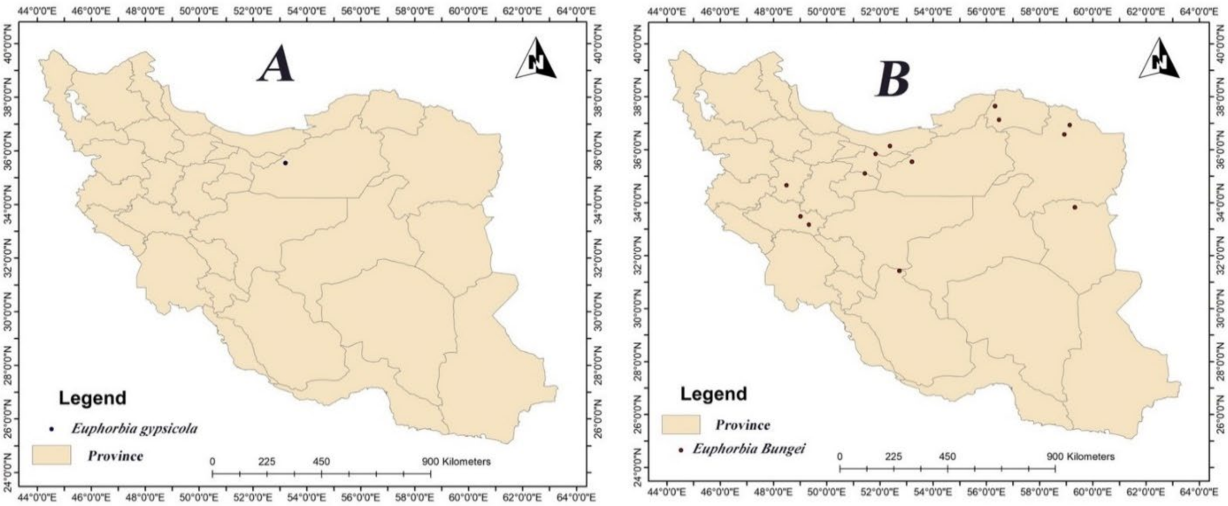


Fig. 1: Distribution map of *E. gypsicola* (A) and *E. bungei* (B) in Iran.

Despite Euphorbia's high morphological diversity and a large number of species, its members can be easily distinguished by their unique morphological synapomorphy, the cyathium, which is a pseudo-inflorescence (pseudanthium), (Steinmann & Porter 2002; Horn & al. 2012; Nasseh & al. 2018). A number of characteristics are important when distinguishing species in the genus, including the structure of the cyathium, capsule shape and surface, seed shape, seed size, ornamentation, and also seed caruncles (Salmakia & al. 2011; Pahlevani & al. 2015). The diversity of *Euphorbia* is centered in southwest Asia with 102 species in Turkey, 92 in Iran, and 50 in Syria (Pahlevani & al. 2020). Members of this genus are compatible with very specific ecological niches and play an important role in different vegetations such as halophytic, gypsophytic, and xerophytic communities (Pahlevani & Akhani 2011; Pahlevani & al. 2017). Some species are often dominant in their respective communities, while some endemic species are rare and limited to a small number of individuals in their habitat, and have been reported exclusively from one location (Pahlevani & al. 2015; Pahlevani 2017; Pahlevani & Amini Rad, 2019).

Subgenus Esula Pers. with approximately 490 species and 21 sections is the most diverse lineage within the genus *Euphorbia* in the temperate regions of the Northern Hemisphere in the Old World and the most diverse in the Irano-Turanian and Mediterranean regions (about 290 species), (Riina & al. 2013; Geltman 2015; Pahlevani & al. 2015; Pahlevani & al. 2017). This subgenus with 19 sections and 184 taxa (176 species) forms the dominant group in Southwest Asia, and most species of the genus in Iran (76 species) belong to this subgenus (Pahlevani & al. 2020). Section *Pithyusa* (Raf.) Lázaro, with approximately 60 species, is the third largest section of subgenus Esula, which is mainly distributed in mountainous and desert areas with limestone beds (Riina & al. 2013; Pahlevani 2017). In addition to conical and smooth capsules, these species have ovoid seeds and irregular cavities decorated with granular elements on the surface, as well as conical caruncles (Pahlevani & Akhani 2011; Pahlevani & al. 2015). More than half of the species in this section are found in the Irano-Turanian region with the highest species richness, followed by the Mediterranean region (Geltman, 2015). Iran with 23 species and 15 endemics is the most species-rich country both in the number of species and endemics for *Euphorbia* section *Pithyusa* (Pahlevani & al. 2020).

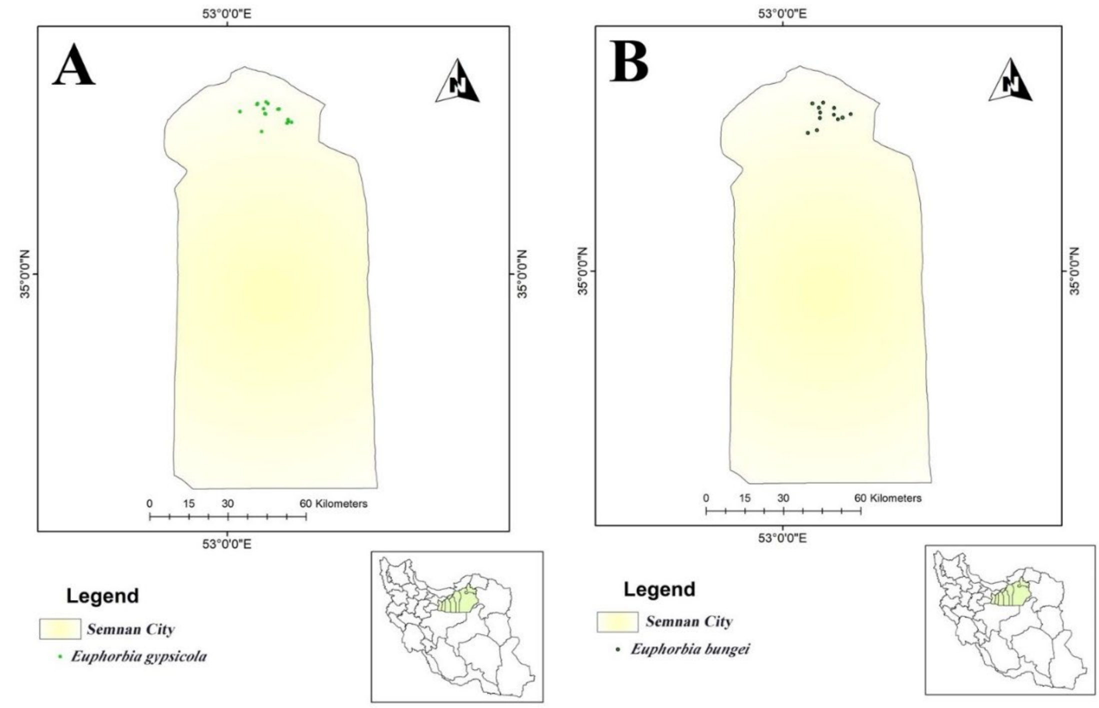


Fig. 2: Distribution of *E. gypsicola* (A) and *E. bungei* (B) in Semnan Province.

Euphorbia gypsicola Rech.f. & Allen*,* of section *Pithyusa,* is an Iranian local endemic, which grows on the gypsum hills of north-central Iran in Semnan province. (Akani 2004; Pahlevani & Amini Rad 2019).

Euphorbia bungei Boiss. belongs to subgenus Esula, section *Sclerocyathium* (Prokh.) Prokh, (Pahlevani & al. 2015; Nasseh & al. 2018). *Euphorbia bungei* is not restricted to the Semnan gypsum soil, it also occurs in the north: Siah Beisheh, between Amol and Damavand; West: Hamadan, Lorestan; Central province: between Isfahan and Abadeh, Ushtrankooh; Northeast: heights between Bojnord and Maravetepe, Hazar Mosque heights, between Mashhad and Qochan, Neishabur mountain, Torbat-Hydrieh, between Birjand and Qain; Semnan Province: Shahroud, Bastam, Damghan, Semnan and Tehran: province (Rechinger & Schiman-Czeika, 1964; Parsa, 1950; Mobayen, 1979; Ghahreman, 2006).

In this study, *E. gypsicolas,* and *E. bungei* were examined in the gypsum habitats of west Semnan Province; using morphological, anatomical, soil characteristics, and environmental factors, and discover the importance of these traits on their adaptability in gypsum habitats.

MATERIALS AND METHODS

This study examined the gypsum habitats in the northern strip of the desert plain in Semnan City, around Lasjard, Aftar, and Sorkheh. These habitats are located between 34º 35' to 37º 35' North and 15º 53' to 17º 53' East, covering an area of approximately 30,000 hectares. The area lies between 1200 and 2100 meters above sea level. It has a hot, dry climate and is mostly covered with gypsophytes, and forms special gypsophyte communities. Ecological data and plant sampling were collected from 35 stations at distances of 2 kilometers from each other. In each station, 2-3 plots were made with a distance of 500 meters from each other and the size of each plot was 25 x 25 meters, and the altitude was measured in each plot. The ecological information of the habitats of the two *Euphorbia* species was recorded in 70-105 plots of the studied area. The distribution of *E. gypsicola* and *E. bungei* in Iran and Semnan provinces is shown in Figs. 1 and 2.

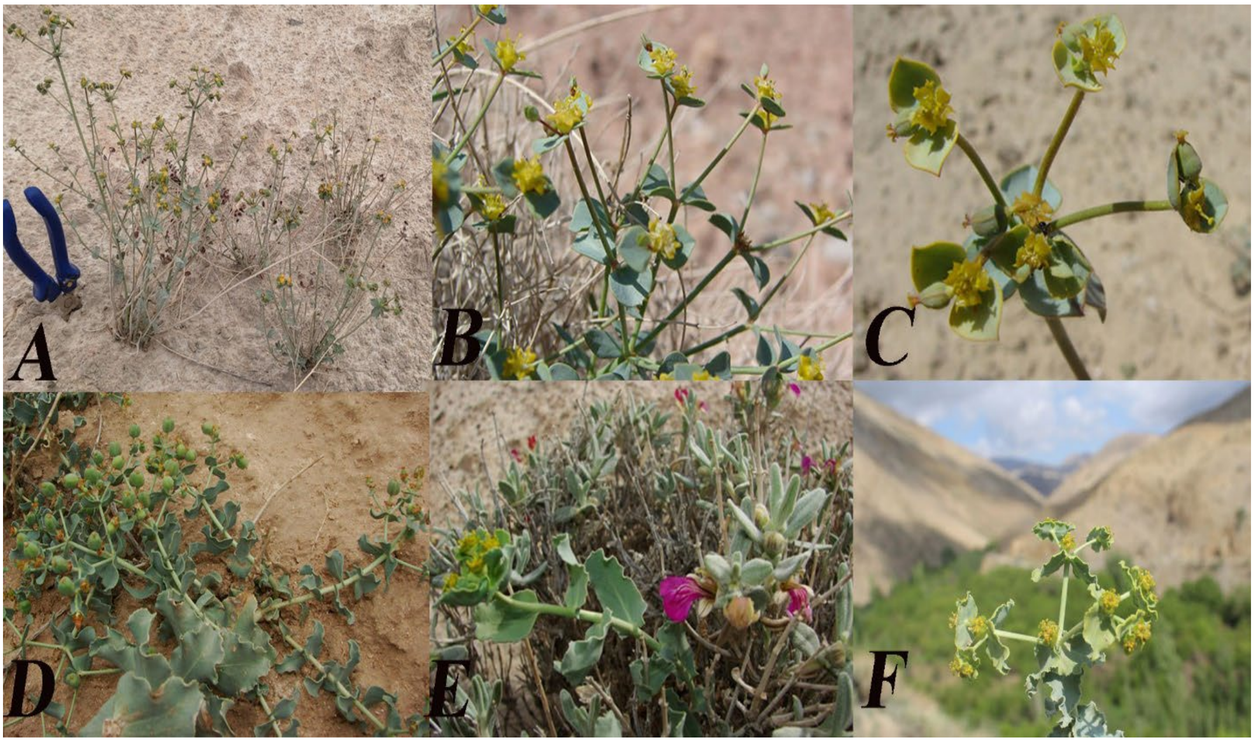


Fig. 3. A-C, *Euphorbia gypsicola,* and D-F, *E. bungei* in the studied habitat. Photos courtesy of Fatemeh Rabizadeh.

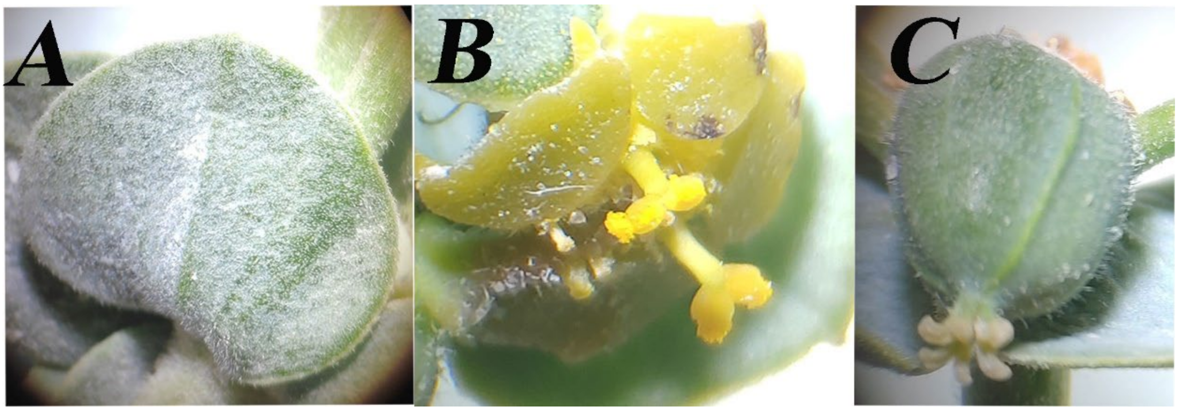


Fig. 4. *Euphorbia gypsicola*, A, leaf; B, flower; C, capsule.

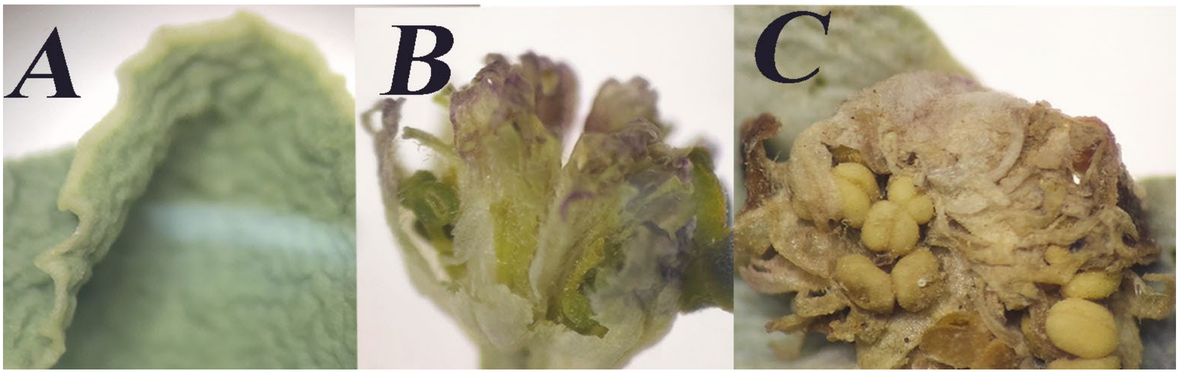


Fig. 5. *Euphorbia bungei.* A, leaf; B, flower; C, capsule.

Soil samples were collected from a depth of 50 cm in each plot and their ingredients including the amount of organic carbon, calcium carbonate ($CaCO_3$), calcium (Ca), potassium (K), sodium (Na), magnesium (Mg), pH and electrical conductivity (EC) were measured using standard methods in the soil science laboratory of Semnan Natural Resources Department.

Fresh leaves of each species (*E. gypsicola* and *E. bungei*) were collected from at least 3 individuals in mid-April. Leaves from individuals of each species were mixed together, and cuttings were taken directly from the leaves. Five leaves from each individual were studied. Leaf anatomical study was based on hand-made cross sections colored with Carmen Zaji and Methylene Blue (Sotoodehnia-Korani 2020). Photographs were prepared using an optical microscope (Leitz model Wetzlar, Nikon camera model Coolpix) with different magnifications. For leaf blade surface analysis, samples were mounted on SEM stubs and spray-coated with gold (ca. 25 nm) and analyzed using an SEM (Tescan, Vega-3 LMU) at an accelerating voltage of 15-22 kV at Semnan University.

The ordination analyses were performed with Canoco 4.5 for Windows, such as RDA (Redundancy Analysis) and CCA (Correspondence Analysis). In this study, RDA shows the correlation of environmental factors associated with the distribution of *E. gypsicola* and *E. bungei* species, and Correspondence Analysis (CCA) was used to analyze the positive and negative impact of the measured environmental variables on the distribution of *E. gypsicola* and *E. bungei.*

RESULTS

Euphorbia gypsicola Rech.f. & Allen is a perennial herbaceous, hairy species; leaves are ovate, hairy, sessile, and entire. Cyathiums are long, capsules subspherical (Figs. 3A-C and 4).

E. bungei Boiss. is a perennial, herbaceous, glabrous, prostrate green to bluish, with many branches. The leaves are dense and thick, succulent, ovate, sessile, amplexicaul, with dentate, and sinuate margins. Cyathiums have green flowers with compound inflorescences, which are placed in umbels of three or in small umbels. Capsule hemispherical, seeds egg-shaped (Fig. 3: D-F and 5).

The average leaf length and width, stem length, and stem diameter in *E. gypsicola* and *E. bungei* are shown in Table 1. The cross-sections of the leaf and stem in the *E. bungei* and *E. gypsicola* clearly showed gypsum crystals in the leaves of *E. gypsicola* (Fig. 6 A) and *E. bungei* (Figs. 6 B, C), and the stem of *E. bungei* (Figs. 6 G, H, I). In the epidermal parenchyma of the stem of *E. gypsicola*, sclerenchyma cells were seen in clusters (Fig. 6 E, F) and in some cases, the pith of the stem was absent in the samples belonging to *E. bungei* (Fig. 6 G, H).

Using electron microscopes, morphological analyses of the leaf and stem surfaces of these two species revealed differences in terms of hair and stomata. In examining the surface of the leaf and stem of *E. gypsicola*, very clear and long hairs with an average length of 224.5 micrometers were observed (Fig. 7 A, B; Table 2); while the surface of *E. bungei* leaf and stem is smooth and glabrous (Fig. 7 C, D; Table 2). In the leaves of *E. gypsicola*, the size of stomatal guard cells is on average 17.5 µm long and 10 µm wide, and the average distance between stomata is 71 µm (Fig. 7 A & B; Table 2), while the size of stomatal guard cells in *E. bungei* is on average 9 µm long and 3 µm wide, and the apertures are located at an average distance of 66 µm (Fig. 7 C, D; Table 2). In general, the stomatal guard cells in *E. gypsicola* are bigger but much deeper compared to *E. bungei* (Fig. 7; Table 2).

Table 1. Measurements of leaves and stems in *E. gypsicola* and *E. bungei*.

	E. gypsicola			***E. bungei***		
	Mean	Std. Deviation	Std. Error Mean	Mean	Std. Deviation	Std. Error Mean
Leaf length (mm)	11.94	1.590	0.53	13.39	2.21	0.74
Leaf width (mm)	9.44	2.07	0.69	7.83	0.61	0.20
Stem length (mm)	145.44	34.76	11.59	97.56	39.61	13.20
Main stem diameter	7.24	0.75	0.259	4.46	0.62	0.21
lateral stem diame-	4.26	0.56	0.19	3.68	0.47	0.16

Table 2. Summary of the observed variation in leaf anatomy and epidermal features of *Euphorbia gypsicola* and *E. bungei.*

Taxon	**Mean Stomata size (width× length µm)**	**Mean Stomata distance µm**	**Mean gypsum crystals size (width× length µm)**	**Mean gypsum crystals distance(µm)**	**Crystal druses in mesophyll**	**Mean hair length (µm)**	**Hair density**
E. gypsicola	10×17.5	71	2.5×12	12.2	dense	224.5	sparse
E. bungei	9×3	66	1.8×10	13.5	dense	-	smooth and glabrous

In the study area, *E. gypsicola* was found in 45% of the plots at altitudes of 1360 to 1975 m. a.s.l. and *E. bungei* in 33 % of the plots and at altitudes of 1400 to 1970 m. a.s.l. Twenty percent of the plots contained both species (Table 3). Based on the physical analysis of the soil, it was determined that 74.67% of the soil content is sand. Mean with a standard deviation of ecological climatic and edaphic factors in the study area are 3.7 mEq/L sodium and 6.7 mEq/L magnesium, calcium 30.5 (mEq/L), potassium 73.3 (mg/kg), gypsum 9.3% and lime 20.6% (Table 4).

Table 3. Habitat, geographical coordinates, and soil elements content in plots containing *E. gypsicola* and *E. bungei.*

Plot Number	*E. gypsicola*	*E. bungei*	Slope Direction	altitude (m)	N	E	Sand (%)	EC (d.s/m)	pH	Ca (mEq/L)	Mg (mEq/L)	TNV (%)	Gypsum (%)	K (mg/kg)	Na (mEq/L)
2072	✓	×	Plain	1400	35	53	86	2/5	7/8	28.4	7.2	3	23	50	7
					31.76	12.452									
2073	✓	×	Plain	1400	35	53	82	2/2	7/5	26.4	7.6	4	20	50	7
					31.75	12.440									
2081	✓	×	E	1361	35	53	80	2/3	7/5	30	3.2	5	21	60	7
					31.955	13.406									
2082	✓	×	Plain	1423	35	53	80	2	7/4	25/2	10	6	23	50	6
					32.088	12.739									
2083	✓	×	Plain	1474	35	53	88	2	7/4	25.2	10	6	20	50	6
					32.474	12.682									
3011	×	✓	Plain	1492	35	53	69	4	7/6	21.6	13.6	1	14	150	28
					29.413	5.269						3			
3021	✓	✓	Plain	1600	35	53	88	2/8	7/6	38.4	8.8	7	24	70	2
					29.968	7.193									
3051	×	✓	Plain	1493	35	53	82	2/5	7/4	26	12.8	1	23	50	1
					32.307	11.553						1			
3061	×	✓	Plain	1567	35	53	82	2/7	7/5	26.8	9.6	9	20	50	2
					32.688	12.514									
3062	×	✓	Plain	1457	35	53	76	2/8	7/5	29.2	9.2	9	21	80	2/5
					33.386	14.254									
4051	×	✓	W	1602	35	53	81	2/4	7/8	34	6.4	3	20	50	1/3
					33.277	10.754									
5011	✓	✓	NS	1663	35	53 7.8	69	2/4	7/6	27.2	10.4	4	10	90	1/3
					32.575										
5041	✓	×	NE	1738	35	53	45	2/6	7/8	22	14	2	12	150	3
					34.707	10.565									
5042	✓	✓	Plain	1724	35	53	45	2/5	7/8	16.8	21.6	1	9	70	1/5
					34.753	10.792						3			
6011	✓	✓	E	1776	35	53	83	2/2	7/6	12	23.2	1	23	50	1/1
					33.778	7.865									5
6012	✓	×	Plain	1680	35	53	65	2/4	7/7	12	23.2	7	24	80	2
					33.667	8.013									
7011	✓	✓	SW	1727	35	53	79	2/3	7/6	31.4	4	6	25	50	1
					34.763	7.595									
7021	✓	✓	N	1796	35	53	77	2/4	7/5	32.4	5.2	3	15	80	1/8
					35.869	8.504									
7022	✓	×	SW	1760	35	53	47	2/7	7/4	32.8	6	1	17	230	4/5
					36.179	8.072						5			
8011	✓	✓	N	1969	35	53	63	2/5	7/4	33.2	4.4	5	23	130	2/8
					35.693	6.218									
8012	✓	×	N	2254	35	53	53	2/5	7/5	30.4	7.2	1	21	50	2/2
					34.184	2.698									
8013	✓	×	N	1840	35	53	87	2.53	7.68	31.2	9.2	3	23	100	3
					35.893	6.336									

Plant growth is influenced by altitude, slope, and temperature at the beginning of its life cycle (Deng & al., 2022). In a comparative study of the vegetative and flowering stages of *E. gypsicola* and *E. bungei* every two weeks in the study area, we observed that these two species appeared earlier on the southern slopes and lower altitudes compared to the northern slopes and higher altitudes. Seedlings emerge from the soil in mid-March, marking the beginning of the growing season for these two species in the studied area. Table 5 shows the different phenological stages of the two species.

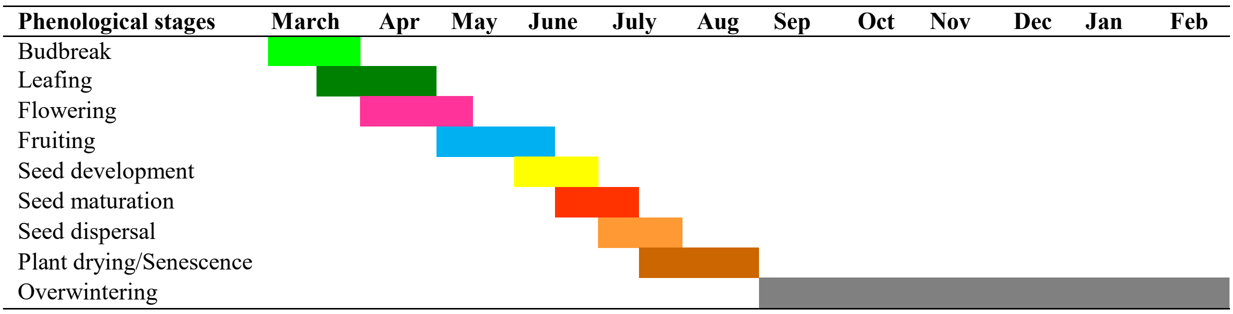
As shown in Table 6, Spearman correlation coefficients are calculated between environmental variables. Based on RDA analysis, the altitude factor had the greatest positive impact on the distribution of *E. gypsicola* species, followed by pH and gypsum. Additionally, the EC factor and lime and calcium contents had the most positive impacts on *E. bungei* distribution. Moreover, most environmental factors affect both species differently; therefore, altitude has the greatest positive effect on *E. gypsicola* and the greatest negative effect on *E. bungei*, and EC factor has the greatest positive effect on *E. bungei* and the most pronounced negative effect on *E. gypsicola.* Additionally, the increase in sodium and magnesium has resulted in a decrease in the abundance of both species (Fig. 8). Cumulative percentage variances for the first and second axes are 66.2 and 37, respectively, compared with 91.4 and 100.4, respectively. As shown in Table 7, the cumulative percentage variance of the relationship between species and environment is 55.8 and 100, respectively. In contrast, it is zero on the third and fourth axes.

In CCA analysis, the correlation between ecological factors and the distribution of two species has been shown. This analysis states that *E. gypsicola* and *E. bungei* are located at two different poles from each other and show little correlation with each other (Fig. 9). Eigenvalues from CCA analysis in the first two axes are 0.45 and 0.11, respectively, and the correlation between species and environmental factors is 0.9 in the first axis and zero in the second axis (Table 8).

Table 4. Mean with a standard deviation of soil analysis in the study area.

Soil analysis	Mean ± SD
Sand (%)	76.00±06.02
Silt (%)	15.20±1.00
Clay (%)	9.33±0.01
EC (d.s/m)	2.50±0.01
pH	7.06±0.09
Ca (mEq/L)	30.53±1.53
Mg (mEq/L)	6.27±0.90
Na (mEq/L)	3.70±0.10
K (mg/kg)	73.33±30.41
SAR	0.75±0.01
P (mg/kg)	2.97±.58
N (%)	0.010±0.00
OC (%)	0.11±0.02
CaCO3 (%)	9.30±2.15
Gypsum (%)	20.67±0.12

Table 5. Phenological stages of *E. gypsicola* and *E. bungei* in the study area.



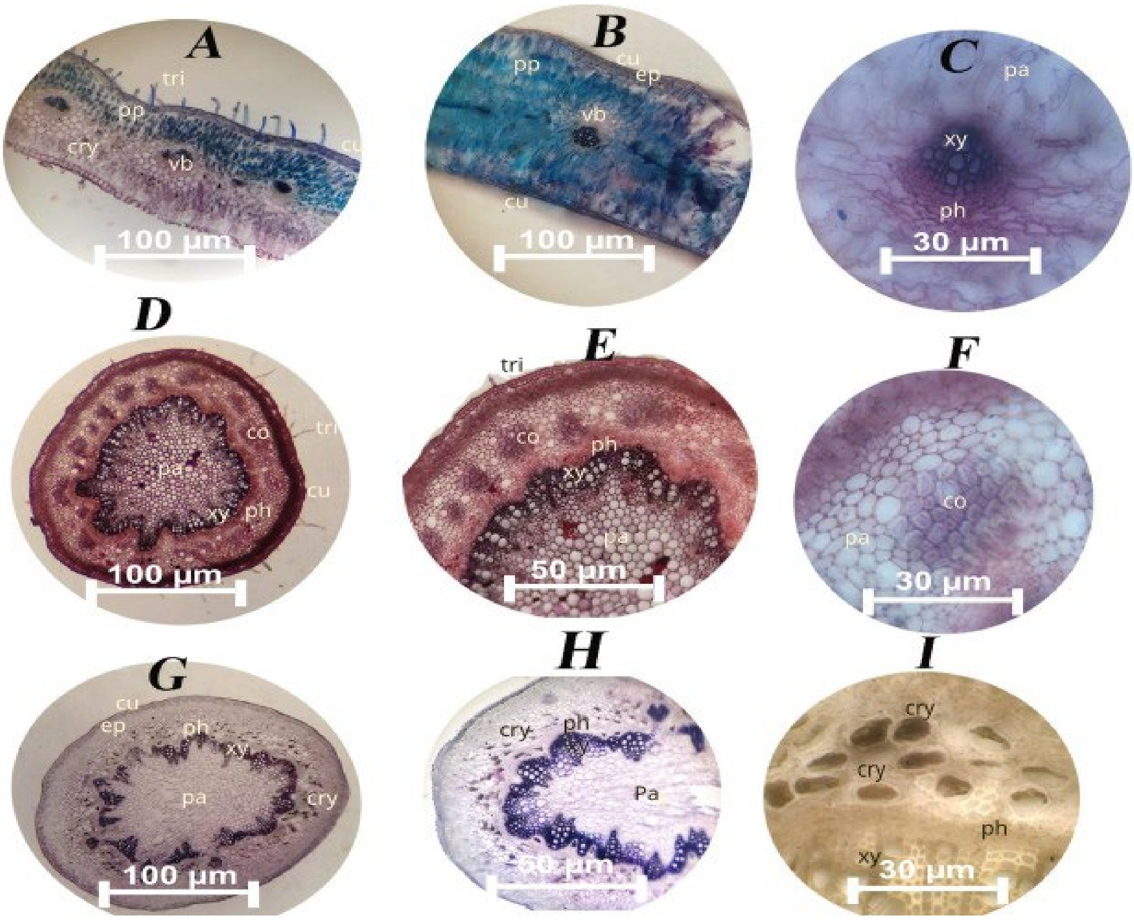


Fig. 6. The cross-sections of the leaf and stem in *E. bungei* and *E. gypsicola*. A, leaf of *E. gypsicola*; B- C, leaf of *E. bungei*; D-F, stem of *E. gypsicola*; G-I, stem of *E. bungei*; cu.: cuticle, ep: epidermis, pp: palisade parenchyma, vb: vascular bundle, tri.: trichome, co: collenchyma, ph: phloem, xy: xylem, pa: parenchyma, cry: crystal.

DISCUSSION

According to this study, *E. gypsicola* and *E. bungei* have adapted to the gypsum soils of Semnan. Approximately 60% of the plots contained both species. *Euphorbia gypsicola,* the gypsum-loving species grows only in Semnan Province, but *Euphorbia E. bungei* is not restricted to the Semnan gypsum soil, it also occurs in other provinces with gypsum or sandy limestone soils. Semnan's gypsum soils represent a high abundance of this species. The morphological, micromorphological, and anatomical features of these two species make them adaptable to the hot and dry climate of the Semnan with high evaporation and low rainfall. These adaptations include small, hairy leaves in *E. gypsicola* and fleshy leaves in *E. bungei*, as well as crystals in the parenchyma of *E. gypsicola* leaves and in the stem of *E. bungei*. The two species have clear idioblasts.

According to the correlation analysis of RDA (Fig.8), soil gypsum ($CaSO_4$) has a positive impact on *E. gypsicola* distribution, and its presence is completely dependent on soil gypsum. In addition to electrical conductivity and calcium and lime levels in soil, the distance between magnesium and sodium is significant in determining the presence of *E. bungei*. The *E. bungei* is actually a halophobe species.

Semnan's soils tend to be saline, containing a high amount of sodium and magnesium salts. The presence of gypsum in the soils of this region solves this problem with calcium cations. It also improves the ratio of calcium to magnesium in the soil and protects plants from high soil alkalinity (Jafari & Tavili 2012). Therefore, the presence of gypsum in the soil of this region has a positive effect on the distribution of *E. bungei*. Gypsum facilitates the exchange of sodium ions and an increase in electrical conductivity.

Table 6. Spearman correlation coefficients between explanatory variables

	Elev	Sand	EC	pH	Ca	Mg	TNV	Gypsum	K	Na
Elev	1									
Sand	0.50	1								
EC	0.29	-0.21	1							
pH	0.02	0.04	0.06	1						
Ca	0.07	0.14	0.46	-0.37	1					
Mg	-0.07	-0.17	-0.30	0.45	-0.92	1				
TNV	-0.11	-0.46	0.37	-0.28	0.11	-0.06	1			
Gypsum	0.06	0.48	-0.11	-0.23	0.10	-0.06	-0.23	1		
K	0.16	-0.30	0.53	-0.12	0.33	-0.32	0.27	-0.22	1	
Na	-0.56	0.49	-0.26	-0.33	0.11	-0.24	-0.12	0.18	0.07	1

Table 7. Eigenvalues and species correlation and environmental factors of the first four axes of RDA

Axes	**1**	**2**	**3**	**4**	**Total variance**
Eigenvalues	0.37	0.29	0.25	0.09	1
Species-environment correlations	0.78	0.86	0	0	
Cumulative percentage variance of species data	37	66.2	91.4	100	
Cumulative percentage variance of species-en-	55.8	100	0	0	
The sum of all eigenvalues					1
The sum of all canonical eigenvalues					0.66

Table 8. Eigenvalues and species correlation and environmental factors of the first two axes of CCA.

Axes	**1**	**2**	**Total variance**
Eigenvalues	0.45	0.11	0.56
Species-environment correlations	0.9	0	
Cumulative percentage variance of species data	80.7	100	
Cumulative percentage variance of species-en-	100	0	
Sum of all eigenvalues			0.56
Sum of all canonical eigenvalues			0.45

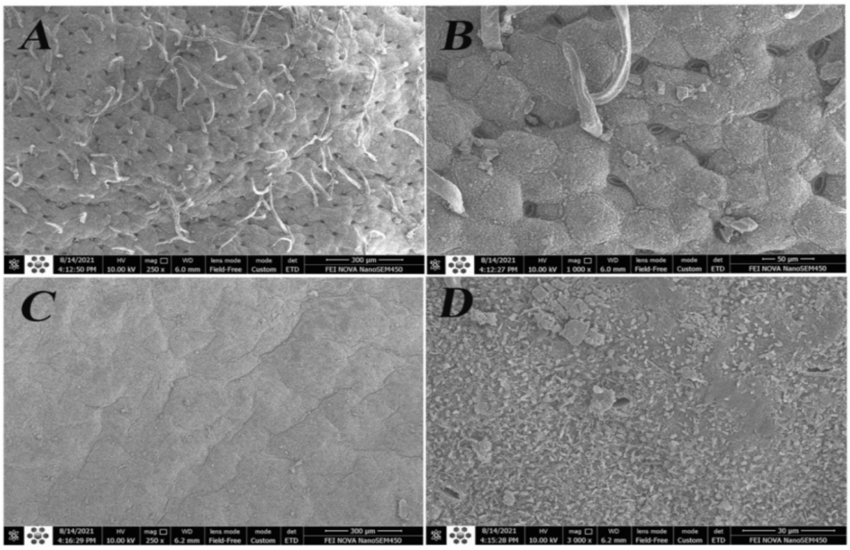


Fig. 7. Scanning electron micrographs of the lower surface of the leaf in *Ephorbia gypsicola* and *E. bungei*. A-B, leaf surface of *E. gypsicola*; C-D, leaf surface of *E. bungei*.

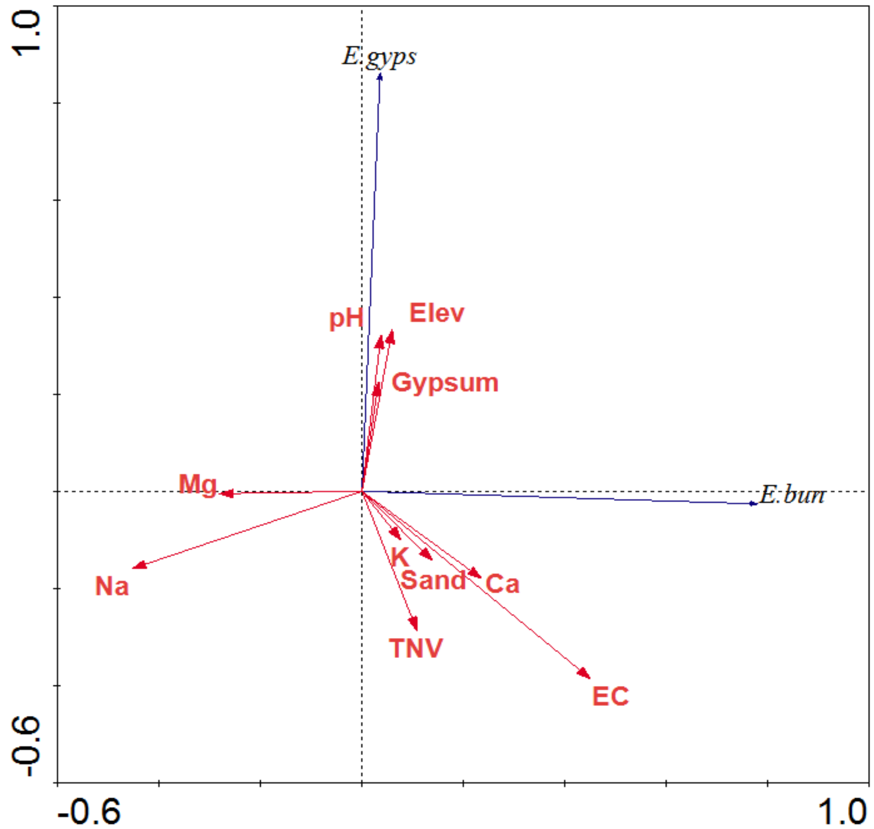


Fig. 8. RDA correlation analysis showing the impact of environmental factors associated with the distribution of species *E. gypsicola* and *E. bungei*.

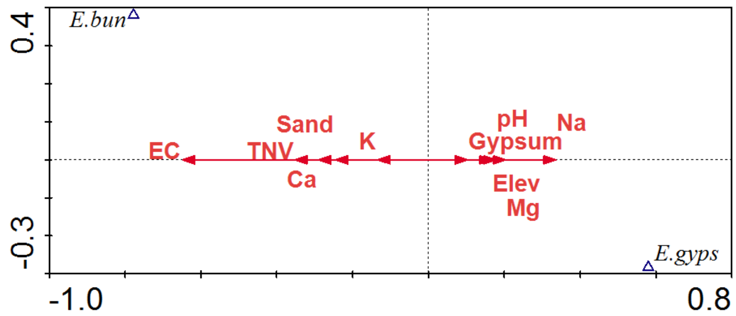


Fig 9. CCA analysis shows the correlation between environmental factors and the distribution of *E. gypsicola* and *E. bungei*.

The presence of crystals in these two *Euphorbia* species plays a significant role in removing excess gypsum from these plants as a result of their ability to store crystals and adapt to gypsum soils that protect them from µm. Crystals play a key role in calcium regulation, metal detoxification, and photosynthesis, in addition to causing plant adaptation to harsh environments (Gomez-Espinoza & al. 2021).

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