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Jipsli Habitatlarda Doğal Olarak Yetişen *Onobrychis germanicopolitana* Hub.-Mor. & Simon (Fabaceae)'nın Ultrayapısal Bulguları ve Uyarlanabilir Yanıtları

Ayşenur KAYABAŞ[1*]

ÖZET: *Onobrychis germanicopolitana* Hub.-Mor. & Simon, Çankırı'da jipsli alanlara endemik çok yıllık bir bitkidir. *O. germanicopolitana*'nın vejetatif ve generatif organları üzerindeki taramalı elektron mikroskobu (SEM) gözlemleri, bitkinin jipsli habitatların belirli koşullarına adaptasyonlarını gösteren ultrayapısal özellikler hakkında ayrıntılı bilgiler sunmuştur. Yaprakların ultrayapısal çalışması, üst yüzeyde daha fazla stoma varlığı ile amfistomatik olduklarını, yoğun yüzey tüylerine sahip olduklarını ve palizat parankimasının (~110 µm) sünger parankimasından (~60 µm) daha geniş olduğunu ortaya çıkarmıştır. Gövdenin enine kesitlerinde sekonder yapı görülmüş ve kambiyum açıkça ayırt edilmiştir. Kök, bir korteks ve periderm ile çevrili büyük vasküler silindirin varlığını göstermiştir. Kaliksin tabanındaki tüy yoğunluğu oldukça fazladır ve kaliks epidermisinin dış yüzeyi altıgen hücrelerden oluşmuştur. Veksillumun dış yüzeyi girinti ve çıkıntı şeklinde kutikular çizgilere sahiptir. Diğer adaptasyon özelliklerine ek olarak, yaprak, gövde ve meyvelerde bol miktarda bulunan tüyler papiller çıkıntılara sahiptir. *O. germanicopolitana*'nın detaylı betimsel ultrayapısal özellikleri ilk kez bu çalışmada bilgi ve referans kaynağı olarak verilmiştir.

Anahtar Kelimeler: Endemik, Jipsofit, Kuraklık, *Onobrychis germanicopolitana*, SEM analizi

Adaptive Responses and Ultrastructural Findings of *Onobrychis germanicopolitana* Hub.-Mor. & Simon (Fabaceae) Growing Naturally at Gypsum Habitats

ABSTRACT: *Onobrychis germanicopolitana* Hub.-Mor. & Simon is a perennial plant endemic to gypsum areas in Çankırı, Turkey. Scanning electron microcopy (SEM) observations on vegetative and reproductive organs of *O. germanicopolitana* presented detailed information of ultrastructural properties, illustrating adaptations to specific conditions of gypsum habitats. The ultrastructural study of the leaves revealed them to be amphistomatic, with more stomata than the upper surface, to have densely surface hairs, and the palisade parenchyma (~110 µm) covers more area than sponge parenchyma (~60 µm). The secondary structure was sighted in the transverse sections of stem and cambium was clearly distinguish. The root showed the presence of large vascular cylinder surrounded by a cortex and periderm. Hair density at the base of the calyx is quite high and the outer surface of the calyx epidermis consists of hexagonal cells. The outer surface of the vexillum has cuticular lines in the form of indentations and protrusions. In addition to other adaptive features, the hairs abundantly found on leaves, stems and fruits had papillary protrusions. The ultrastructural properties of *O. germanicopolitana*, were given for the first time in this study revealing detailed descriptive ultrastructures that as a source of information and reference.

Keywords: Endemic, Gypsophyte, Drought, *Onobrychis germanicopolitana*, SEM analysis

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INTRODUCTION

Fabaceae, also known as Leguminosae or the bean/pea family, is one of the largest plant families and it ranks second to Poaceae in terms of economic importance (Shaheen et al., 2020; Abusaief and Boasoul, 2021; Alimi et al., 2021). Fabaceae, the third largest Angiosperm family after Asteraceae and Orchidaceae, contains 765 genera and ~20000 species (Azani et al., 2017). Turkey includes 72 genera and 1228 taxa belonging to Fabacaea family, 383 of which are endemic, and the endemism rate ~31% (Güner et al., 2012; Latifi and Akan, 2020). Legumes also have the ability to contribute to soil fertility through biological nitrogen fixation for natural and agro-ecosystems. Legumes increase their tolerance to various biotic and abiotic stresses by establishing mutual relationships with arbuscular mycorrhizal fungi (AMF), which helps them meet their nutrient requirements in soils with low phosphorus content (Talebi et al., 2020; Alimi et al., 2021).

The distribution of the genus *Onobrychis* Adans., belonged to the Fabaceae family, is limited to the Paleartic region, including the centers of diversity in Turkey (74 taxa), Iran (52 taxa) and Caucasia (39 taxa) (Güner et al., 2012; Talebi et al., 2020). The rate of plants belonging to the Fabaceae family is high in arid habitats (Shaheen et al., 2020).

Çankırı province, which has been known as a settlement since the Neolithic period (7000-5000 BC), was first named Germanikopolis, then Gangra, and later Çankırı, known as Kengri (TR North Anatolian Development Agency, 2021). There are 1502 plant taxa in Çankırı province, 231 of which are endemic. According to the IUCN Red List by the International Union for Conservation of Nature, there are also 5 critically endangered (CR), 9 endangered (EN) and 15 vulnerable (VU) plant species in Çankırı (Anonymous, 2021a). Plant species growing in Çankırı and taking their epithet from 'Germanikopolis': *Centaurea germanicopolitana* Bornm., *Gypsophila germanicopolitana* Hub.-Mor., *Onobrychis germanicopolitana* Hub.-Mor. & Simon, *Tanacetum germanicopolitanum* (Bornm. & Heimerl) Grierson, *Sideritis germanicopolitana* Bornm., *Helianthemum germanicopolitanum* Bornm. and *Reseda germanicopolitana* Hub.-Mor. *O. germanicopolitana* is a perennial endemic medicinal plant found only in Çankırı (İnan et al., 2012).

Gypsum, the most common sulfate mineral in the world, is also found on Mars (Reiss et al., 2021). The relative area of gypsum soils can be quite high across continents, including 54.7% of Africa, 19.5% of Southern Asia, 25.3% of Central Asia, 0.4% of Europe (Spain and Turkey) and 0.1% of North America. According to the soil map of the world, the climate in gypsum soils is generally Mediterranean, continental and sub-tropical, desertic tropical and sub-tropical, tropical arid and dry (FAO, 2021). In Turkey, the Mediterranean climate is dominant in the provinces with gypsum soils (Çankırı, Çorum, Ankara, Eskişehir, Afyon, Malatya, Kayseri, Sivas and Erzincan) (Özdeniz et al., 2016). The subsidence dolines in Çankırı are one of the most important geological formations, representing a geological heritage with their rare karstic formations, unique landscapes and high diversity (Özcan and Aytaş, 2019).

Since the plasticity of gypsum soil is low, soil particles do not stick together resulting in a high incidence of erosion. At the same time, plant growth can be inhibited in soils with a gypsum content of more than 25% (FAO, 2021). Plants develop morphological adaptations to deal with these extreme conditions. Adaptation, which can be genetic or developmental in origin, includes both physiology and morphology traits (Beyschlag and Zotz, 2017). Morphology has always been important issue to taxonomy, for understanding plant growth and the reflection of abiotic stress on the plant and inferring phylogeny.

Gypsum and saline soils are among the major soils of arid and semi-arid regions of the world (Moghiseh and Heidari, 2012). Gypsum habitats are also often saline. Salinity induces changes in plant organ morphology, anatomy, and ultrastructure. The appearance and cell/tissue structure of root, shoot, leaf, fruit, seed, and flower are affected by stress, or the plant becomes tolerant by adapting via a specific structure (Jahromi et al., 2019).

Drought is considered as one of the most important environmental extremes that constraints to plant survival at gypsum habitats in arid and semi-arid regions. Plants growing under drought conditions have developed a range of anatomical, physiological and/or phenological adaptations to escape or tolerate water stress. Species which escaping drought are generally annual or biennial plants that complete their life cycle in a short time or they acquire permanent structural properties such as bulbs, rhizomes or runners, which remain hidden until conditions are right again (Rivera et al., 2017). The presence of xeromorphic features such as thick epidermis and sclerenchyma, developed bulliform cells, excretory structures, trichome density and succulence seen in drought-adapted plants are the best evidence of adaptation to arid conditions (Abd El-Maboud and Abd Elbar, 2020).

Studies on vegetation and plant ecology in the context of the morphological features that arise in relation to the substrate and arid climatic conditions in gypsum habitats are insufficient. Vegetation in extreme habitats is reflected in the ecomorphological characteristics (physiognomy) of dominant plants. Such traits are thought to be a response to abiotic factors such as substrate and climate. The term eco-morphological traits has been used to name traits of organisms that have a clear adaptive role with respect to environmental conditions. While several modern authors apply it to plants, this term is used more frequently in zoology (Loidi, 2018).

Micromorphological data play a significant role in the classification of Angiosperms (Zareh et al., 2017). While investigating the morphological and anatomical features of vegetative plant parts, especially leaves, by microscopy techniques the adaptive capacity of the species can be observed (Hu et al., 2020; Leshcheniuk and Chipilyak, 2020; Silva et al., 2020). Microscopy creates high-resolution images with high sensitivity that are widely used in morphological microanalysis of plant tissues (Chavarria et al., 2020). Plant parts of Fabaceae are highly variable, especially for epidermal cell types, and it is important to use micromorphological features in the differentiation of some taxa (Abusaief and Boasoul, 2021). The flower is a reproductive organ of evolutionary and systematic importance, and there is a great deal of literature on flower characteristics and ontogeny of flower organs in Fabaceae (Rather et al., 2021).

Scanning electron microcopy (SEM) is a valuable instrument in observing the surface micromorphology of plant parts due to the depth of field and high resolution not easily obtained with light microscopy, significantly increasing the use of microstructural characters in plant morphological studies (Shah et al., 2019). SEM, together with the light microscope, is widely used today to analyze the micromorphological features of most plant species (Majeed et al., 2020; Akhtar et al., 2021; Rosa et al., 2021). SEM provides more clear images of anatomical characters as compare to the LM at some species of family Fabaceae (Shaheen et al., 2020).

Supporting macromorphological features with LM and SEM microscope-based processes is very beneficial in interpreting the response of plants to environmental stress (Kim, 2018; Khan and Khan, 2020; Sadia et al., 2020; Han et al., 2021; Mani et al., 2021; Nazir et al., 2021; Noor and Ahmad, 2021). There are recent studies with SEM of members of the Fabaceae family, but SEM studies that interpret adaptation to abiotic stress are insufficient (Bacchetta et al., 2020; Shaheen et al., 2020; Abusaief and Boasoul, 2021; Guerrero et al., 2021; Kashyap et al., 2021; Soares et al., 2021).

The adaptive responses of the plant to abiotic conditions such as drought and salinity stress were interpreted with the anatomical and ultrastructural structure of the halophyte *Limoniastrum monopetalum* (L.) Boiss. by Abd El-Maboud and Abd Elbar (2020). In several reports, xerophytic features such as the organization of leaves, the presence of a protective outer layer of the epidermis and cuticle and the characteristics of stomata, the presence of gypsum crystals on stems, trichomes and glandular hairs as well as leaves have been interpreted by the anatomical and ultrastructural features of the plant, in the adaptation of gypsophiles to changing environmental conditions (Rabizadeh et al., 2019; Rabizadeh, 2020a; Rabizadeh, 2020b).

In this study, *O. germanicopolitana* plants growing naturally on gypsum habitats of Çankırı (Turkey) were examined. There are no studies by SEM on vegetative and reproductive plant parts of *O. germanicopolitana* up to now. This study seeks to answer the question of which adaptive responses are made to the ultrastructures on the plant parts of *O. germanicopolitana* with respect to drought conditions and gypsum soils.

MATERIALS AND METHODS

Morphology of Plant Samples

O. germanicolitana is a perennial plant that is woody at the base (Figure 1a). Flowering stems are herbaceous and procumbent or arcuate-ascending. Its leaves are linear oblong or elliptic and very closely pilose on both surfaces, eventually glabrescent (Figure 1b). Peduncles are axillary, very short or absent. Calyx lobes are subulate, subequal and densely pilose (Figure 1c). Corolla is rose colored; standard suborbicular, scarcely emerginate (Figure 1c). The legume is semiorbicular, with very short, very closely adpressed hairs, crest 1-3- denticulate (Figure 1d). Flowering term is in June on gypsum hills (Davis et al, 1988; Figure 1e).

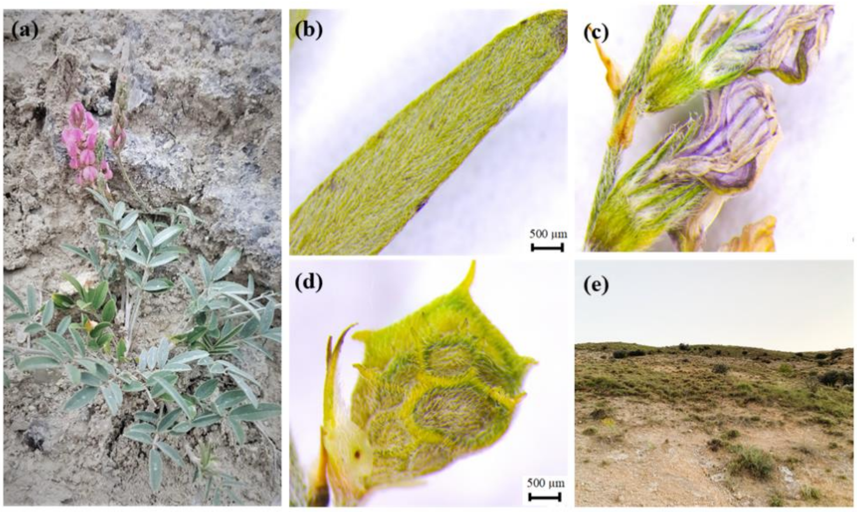

Figure 1. Morphology of *O. germanicopolitana* Hub.-Mor. & Simon (from Çankırı province) observed by using a stereomicroscope (Photos by A. Kayabaş). (a) Plant habit. (b) Leaf surface. (c) Calyx and corolla surfaces. (d) Fruit surface. (e) Gypsum habitat where the species grows. (Scale bars; b, c, d= 500 µm)

The Study Area and Collection of Samples

Geographically, Çankırı province, which is 130 km from Ankara, is located in the Central Anatolia and is in the A4 square according to the grid system of Davis (1965-1985). Çankırı province is covered by Oligo-Miocene deposits (Sönmez and Celik, 2017) and Dolines are the most common gypsum formations (Özcan and Aytaş, 2019). Topographically, the study area is covered with small hills and soft valleys with gypsum soils covering most of the area.

O. germanicopolitana was collected from gypsum habitats located in Süleymanlı village (700-750 m a.s.l., 40°48'18.6" N, 33°64'69.0" E, Çankırı, Turkey) in May-June 2020. Taxonomical identifications of the *O. germanicopolitana* were made according to Flora of Turkey and the East Aegean Islands (Davis et al., 1988). The plant material identification was determined by the author. Samples have been stored in Çankırı Karatekin University as a personal collection. Plant samples were taken individually from their natural habitat cleaned of soil and dirt using a fine paintbrush or by gently blowing on the sample followed by a wash with distilled water.

Climatological and Meteorological Analyses

Long term meteorological data recorded at the Çankırı station for the period of 1930 to 2020 were obtained from the databases of the Turkish State Meteorological Service (TSMS, 2020). Precipitation in the study area is low from June to October (drought period), which is typical of the Mediterranean climate. Based on long-term meteorological data, the maximum precipitation occurred in December (~45 mm), while the minimum precipitation occurred in September (~18 mm). When multi-annual average of precipitation (mm) is analyzed seasonally, the rainfall regime is Sp.W.Sm.A (spring, winter, summer, autumn, respectively 141.1, 124.9, 82.3 and 69.9 mm) type. The coldest month, with a long-term average temperature of -25 °C, is January, and the warmest, with an average of 42,4 °C, is July. The ombrothermic climate diagram (Figure 2) for Çankırı was drawn using Gaussen's method in the Excel software (Akman, 2010). The climate of the study area in which the steppe vegetation is dominant is characterised by cold winters and hot summers with drought periods (Figure 2). This type of climate can be classified as a cold, semi-arid variant of Mediterranean type, particularly predominant in Central Anatolia (Akman, 2010; Table 1).

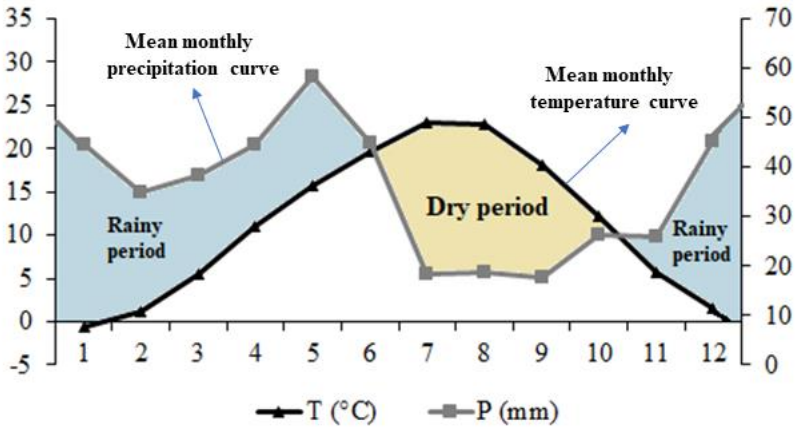

Figure 2. Ombrothermic diagram of Çankırı province

Table 1. Bioclimatic synthesis of Çankırı

Altitude (m)	P (mm)	M (°C)	m (°C)	PE	Q	S	Rainfall regime	Bioclimate
755	418.2	23.0	-0.5	82.3	62.6	3.58	Sp.W.Sm.A	Semi-arid cold Mediterranean climate

P: Average annual precipitation (mm). M: Average of maximum temperatures of the warmest month (°C). m: Average of minimum temperatures of the coldest month (°C). Q: Emberger's pluviometric quotient (Q=2000.P/M2-m2). S: Emberger's index of aridity (S=PE/M)

The Mediterranean climate becomes arid as the quotient value decreases and if the Q value is between 32 and 63, the climate type is semi-arid (Emberger, 1930). Since the Q value is 62.6 in the Çankırı province, the climate type is semi-arid (Emberger, 1930). The water budget for the province of Çankırı was calculated using Thornthwaite's (1948) climate classification and diagram was drawn in the Excel software (Figure 3). While the soil moisture is high in the winter months, there is a water deficit between June and October (Table 2; Figure 3). The months when the water deficit is most evident are July and August. When the details of the water budget of the study area are examined in detail (Table 2), there is an excess of soil moisture (150.40 mm in total) from November to March and a water deficit period (total 324.20 mm) from June to October.

Table 2. Thorntwaite water budget of the Çankırı station for 1990-2020

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec	Total
T	-0.5	1.2	5.6	11.1	15.8	19.7	23.0	22.8	18.1	12.1	5.7	1.5	
i	0.00	0.11	1.19	3.41	5,88	8.26	10.32	10.35	7.25	3.9	0.11	0.15	*I*= 50.93
UPE	0.0	2.4	18.1	43.7	68.9	91.6	111.9	110.6	82.1	48.9	18.5	3.3	
n	0.84	0.83	1.03	1.11	1.24	1.25	1.27	1.18	1.04	0.96	0.83	0.81	
APE	0.0	2.0	18.6	48.5	85.4	114.5	142.1	130.5	85.4	46.9	15.4	2.7	692.00
P	44.7	34.9	38.3	44.6	58.2	45.0	18.4	18.9	17.6	26.4	25.9	45.3	418.20
ST	100.0	100.0	100.0	96.1	68.9	0.0	0.0	0.0	0.0	0.0	100.0	100.0	
AE	0.0	2.0	18.6	48.5	85.4	113.9	18.4	18.9	17.6	26.4	15.4	2.7	367.80
S	44.7	32.9	19.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.5	42.6	150.40
D	0.0	0.0	0.0	0.0	0.0	0.6	123.7	111.6	67.8	20.5	0.0	0.0	324.20

T: Mean monthly temperature (°C). i: Monthly heat index. I: Heat index. UPE: Unadjusted potential evapotranspiration (mm). n: Amplitude index. APE: Adjusted potential evapotranspiration (mm). P: Monthly precipitation (mm). ST: Soil moisture storage (mm). AE: Actual evapotranspiration (mm). S: Soil moisture surplus (mm). D: Soil moisture deficit (mm)

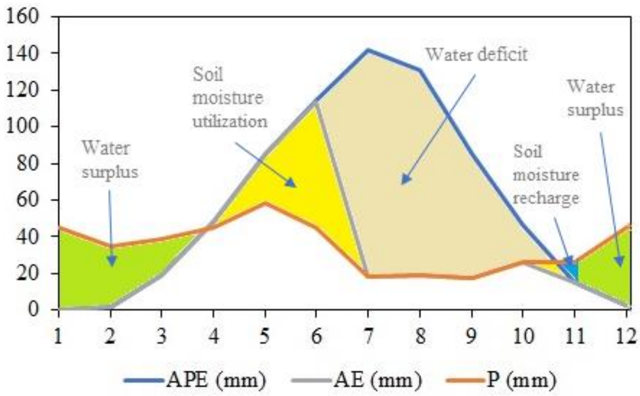

Figure 3. Thorntwaite water budget diagram of the Çankırı province

Sample Preparation for Scanning Electron Microscopy

The morphological characters were examined by SEM. The of vegetative and reproductive parts were studied by using a stereomicroscope and SEM (Gul et al., 2019; Khan et al., 2021). For this purpose, dried plant parts were cut into small pieces and SEM stubs were placed. Firstly, the plant parts were attached using double-sided adhesive tape and plated with gold in a Polaron SC 502 sputter coater. Secondly, the plant parts were investigated at varying magnifications using SEM (JEOL JSM 6060 LV) at 10 kV. Microphotograps were shot at Gazi University. Also, plant parts were photographed with a Leica M125 stereomicroscope.

RESULTS AND DISCUSSION

Vegetative Plant Parts Ultrastructure

Leaf anatomical features of *O. germanicopolitana* indicated the presence of xeromorphic structures. Its leaves have a densely adpressed pilose indument on both surfaces (Figure 4a,5a). These trichomes have protruding papillae on their surface (Figure 4b,5b). The presence or absence of papillae on the hair surface is important for phylogeny (Zarre, 2003). Upper epidermis consist of square and rectangular oval shaped cells (Figure 4c). Lower epidermis consist of rectangular oval occasionally irregular shaped cells (Figure 5c). Zareh et al. (2017) have documented cell wax and epidermal cell shape for the stem and leaf at micromorphological study on the genus *Lotus* L. (Fabaceae). Stomata of xeromorphic type were observed more on the lower surface of the leaf than on the upper surface in *O. germanicopolitana* (Figure 5d). These xeromorphic stomata are found in stomatal crypts. Although the stomata appear to be elevated in the surface view of the leaf (Figure 5d), the guard cells are actually sunken, with raised neighboring cells. These properties, which were interpreted by Yiotis et al. (2006) as retarding the water loss of the plant, were also observed in *O. germanicopolitana*. Its leaves are amphistomatic with the stomata lengths ~15 µm. Amphistomaty, more common in xeric habitats, shortens the distance of CO_2 diffusion to mesophyll cells (Yiotis et al., 2006). The presence of small and non-succulent amphistomatic leaves of *O. germanicopolitana* indicates its tolerance to arid gypsum habitats. Zhu et al. (2019) reported that the leaves of lettuce plants under drought stress decreased in size and that stomatal density increased. Crystalline epicuticular waxes were observed on surface of the leaves (Figure 4d). Epicuticular waxes, which cover the leaves, flowers, fruits and non-woody stems of terrestrial plants, appear as small crystals under the microscope, creating a jagged appearance (Gorb et al., 2008). Gorb et al. (2008) reported that both leaflet surfaces of *Pisum sativum* L. are densely covered with crystalline epicuticular waxes. Shaheen et al. (2020) investigated the leaves of endemic Fabaceae species and reported that epidermal cells, stomata and trichomes are of good taxonomic utility in the Fabaceae.

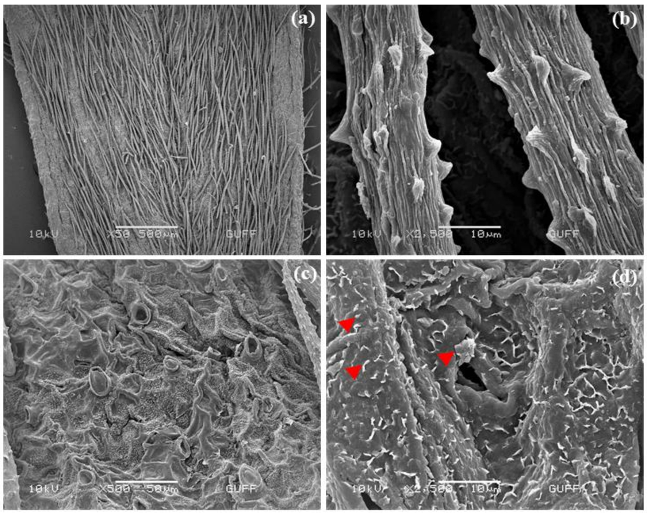

Figure 4. SEM images of the upper surface of *O. germanicopolitana* leaf. (a) The general view of the upper surface, Scale bar = 500 µm. (b) Leaf hairs, Scale bar = 10 µm. (c) Surface shape of the upper epidermis, Scale bar = 50 µm. (d) Crystals (►), Scale bar = 10 µm

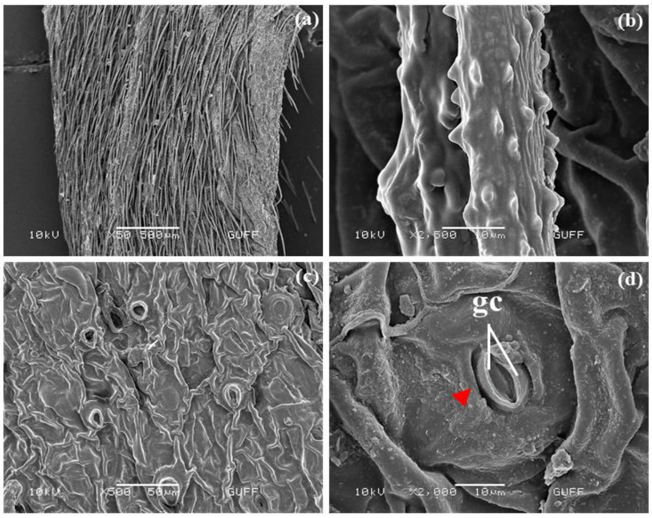

Figure 5. SEM images of the lower surface of *O. germanicopolitana leaf*. (a) The general view of the lower surface, Scale bar = 500 µm. (b) Leaf hairs, Scale bar = 10 µm. (c) Surface shape of the lower epidermis, Scale bar = 50 µm. (d) An open stomata (►), with two guard cells, gc: guard cells, Scale bar = 10 µm

Transverse leaf sections were observed to be dominated by the palisade parenchyma compared to the sponge parenchyma and the mesophyll type was dorsiventral (Figure 6a). The leaves are surrounded by single layered epidermis on the upper and lower surfaces (Figure 6b). Underneath of upper epidermis, there are palisade parenchyma cells (Figure 6b). Their mesophyll thickness was ~160 µm, and the palisade parenchyma (~110 µm) covers a larger area than the sponge parenchyma (~50 µm) (Figure 6c). Palisade parenchyma cells are long and cylindirical or rectangular shaped in *O. germanicopolitana* (Figure 6b). Spongy parenchyma cells are composed of irregularly circular shaped cells with large intercellular spaces (Figure 6c). Since it is believed that abundant palisade tissue also increases the CO_2 absorbing surface of the mesophyll (Terashima et al., 2005), leaf ultrastructure should be examined in detail in order to understand the adaptation mechanisms of plants grown in arid environments.

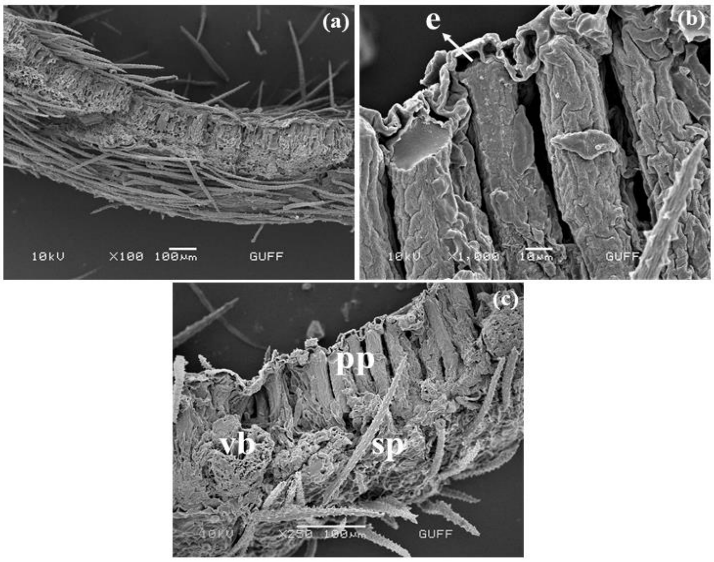

Figure 6. SEM images of transverse section of *O. germanicopolitana* leaf. (a) The general view, Scale bar = 100 µm. (b) Epidermis, e: one layer epidermis, Scale bar = 10 µm. (c) Mezophyll, pp: palisade parenchyma cells, sp: spongy parenchyma cells, vb: vascular bundles, Scale bar = 100 µm

The secondary structure of the stems was observed in the transverse sections, and it is shown to progress from the epidermis on the outside to the cortex (parenchyma cell layer), central cylinder, xylem and phloem, and then pith on the inside (Figure 7a,b,c,d). The epidermis consisted of square or rectangular oval cells in *O. germanicopolitana* (Figure 7b). Underneath the epidermis there was collenchyma with 1-2 layered cells while collenchyma in the cortex was multilayered. The cortex consist of parenchyma cells and cells of cortex were oval and occasionally irregular in shape (Figure 7c). The vascular cambium was clearly distinguish with as a region of 2-5 layers situated between the pith and cortex. Tekin and Yılmaz (2015) noted that in the cross section of the stems of four rare *Onobrychis*, the epidermis cell shapes were square, rectangular or rectangular oval, and that the cortex in these species was surrounded by parenhyma cells.

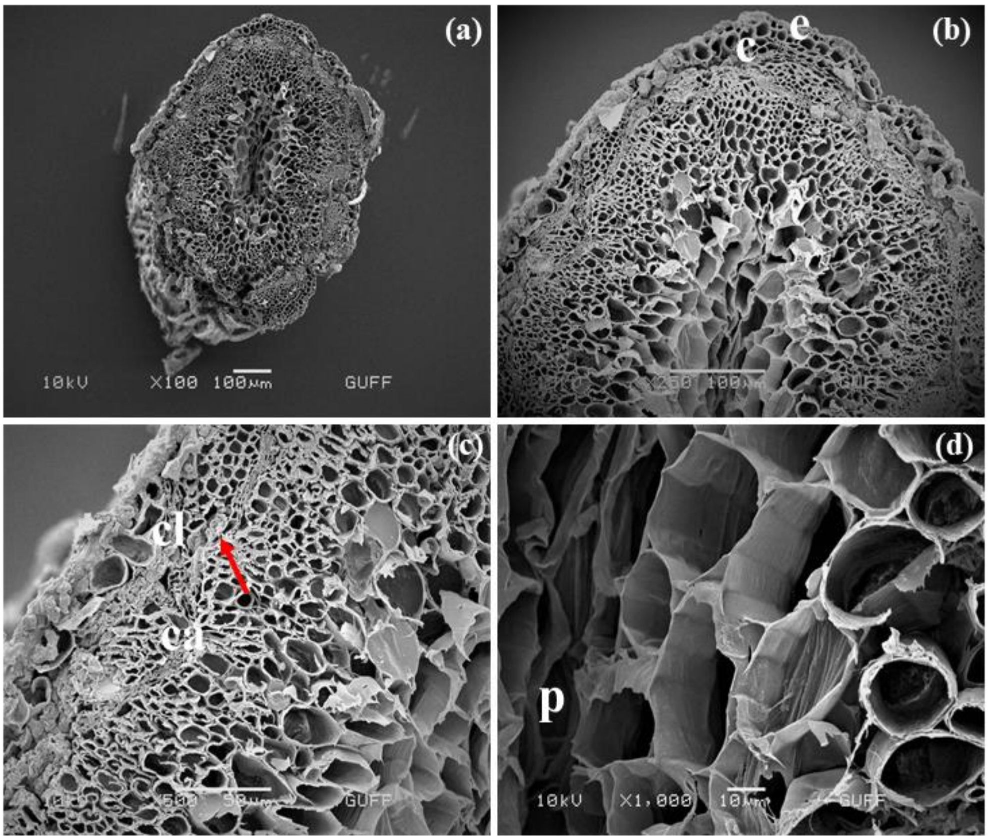

Figure 7. SEM images of transverse section of *O. germanicopolitana* stem. (a) The general view, Scale bar = 100 µm. (b) Parts of stem, e: epidermis, c: cortex, Scale bar = 100 µm. (c) ca: kambiyum, cl: collenchyma, sclerenchyma (→), Scale bar = 50 µm. (d) p: pith, Scale bar = 10 µm

Roots were ~1.3 mm in diameter with a uniform periderm (~11 µm) all around the root (Figure 8a,b). Electron micrographs of the root cross section showed the presence of large vascular tissue (~0.9 mm) surrounded by the cortex and periderm (Figure 8b). In roots of *O. germanicopolitana,* the xylem is found in central core of the vascular cylinder with elements forming radii. The protoxylem vessels were smaller than of the metaxylem vessels (Figure 8c). Tekin and Yılmaz (2015) documented that the stems and roots of *Onobrychis* sp. display a a similar composition.

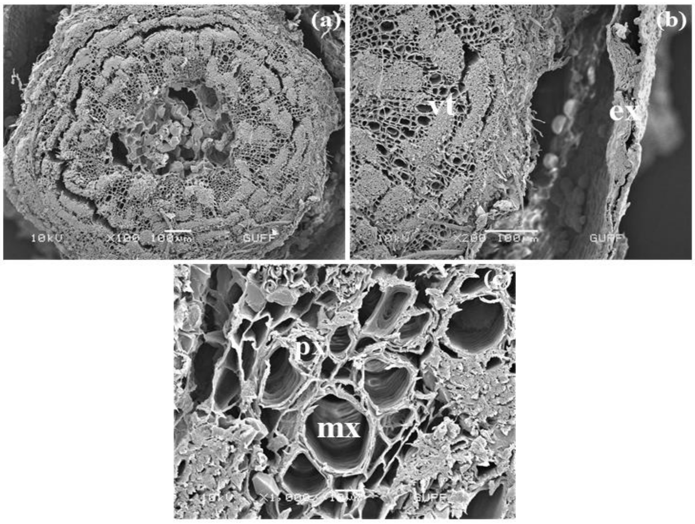

Figure 8. SEM images of transverse section of *O. germanicopolitana* root. (a) The general view, Scale bar = 100 µm. (b) e: exodermis (periderm), vt: vascular tissue, Scale bar = 100 µm. (c) Xylem tissue, mx: metaxylem, px: protoxylem, Scale bar = 10 µm

Reproductive Plant Parts Ultrastructure

The flowers of the plants in the papilionoid Fabaceae are complex. The petals are modified into a vexillum, wings and keel, with the reproductive structures being enclosed by the keel (Etcheverry et al., 2008). The flower of *O. germanicopolitana* (Figure 9a,b,e) is markedly asymmetric except for the calyx, which is zygomorphic. The calyx is tubular and encloses the bases of the petals, taking the shape of a tube. As with the leaf, there are hairs with protruding papillae on the surface of the calyx (Figure 9b,c). Hair density at the base of the calyx is quite high and the outer surface of the calyx consists of hexagonal cells (Figure 9d). The corolla has five petals that form a functional tube enclosed by the elongated tubular calyx and has a dark purple bands (Figure 1c). The outer surface of the vexillum has cuticular lines in the form of indentations and protrusion (Figure 9f). Kochanovski et al. (2018) reported that the presence of protrusions on the surface of the anthers, sepals and petals of *Hymenaea verrucosa* Gaertn. belonging to the Fabaceae family, and that the surface structure of the calyx epidermis showed indented features.

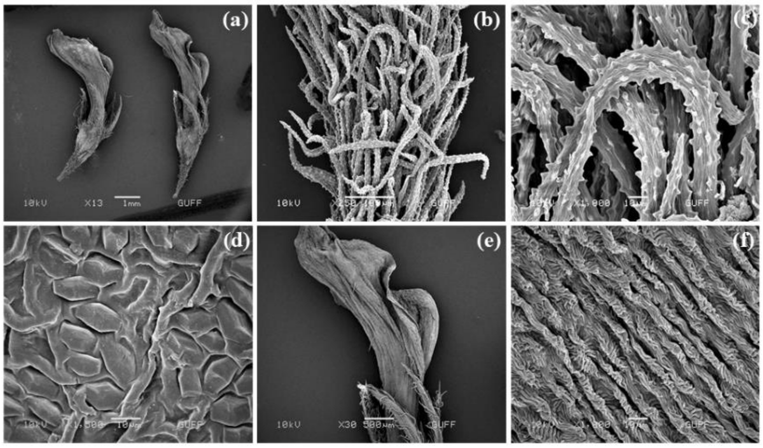

Figure 9. SEM images of *O. germanicopolitana* flower. (a) The general view, Scale bar = 1 mm. (b) Calyx base, Scale bar = 100 µm. (c) Calyx hairs, Scale bar = 10 µm. (d) Calyx surface, Scale bar = 10 µm. (e) Corolla, Scale bar = 500 µm. (f) Corolla surface, Scale bar = 10 µm

The legume in *O. germanicopolitana* is semiorbicular (Figure 10a), with very short, closely adpressed hairs (Figure 10b), crest 1-3- denticulate. Etcheverry et al. (2008) documented that the thickness and shape of sepals and petals, and the joint function of keel-wings showed are directly related to the size of the pollinators of *Vigna caracalla* (L.) Verdc. They also interpreted that the presence of cuticular lines in the form of indentations and protrusion on the outer surface of the vexillum could serve as both a tactile guide for nectar and a foothold for visitors.

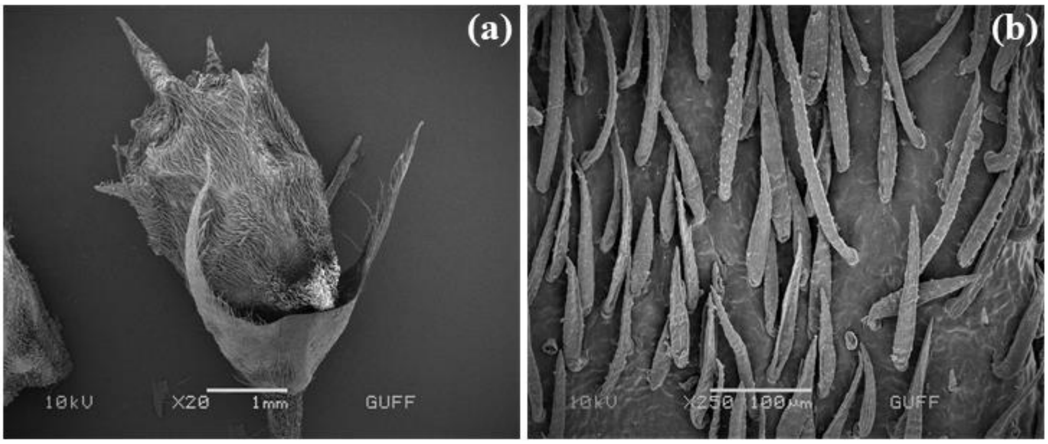

Figure 10. SEM images of *O. germanicopolitana* fruit. (a) The general view, Scale bar = 1 mm. (b) Fruit surface with hairs, Scale bar = 100 µm

CONCLUSION

As a result of a suite of anatomical features, gypsophytes are resistant to saline soils and drought characteristic of gypsum. *Onobrychis germanicopolitana* is a good example of such a gypsophyte. There are also plants such as *Achillea gypsicola* Hub.-Mor., *Alyssum nezaketiae* Aytaç & H. Duman, *Helianthemum germanicopolitanum* Bornm., *Thymus leucostomus* Hausskn. et Velen var. *gypsaceus* Jalas, *Paracaryum paphlagonicum* (Bornm.) R. Mill and *Salvia absconditiflora* Greuter & Burdet in the habitat of the *O. germanicopolitana.* It can survive in extreme environments and face multiple stresses associated with gypsum habitats. Under such extreme conditions, their morphology and ultrastructure provide clues about their adaptive response. It can be concluded that *O. germanicopolitana* has the ultrastructure of a gypsophyte. Here it can be reported that the first ultrastructural investigation of *O. germanicopolitana,* providing a broader overview of the morphology and additional characters relevant to the taxonomic status of this species. In gypsum areas where the species grows, the dry season is quite long and drought severity is high. In order to prevent or reduce water loss during the dry period, plants like *O. germanicopolitana* show morphological and anatomical adaptations of their vegetative structures. Gypsum habitats are used as models for adaptation to extreme habitats. Particularly, the ultrastructure of the endemic *O. germanicopolitana* emphasizes its anatomical adaptations to withstand dryness and atypical mineral soils. Conservation strategies of the species are very important as *O. germanicopolitana*, whose only habitat is in Çankırı province, is point endemic. *O. germanicopolitana* is in the EN category according to the Red Data Book of Turkish Plants (Ekim et al., 2000). *O. germanicopolitana* faces anthropogenic pressures such as grazing, agriculture, and road work in its habitat. Conservation strategies should be developed and extinction should be ensured by further research on the ecological characteristics and habitat preferences of the species. No literature data has been found so far that *O. germanicopolitana* was collected in Turkey or even outside of Çankırı. Therefore, the position and importance of the plant in Turkey also reflects its position in the world.

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Conflict of Interest

I declare that there is no conflict of interest during the planning, execution and writing of the article.

Author's Contributions

I hereby declare that the planning, execution and writing of the article was done by me as the sole author of the article.

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