



Research Article

Leaf Microstructure and Adaptation Relationships in Ten Woody Species from the Semi-Arid Forests

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Abstract

Micro-morphological characteristics of leaves such as stomata and trichomes are reliable indicators of plant response to environmental conditions. The aim of this study was to determine the adaptation strategies of 10 woody species growing in semi-arid Zagros forests in western Iran based on leaf microstructures, focusing on trichomes and stomata using light and scanning electron microscopy. Different types of trichomes and stomata were recognized. Stomata were generally more visible on the abaxial sides of leaves, a feature that helps reduce water loss from leaf surfaces exposed to direct airflow and radiation. Trichomes were classified into two different types: glandular trichomes and non-glandular trichomes with subgroups such as solitary, two-armed T-shaped unicellular, crypt, hooked hairs with cystoliths, fasciculate, stellate, long coiled, tufted stellate, long multiradiate, short bristles with cystoliths, peltate scales, and dendritic hairs. The abundance of trichomes on the leaves indicates their role as biological control parameters. *Eucalyptus camaldulensis* was the only introduced species studied that lacked trichomes on both sides of the leaf. The most frequently observed types of stomata were "Anomocytic" and "Paracytic". The striking differences between the micro-morphological characteristics of the leaves of different species are an expression of the plants adaptations to the environmental ecological conditions.

Keywords: Leaf micro-morphology, Glandular trichomes, non-glandular trichomes, Stomata, environmental conditions, Semi-arid plants.

1. Introduction

Plant functional features, generally considered to be indicators of parameters with key roles in the distribution of species, may be used to predict the responses of trees in forest ecosystems to climate change (Diaz et al., 2007; Birks, 2020). Anatomical and micro-morphological features of plants are the most common features examined in plant studies (Song et al., 2020). Due to ease of measurement of

functional traits, leaves are the first structures considered in research on plants (Cornelissen et al., 2003; Liu et al., 2019). Wang et al. (2017) stated that leaf traits are reliable indicators for whole plants. Leaf is of fundamental importance in plant growth and function, and in long-term environmental adaptations (Royer et al., 2008). As a consequence, adaptive strategies against environmental conditions may be observed in leaf

features (Vendramini et al., 2002; Derroire et al., 2018).

The wide variations in leaf anatomy known from observation of anatomical features such as cell shape, size, and structure, are determined genetically, but influenced phenotypically by environmental factors related to climate, light, precipitation, soil and altitude (Boeger et al., 2004; Kenzo et al., 2004; Sosnovsky, 2015). Leaf microstructures can function as a primary barrier to pests and diseases, but mainly serve to reduce water loss (Yu et al., 2018). In particular, these anatomical features on leaf surfaces increase the ability of plants to cope with environmental conditions, especially drought, as well as functioning in the adsorption of particulate matter in the atmosphere (Rai, 2016).

Trichomes and stomata are of great interest to ecologists and physiologists examining drought-resistance in plants (Karabourniotis et al., 2020). Trichomes have a physical protective role in plant defense against environmental stresses such as drought and potentially damaging solar radiation (Agrawal & Fishbein, 2006). The type and density of trichomes vary between different genera and species, sometimes within species (Uphof, 1962). Trichomes are in single- or multi-celled, and tubular structures (Fahn, 2000; Kolb & Muller, 2004).

The type of stomata is determined by the number of surrounding cells, which play an important role in opening and closing mechanisms (Oyeleke et al., 2004). The morphology and distribution of stomata are influenced by phenotypic and genetic characteristics, and show the long-term adaptation of plants to growing conditions (Hetherington & Woodward, 2003; Bresson et al., 2011).

Several recent publications describe leaf stomata and trichome structures in trees. Examination of leaf stomata of *Betula spp.* using light and scanning electron microscopy (SEM) showed that paracytic and anomocytic stomata were dominant (Kordalivand et al., 2015). Micro-morphological examinations of stomata in three species of hornbeam in the Hyrcanian forest, *Carpinus betulus*, *C. schuschaensis* and *C. orientalis*, were in paracytic, anomocytic, and anisocytic, although there were differences in density and size between the species (Chapolagh Paridari et al., 2012). Five different types of non-glandular trichomes were recognized on the adaxial and abaxial surfaces of leaves of Persian oak

(*Quercus brantii* L.): simple-uniseriate, solitary, fasciculate, multiradiate and stellate (Panahi et al., 2012c). On *Fagus orientalis* Lipsky in Hyrcanian forests, there were three types of trichomes on both sides of the leaf including uniseriate, solitary and unicellular conical (Panahi et al., 2017).

Over 180 woody species occur in the semi-arid Zagros forests in the west of Iran, which cover approximately 5 million ha. These forests are unique due to a rich plant diversity and varied climatic conditions (Sagheb Talebi et al., 2014). The forests occur in a semi-arid temperate climate, with a range of annual precipitation from 400 to 800 mm. The greatest precipitation falls during winter and spring. The summers are extremely arid with a maximum temperature over 45 °C. However, winters are cold, with minima sometimes below -25 °C (Akhani & Ghorbanli, 1993).

So far, little attention has been paid to variations in leaf micro-structure in Zagros forest trees in relation to the environmental conditions. The aim of this work was to examine variations in the microstructures of leaf trichomes and stomata to identify plant adaptation mechanisms in different ecological conditions of natural and plantation forests of the Zagros Mountains, using a combination of light and electron microscopy. This type of fundamental research will assist in elucidating the compatibility features of these tree species that enable survival and vigour in semi-arid conditions, and provide a basis for further eco-physiological studies.

2. Materials and Methods

2.1. Study area

The micro-morphology of leaf traits, stomata and trichomes of 10 woody tree species as mentioned in Table 1 growing in the Zagros forest, was examined on five healthy individuals of each species, with no apparent signs of damage, disease or dieback. These 10 tree species are a mixture of the introduced and endemic woody species as well as the important species and that can be seen widely across the region. Ten fully-matured, sun-light exposed leaves were selected from the outer-middle part of the tree crowns facing the site slope, returned to the laboratory and fixed in FAA solution (Formaldehyde: Alcohol: Acetic Acid, 10%:50%:5% + 35% water, v/v/v).

The study areas are located in the Zagros forest ecosystem, which ranges from northwest to southeast Iran, and is categorized as a semi-arid temperate forest (Sagheb-Talebi et al., 2014). The

sampling areas were in three forest regions: Ilam and Chavar as temperate semi-arid and Saleh Abad as semi-tropical areas, all in Ilam Province, Western Iran (Figure 1). In Figure 1 and Table 1,

the basic information on sampled trees, site characteristics and geographical features are shown.

Table 1. List of species examined and basic information for each sampling site.

Species Name	Sampling Site	Latitude	Longitude	Altitude (m)	Collector
<i>Acer monspessulanum</i>	Ilam	0635518	3726774	1738	H.R. Naji
<i>Cercis griffithii</i>	Ilam	0637705	3728281	1507	F. Soheili
<i>Cratageus aronia</i>	Ilam	0635546	3726807	1733	H.R. Naji
<i>Eucalyptus camaldulensis</i>	Saleh Abad	0637788	3728180	890	F. Soheili
<i>Fraxinus excelsior</i>	Ilam	0627721	3724618	1365	F. Soheili
<i>Nerium oleander</i>	Chavar	0627710	3724778	1370	H.R. Naji
<i>Olea europaea</i>	Ilam	0634817	3719026	1357	F. Soheili
<i>Populus euphratica</i>	Ilam	0605372	3738602	1075	H.R. Naji
<i>Platanus orientalis</i>	Ilam	0627698	3724764	1363	F. Soheili
<i>Quercus brantii</i>	Ilam	0635855	3726553	1840	F. Soheili

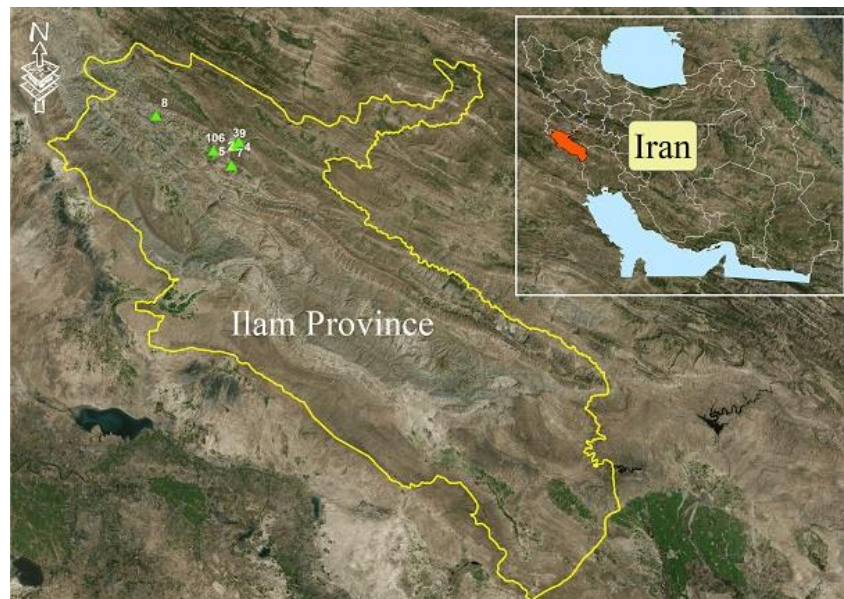


Figure 1: Geographical distribution of sampling site.

2.1. Light Microscopy (LM)

2.1.1. Leaf Sample Preparation

Sampled leaves were boiled in water for approximately 5 min before removing the epidermal layers with a scalpel. To better visualize trichomes and stomata on the abaxial sides, the density of trichomes in some species was reduced using a twin-blade razor and adhesive tape. According to Camargo & Marengo (2011), leaf specimens were placed in sodium hypochlorite

solution (4%) for approximately 5 min. Chlorophylls were removed by immersion in mixtures of (1.5:100 ml) acetic acid (99%) and hydrogen peroxide (30%), respectively at 100 °C for an hour in a Bain-Marie water bath.

After being washed in distilled water for two times, samples were stained in aqueous safranin O, Astra-blue, and Lugol's solution and dehydrated in a series of ethanol 60%, 85%, 95% and absolute for 15 min, before mounting on glass microscope

slides in Canada balsam. Trichomes and stomata were observed under an Olympus CH2 light microscope with an attached digital camera connected to a computer.

2.2. Scanning Electron Microscopy (SEM)

2.2.1. Leaf Sample Preparation

Two leaves from each individual tree were gently washed in distilled water to remove fine dust and solid particles. Fresh tissues were cut from the leaves at a point midway between the main vein and margin, before drying in an oven at $60\pm 3^\circ\text{C}$ for 24 h. and dehydrating in an ascending series of ethanol from 50% to absolute. Specimens were fixed on SEM stubs and again dried in the oven for a further 24 h. Subsequently, specimens were gold-sputtered in a Quorum model Q150R ES (UK) and imaged using a FESEM: TESCAN, model Mira 3 (Czech) SEM.

To measure average pore length and pore aperture size (μm) of stomata on abaxial sides of the leaves, about 30 stomata from each tree species were counted using SEM.

2.2.2. Trichome Identification

Trichomes were classified, based on Esau (1965) and Metcalfe & Chalk (1950), using the following definitions:

1. Solitary: This type of trichome is often present on midribs and veins. The wall is relatively thin consisting of a straight cell (Figures: 3D, F, Q, S; 4A, B, C, G, R).

2. Two-armed (T-shaped unicellular): relatively long trichomes with two arms which are centrally attached to the surface of the epidermis (Figure. 3B).

3. Glandular multi-dimension: has a large (swollen) tubular base with a number of short/long arms (cells) with thick walls (Figure. 3J).

4. Glandular peltate: consists of a basal cell, with a short cylindrical stipe and a wide head of eight and more secretory cells located in a circle (Figures. 3K, N, V, W, X; 4J, K, L, P, V, X).

5. Crypt trichomes: have numerous uniseriate arms, each with a distinct base that collectively is attached to the circumference of the crypts at the surface of the epidermis. These trichomes have (relatively) both short and long arms twisted inward towards the crypt (Figures. 3L and 4M).

6. Hooked hairs with cystoliths: have a tubular base from which an arm or a long cell arises. The trichome emerges from the leaf surface and the cell wall is often thick (Figures. 3L and M; 4M and N).

7. Fasciculate: have very long arms and thick

walls. These trichomes are rarely observed (Figures. 4T).

8. Stellate: Usually thick-walled trichomes with long-slender arms emerging horizontally from the main base. The number of trichomes arising from the base is often 8-12 (Figures. 3S, T, U; 4S, T).

9. Long coiled: has a long curved and twisted arm (Figure. 4T).

10. Tufted stellate: thick-walled trichomes with short or medium arms. In some types, the 4-8 arms are raised or horizontally aligned along the main base (Figures. 3S; 4T).

11. Long Multiradiate: have thick walls and long arms (Figure. 4U).

12. Short bristle with cystolith: short with a thick wall; no obvious base – the trichome flattens out to the surface of the epidermis (Figures. 3Q; 4Q).

13. Non-glandular Peltate scale: circular and large scutiform; a number of arms emerge from the circumference to make a non-tubular trichome (Figures. 3N; 4O, P).

14. Dendritic Hair: made of a raised cell with several radiating small arms emerging from. The trichome has a dendritic structure (Figure. 3X).

2.3. Stomata Identification

Stomata were classified according to the descriptions of Esau (1965) and Metcalf & Chalk (1950):

1. Anomocytic type: usually placed at a lower level than the leaf epidermis. Cells beyond the guard cells are usually similar to the epidermal cells (Figure. 2A).

2. Anomocytic with an undulate wall: As for stomata type 1, but with undulate (corrugated) cell walls (Figure. 2B).

3. Paracytic Type: usually located above the level of the epidermal cells. The long axis of subsidiary cells is parallel to the long axis of guard cells (Figure. 2C).

4. Anisocytic type: raised above the leaf epidermal cells. The stomata are surrounded by approximately three subsidiary cells of different sizes (Figure. 2D).

5. Cyclocytic type: surrounded by over four subsidiary cells arranged in a cyclic manner (Figure. 2E).

3. Results

Trichomes of varying densities were observed on both adaxial and abaxial surfaces of leaf from the examined species. Stomata were present in all

species. Their shapes were usually elliptical and higher densities occurred on the abaxial sides of the leaf. Table 2 shows different types of trichomes

and stomata on adaxial and/or abaxial sides of the taxa examined in this work.

Table 2. Different types of trichomes and stomata on adaxial and abaxial surfaces of the species examined

Species	Type of trichomes														Type of stomata				
	S	T-A	GMD	GP	Crypt	HHC	F	S	LC	TS	LM	SBC	NGPS	DH	A	B	C	D	E
<i>Acer monspessulanum</i>	+/+	+/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-			✓		
<i>Cercis griffithii</i>	+/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-					✓
<i>Crataegus aronia</i>	+/+	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	✓				
<i>Eucalyptus camaldulensis</i>	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-		✓			
<i>Fraxinus rotundifolia</i>	-/-	-/-	-/+	+/+	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-				✓	
<i>Nerium oleander</i>	-/-	-/-	-/-	-/-	-/+	+/+	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	✓				
<i>Olea europaea</i>	-/-	-/-	-/-	+/+	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	+/+	-/-	✓				
<i>Populus euphratica</i>	+/+	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	+/+	-/-	-/-			✓		
<i>Platanus orientalis</i>	-/-	-/-	-/-	+/+	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	+/+			✓		
<i>Quercus brantii</i>	+/+	-/-	-/-	-/-	-/-	-/-	+/+	+/+	+/-	+/+	+/-	-/-	-/-	-/-	✓				

(adaxial/abaxial; += present, - = absent: A= Anomocytic; B= Anisocytic; C= Paracytic; D=Anomocytic with undulate wall; E= Cyclocytic). S, Solitary; T-A, Two-arms; GMD, Glandular-multi dimensions; GP, Glandular peltate; HHC, hooked hairs with cystoliths; F, Fasciculate; S, Stellate; LC, Long coiled; TS, Tufted stellate; LM, Long Multiradiate; SBC, Short bristle with cystolith; NGPS, Non-glandular Peltate scale; DH, Dendritic Hair.

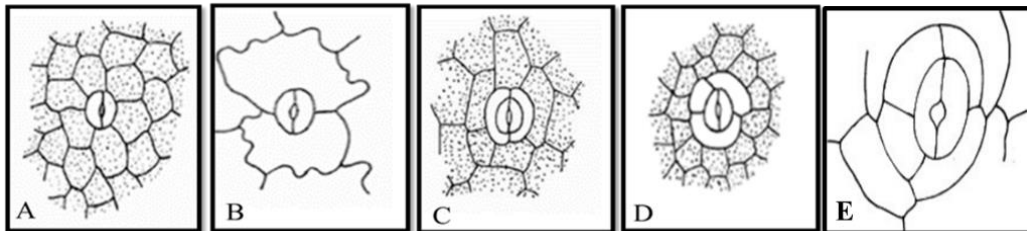


Figure 2: Different types of stomata. A: Anomocytic, B: Anomocytic with undulate wall, C: Paracytic, D: Anisocytic, E: Cyclocytic.

3.1. Micro-morphological characteristics of trichomes and stomata in leaves

***Acer monspessulanum* L.**

Trichomes were rare and scattered on the adaxial side of the leaves, but abundant on the abaxial side. Trichome types identified were: Two armed T-shaped unicellular (Figure. 3B) and solitary (4A- C). Stomata were observed only on the abaxial side. Stomata were paracytic, level with the surrounding epidermal cells (Figures. 3A; 4A).

***Cercis griffithii* Boiss.**

The adaxial side of the leaf had a low density of solitary trichomes, whereas no trichomes were

detected on the abaxial epidermis (Figure. 3D). Stomata occurred on the both adaxial and abaxial sides, but mostly observed in greater numbers on the abaxial side. Stomatal cells were depressed below the epidermal cells; cells surrounding the stomata were similar to the other epidermal cells with undulate walls. The densely pitted anticlinal walls were categorized as paracytic (Figures. 3C; 4D, E).

***Crataegus aronia* C. Koch**

In this species, trichomes were detected on both adaxial and abaxial sides, but density and distribution were higher on the adaxial than on the

abaxial side. Trichomes on both sides of the leaf were unicellular simple and solitary glandular (widening at the base). The trichomes were also distributed on the midribs and veins (Figures. 3F; 4G). Stomata were present on both the adaxial and abaxial sides, but the density was greater on the abaxial side with an average of 68 mm^{-1} . Stomata were sunken in the epidermal surface; the subsidiary cells were all similar, and the stomata were categorized as cyclocytic (Figures. 3E; 4F).

***Eucalyptus camaldulensis* Dehnh.**

No trichome was identified on either side of the leaf. Stomata were present on both sides, but at a lower density on the adaxial side. The stomata were slightly sunken and completely elliptical, categorized as anisocytic with 4-5 subsidiary cells and the presence of wax deposits in the pores (Figures. 3G, H; 4H, I). Furthermore, the epicuticular wax is visible.

***Fraxinus rotundifolia* Miller.**

Both adaxial and abaxial sides of the leaf had glandular peltate trichomes with a higher density on the adaxial side (Figures. 3K; 4J- L). The abaxial side had a type of glandular multi-dimensional trichome observed just once on the leaf surface (Figure. 3J). The density of glandular peltate trichomes on the midrib and vein on the adaxial side were much higher than on the between midribs. Many gland-like structures were scattered on the epidermis (Figure. 3 K.). An abundance of wax particles was also present on the epidermis. Stomata were abundant on the abaxial side of the leaf, raised (slightly above the leaf surface) and completely elliptical, categorized as anomocytic with an undulate wall (Figures. 3I; 4J).

***Nerium oleander* L.**

Hooked hairs with cystoliths were visible on both adaxial and abaxial sides of the leaf with higher densities on the abaxial side (Figures. 3L, M; 4 M, N). The crypt was observed on the abaxial side (Figures. 3L; 4M) that was entirely covered by trichomes. The mean diameter of crypt was $217.37 \mu\text{m}$. Stomata were hidden on the abaxial side of the leaf, underneath of long trichomes; no stomata were seen on the adaxial side. The stomata were sunken and located inside the crypt of epidermis over the mesophyll.

***Olea europaea* L.**

Trichomes were observed on both adaxial and abaxial sides of the leaf. Scutiform non-glandular trichomes (peltate scales) were visible on both

sides, with much higher densities on the abaxial side (Figures. 3 N; 4O, P). The shapes of trichomes resembled as an umbrella. There were also glandular peltate trichomes on both sides of the leaf, more abundantly on the adaxial surface (Figures. 3N; 4P). Stomata were observed only on the abaxial side of the leaf, while none were visible on the adaxial side. The stomata were prominent and of the anomocytic type (Figure. 3N).

***Populus euphratica* Olivier.**

Both the adaxial and abaxial sides of the leaf had two types of trichomes: solitary, at a higher density on the adaxial than the abaxial side (Figures. 3Q; 4R), and short bristle with cystoliths, in greater abundance on the abaxial side (Figures. 3Q; 4Q). Stomata were present on the both sides of the leaf with higher density on abaxial. Furthermore, the paracytic stomata were lower than the subsidiary cells of epidermal cells sunken in the epidermis (Figures. 3P; 4Q).

***Platanus orientalis* L.**

Glandular peltate trichomes were observed on both sides of the leaf, with more distribution on the adaxial side. Furthermore, the midrib and veins were often covered by trichomes (Figures. 3V and W). Dendritic hairs were occasionally seen on the adaxial surface, but only on a single leaf (Figure. 3W). Stomata were only detected on the abaxial side of the leaf (4V and X). The paracytic stomata were parallel to the subsidiary cells at the same level as the epidermal cells (Figures. 3V; 4V, W). Furthermore, striations were present around the stomata. Subsidiary cells were difficult to detect.

***Quercus brantii* L.**

Trichomes were clearly visible on both the adaxial and abaxial sides of the leaf of this species, with a greater density on the abaxial than the adaxial side. Six types of trichomes were identified on the abaxial side, including stellate, fasciculate, long multiradiate, tufted stellate, long coiled and also a limited number of simple solitary, individual trichomes (Figures. 3R- U; 4S- U). These trichomes were also observed on the adaxial side, but at a lower density. Stomata were present only on the abaxial side of the leaf. The stomata were slightly prominent and elliptical in a shape categorized as anomocytic type occurring in high density on the abaxial side (Figures. 3R, T; 4S).

Table 3 shows the averages of stomata features for the studied trees.

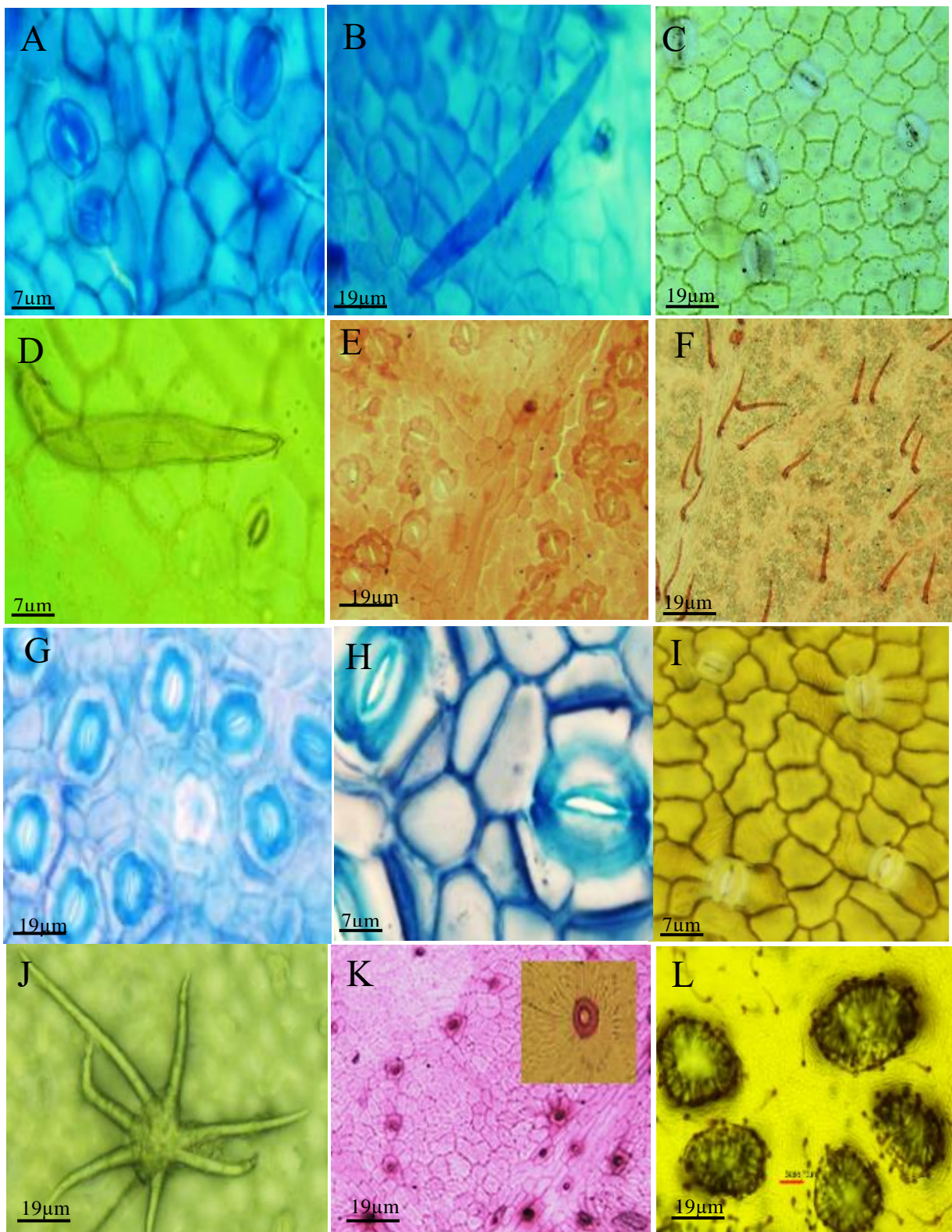


Figure 3. Light microscope images of trichomes and stomata from upper and lower leaf surfaces of the taxa examined. **A-B**, frequent stomata and rare trichome in abaxial and adaxial sides of *Acer monspessulanum*. **C**, great numbers of stomata in abaxial side and epidermal cells with undulate walls in abaxial side; **D**, more less stomata with rare trichome in adaxial sides of *Cercis griffithii*. **E**, high density of stomata in abaxial side and **F**, enormous numbers of unicellular trichomes in adaxial sides of *Crataegus aronia*. **G**, more stomata in abaxial side and **H**, less stomata in abaxial side of *Eucalyptus camaldulensis* with no trichomes in both sides. **I**, more stomata in abaxial side; **J**, infrequent of glandular peltate trichomes in both sides and **K**, gland-like structure in epidermis of *Fraxinus excelsior*. **L**, hooked trichomes with cystoliths on both sides with higher numbers in abaxial.

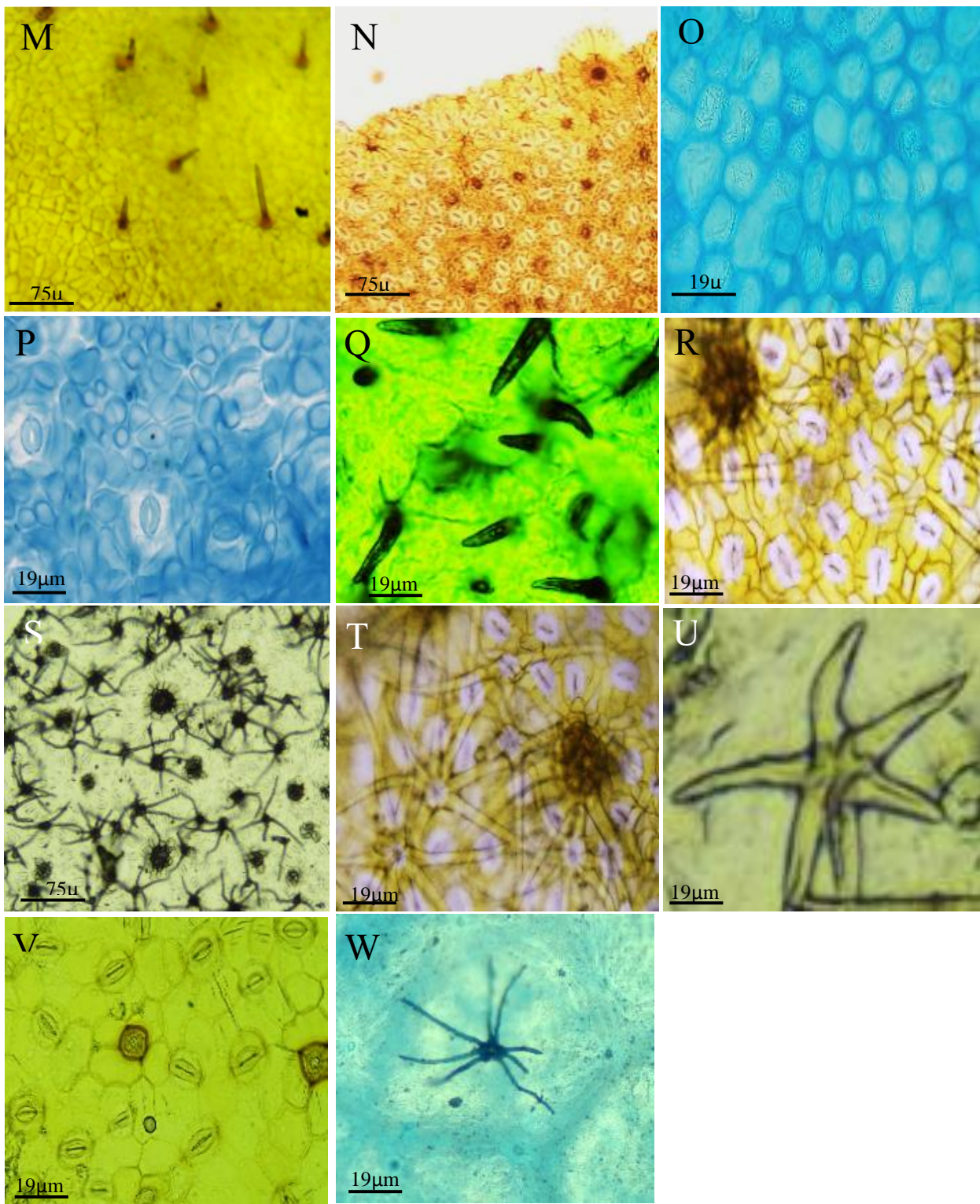


Figure 3. Continued. **M**, more less crypts on adaxial sides of *Nerium oleander*. The stomata were hidden. **N**, scutiform non-glandular trichomes with more stomata in abaxial and **O**, no stomata were observed in adaxial sides of *Olea europaea*. **P**, frequent stomata in abaxial and **Q**, fine trichomes in adaxial sides of *Populus euphratica*. **R**, **S**, and **T**, more frequent of stomata and different types of trichomes in abaxial, and **U**, no visible stomata and a unique branched trichome in adaxial side of *Quercus brantii*. **V**, more stomata in abaxial, and **W**, rare glandular peltate trichomes in adaxial side of *Platanus orientalis*.

Table 3. The average pore (\pm SD) length and aperture from stomata of the leaf of studied trees.

Species Name	APL ¹ μm	APA ² μm
<i>Acer monspessulanum</i>	12.87 (\pm 1.3)	4.90 (\pm 0.47)
<i>Cercis griffithii</i>	8.73 (\pm 0.7)	3.18 (\pm 0.25)
<i>Crataegus aronia</i>	14.24 (\pm 1.7)	4.68 (\pm 0.5)
<i>Eucalyptus camaldulensis</i>	8.88 (\pm 0.6)	3.76 (\pm 0.26)
<i>Fraxinus excelsior</i>	7.46 (\pm 0.8)	3.03 (\pm 0.4)
<i>Nerium oleander</i>	ND	ND
<i>Olea europaea</i>	25.32 (\pm 1.9)	15.50 (\pm 1.98)
<i>Populus euphratica</i>	10.58 (\pm 1.5)	2.47 (\pm 0.32)
<i>Platanus orientalis</i>	13.87 (\pm 1.1)	4.55 (\pm 0.8)
<i>Quercus brantii</i>	8.09 (\pm 1.08)	3.92 (\pm 0.43)

¹ Average Pore Length; ², Average Pore Aperture; ND, not determined.

4. Discussion

The results of the current work on micro-morphological characteristics of the leaf of 10 endemic- and introduced tree species growing in a semi-arid habitat of Ilam showed that stomata and trichome characters are of important parameters in adaptation to the environment. The micro-morphology of the leaf epidermis, focused on the stomata and trichomes, demonstrated great differences among species. Leaf surfaces are crucial in terms of interactions occurring between plants and the surrounding environment, playing central roles in functions such as gas exchange and defence against herbivorous animals (Agrawal et al., 2009). Half of the examined plants had amphistomatic leaves. Generally, the presence of greater numbers of stomata on the abaxial (lower) leaf surface compared to the upper surface could be considered as an adaptation to reduced water loss and, hence, a component in drought tolerance (Metcalf & Chalk, 1950; Aworinde & Ogundairo, 2009).

Stomata commonly occur in high numbers on the abaxial side of the leaves of woody plants, as found with several plants in this work. With *Acer monspessulanum*, the stomata were present only on the lower leaf surface; trichomes were also in greater numbers on the abaxial, compared with the adaxial surface. This morphological trait may be an adaptive strategy to maintain leaf turgor and reduce potential absorption of dust. The low number of stomata (3-5 mm⁻¹) in *A. monspessulanum* populations from the semi-arid forests, compared to other maple species from humid areas such as Hyrcanian forest in Northern Iran, is a consequence of reduced gas exchange that could be an adaptation to drought

conditions (Mohtashamian et al., 2017).

Based on the location of the leaf stomata, three positions were identified: lower, above, and level with epidermal cells. Kordalivand et al. (2015) examining *Betula spp.* growing in the Hyrcanian forests, demonstrated the presence of anomocytic and paracytic stomata; on leaf of this genus the paracytic type was dominant. In terms of stomatal position against subsidiary cells, all stomata were somewhat raised over the epidermal cells. In a similar work, a single anomocytic stomata type was detected on *Castanea sativa* in the Hyrcanian forest (Akbarinia et al., 2011).

Metcalf & Chalk (1950) suggested that subsidiary cells were absent in *Cercis siliquastrum* leaves with anomocytic stomata: in the present study this type of structure was not found on *C. griffithii*. The two cells surrounding the guard cells were clearly similar to other epidermal cells. This stomata apparatus, classified as the paracytic type, agrees with the findings of Zou et al. (2008) on *C. chingii*, which showed clear differences in the shapes of epidermal cells and stomata with other species of *Cercis*. Therefore, it could be stated that the features of *C. griffithii* observed in this work were similar to those of *C. chingii* (Zou et al., 2008) but differed from some other species in this genus. The trichomes of *C. griffithii* were rare on the adaxial epidermis, similar to *C. chinensis* forma *pubescens* and *C. racemosa*. Seven other *Cercis* species as Zou et al. (2008) stated had no trichome on either side of the leaves.

Solitary, unicellular trichomes were observed on the adaxial and abaxial sides of *Crataegus*

aronia leaf, with higher numbers on the adaxial side. Tuyakova et al. (2016) reported unicellular trichomes in *C. ambigua*. Single, simple non-glandular trichomes of differing lengths, with thick cell walls were also detected on both sides of other species of *Crataegus* (Ganeva et al., 2009; Erarslan & Kültür, 2019). The distribution of trichomes in these species was greater on the abaxial side. Furthermore, Bekbolatova et al. (2018) found solitary trichomes on adaxial sides of *C. almaatensis* leaf and on the veins of the leaf blade, in line with the findings in the present research. Based on Meltcafe & Chalk (1950), various structures of simple glandular trichomes are found in different genera of the Rosaceae, such as *Crataegus* and *Malus*. Cyclocytic stomata, surrounded by 5-7 subsidiary cells, were the most common type in *C. aronia*, as was stated by Ganeva et al. (2009) and Erarslan & Kültür (2019) on all investigated species of genus *Crataegus* with the same types of cells surrounding the stomata. In contrast to the results presented here, anomocytic stomata were found in *C. almaatensis* from Kazakhstan (Bekbolatova et al., 2018). Variations in epidermal structure on leaf of different species of *Crataegus* at different sites may represent adaptations to varying ecological conditions. It can be concluded, therefore, that the morphological structure of the trichomes in this genus are of great value in taxonomic and ecological purposes (Ganeva et al., 2009).

Anisocytic stomata were the most abundant type among *Eucalyptus* species with a greater stomatal density on the abaxial side. When stomata are present on both sides, the leaves are known as amphistomatous. This type of leaf occurs in plants with high photosynthetic capacities that grow well in full-sun when sufficient water is available (Mott et al., 1991). In addition, no trichomes were observed on either side of the *Eucalyptus* leaves. Tantawy (2004) reported anisocytic stomata in *E. camaldulensis*, in agreement with our findings. Chathlingathe et al. (2017) showed that both anisocytic and anomocytic stomata occurred on leaf of *Eucalyptus globulus*. Anatomical analyses of seven species of *Eucalyptus* spp. suggested greater numbers of stomata on the abaxial compared with the adaxial sides (Santos et al., 2008). Al-Edany et al. (2012) identified three types of stomata: anomocytic, paracytic, and anisocytic, on species of Myrtaceae such as *E. camaldulensis* and stressed their importance as taxonomic and anatomical features. The development of structural

changes in plant species indicates adaptations to different environments. Epicuticular wax layer with no trichomes, fewer stomata and wax blocking stomatal pores on adaxial sides of eucalypt leaves are clear indicators of species adaptation to arid habitats (Ali et al., 2009; Naz et al., 2009).

The abaxial side of ash (*Fraxinus excelsior*) leaves examined in the current research had a few glandular multi-dimensional trichomes at low densities. Only one single trichome from this type was observed on the lower surface of the leaves. In addition, glandular peltate trichome distribution on the midrib and veins of the adaxial side was more visible than on the surrounding epidermal cells. The epidermal cells were almost even with undulate walls. These characteristics were also described by Hardin & Beckmann (1982) in other *Fraxinus* species, especially *F. americana*. In the current study; however, the stomata were clearly sunken below the epidermal surface, and were categorized as anomocytic with undulate walls. The stomata were restricted to the abaxial side of the leaves. Donselman & Flint (1982) suggested that the frequency of stomata on the two sides of *Fraxinus* spp. leaves represented adaptations in this genus to mesic conditions. Hardin & Beckmann (1982) identified similar trichomes to those observed in the present work on leaves of *Fraxinus* species in the North-east of the US.

In *Nerium oleander*, two different types of trichomes were identified: crypt and hooked hairs with cystoliths. The crypt type occurred only on the abaxial sides of the leaf, whereas the hooked hairs with cystoliths were on both sides. The density and distribution of trichomes were considerably higher on the abaxial side. In many xerophytes, stomata are gathered in sunken epidermal chambers termed "stomatal crypts" (Metcalf & Chalk, 1979). Lakušić et al. (2007) reported trichomes of solitary unicellular on the adaxial and crypt types on the abaxial sides of *N. oleander*. Similarly, Parashuram et al. (2018) did not recognize stomata on the adaxial side of leaf on a range of *Nerium* species; on the abaxial side, however, the stomata inside the crypt, were covered by a high density of trichomes. It was suggested that the main function of crypts and trichomes covering the crypt was to reduce transpiration and increase photosynthesis efficiency (Roth-Nebelsick et al., 2009). The presence of sunken and crypt-occupying stomata covered with trichomes is another adaptive strategy to arid conditions.

Olea europaea (olive) is a common component

of the semi xeric Mediterranean climate with warm and dry weather in summers and rainy and cool winters. This species has thermophilic features well-adapted to tolerate drought and harsh conditions (Guerrero Maldonado et al., 2016). Two types of scutiform glandular and non-glandular trichomes were observed on both adaxial and abaxial sides of olive leaves: the density of glandular trichomes was greater on the adaxial side, whereas the density of non-glandular trichomes was much higher on the abaxial side. Peltate trichomes are the usual types found in different species of *Olea* (Palliotti et al., 1994). The anomocytic type of stomata was only seen on abaxial side, signifying a hypostomatous species.

Similar results were reported by Guerfel et al. (2007) in other species of *Olea* from Tunisia. This genus of plants is well adapted to semi-arid environments and drought conditions (Gimenez et al., 1997). Lakušić et al. (2007) observed a high density of non-glandular scutiform trichomes as well as stomata on the abaxial side of *Olea spp.* leaf. A role for non-glandular scutiform trichomes in resistance to environmental stresses was suggested by Roka et al. (2018). Moreover, Schwabe & Lionakis (1996) stressed the role of trichomes in reducing water evaporation as well as increasing the reflection of solar radiation.

Two types of trichomes (solitary and short bristles with cystoliths) were observed on both sides of leaves of *Populus euphratica*. However, the frequency of solitary was greater on adaxial side of the leaves. Stomata were of the paracytic type and occurred only on the abaxial side. These types of trichomes and stomata were also reported on *P. euphratica* by Zhuang et al. (2011), whereas Liu et al. (2015) recognized solitary non-glandular unicellular trichomes and paracytic stomata on the adaxial side of the leaves. The previously reported findings are in agreement with the results of the current study. High abaxial stomatal density occurred in 12 *Populus* clones, with only a clone of *P. trichocarpa* having hypostomatous stomata (Al Afas & Ceulemans, 2006); again, the results of the present work are in agreement with this previously published work.

Two types of trichomes were detected on leaf of *Platanus orientalis*: glandular peltate trichomes were present on both sides of the leaves; however, dendritic trichomes were observed on the adaxial side only. Both trichome types were abundant on the adaxial side. Carpenter et al. (2005) identified glandular peltate and dendritic trichomes with

whorls of arms in all species of Platanaceae examined, especially *Platanus orientalis*. These trichomes were usually abundant on the veins and midrib, in agreement with the results of the present study. They also reported that three types of stomata including paracytic, anomocytic, and letrocyclic were recognized on the abaxial side of the leaves only. The stomatal types, confined to the abaxial side, as well as striations, were also reported by Kvaček et al. (2001) and Carpenter et al. (2005). Dendritic trichomes of varying density are common features of all species in the Platanaceae. This type of trichome may confer ecological advantages, and also has phylogenetic value (Nixon & Poole, 2003). The abundant trichomes could cover the stomata and play a key role in the control of transpiration (Carpenter et al., 2005).

Leaves of Persian oak (*Quercus brantii*) had six types of trichomes: stellate, fasciculate, long multiradiate, tufted stellate, long coiled and solitary. The trichomes were widely scattered over the abaxial side of the leaves, obscuring the appearance of ground cells and stomata. A high density of trichomes is a well-known feature of *Q. pubescens* (Viscosi et al., 2009). Leaf micro-morphological traits (trichomes and stomata) of oak species and subspecies from Iran have significant implications for systematic studies (Panahi et al., 2012 a-c). Furthermore, five types of trichomes were detected on *Q. brantii* leaf: simple-uniseriate, solitary, fasciculate, multiradiate and stellate from non-tubular trichomes, on both adaxial and abaxial sides of the leaves. The stomata in all species examined were classified as anomocytic, elliptical to round in shape, which agrees with the findings of the present study. On the upper leaf surface of *Quercus petraea* L., three types of trichome were reported including uniseriate, solitary and stellate (Fortini et al., 2009), consistent with the results of the current study. Similar results were reported in *Q. suber* by Simões et al. (2020). The existence of these similar types of stomata and trichomes in various different oak species emphasized the genetic proximity between the species. Furthermore, Pérez-Estrada et al. (2000) stated that high numbers of trichomes are typical for plants in dry and full-sun areas. Therefore, in many oak species the variability is amongst the functional strategies to tolerate drought conditions, rainfall regimes and abiotic stresses (Johnson, 1975). In these species, the high number of trichomes maintains a large leaf boundary layer to reduce water loss.

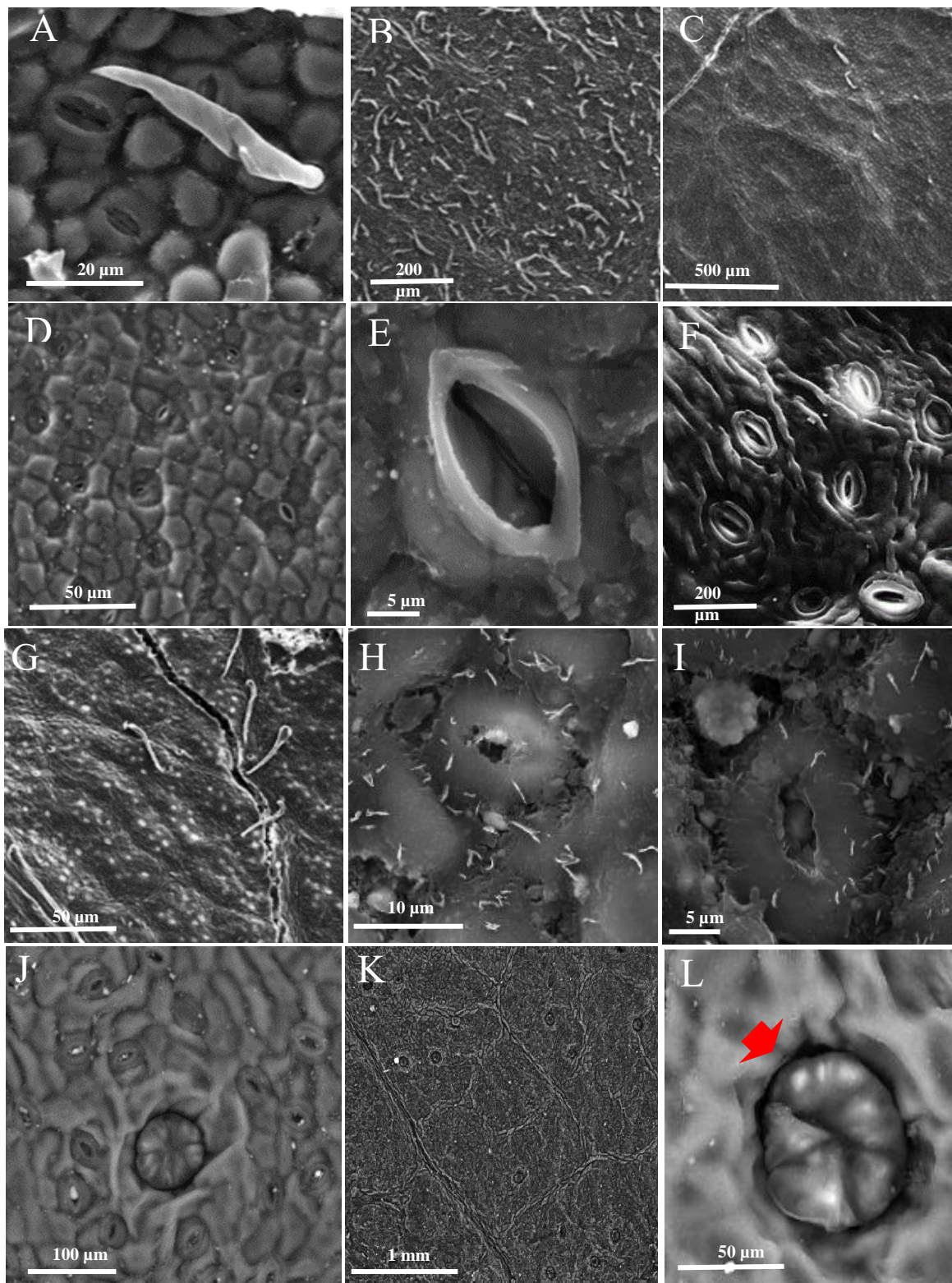


Figure 4. SEM images of trichomes and stomata from both leaf surfaces of the taxa examined. **A** and **B**, fine and solitary trichomes and frequent stomata in abaxial and **C**, rare trichomes and stomata in adaxial side of *Acer monspessulanum*. **D**, dense stomata in abaxial and **E**, infrequent stomata in adaxial sides of *Cercis griffithii*. **F**, great numbers of stomata in abaxial and **G**, numerous figures of solitary trichomes in adaxial sides of *Crataegus aronia*. **H** and **I**, sunken stomata with high amount of wax depositions in both abaxial and adaxial sides of *Eucalyptus camaldulensis*. **J-K**, frequent amount of stomata with gland structure in abaxial and **L**, high magnification of a gland structure (arrowed) in adaxial side of *Fraxinus excelsior*.

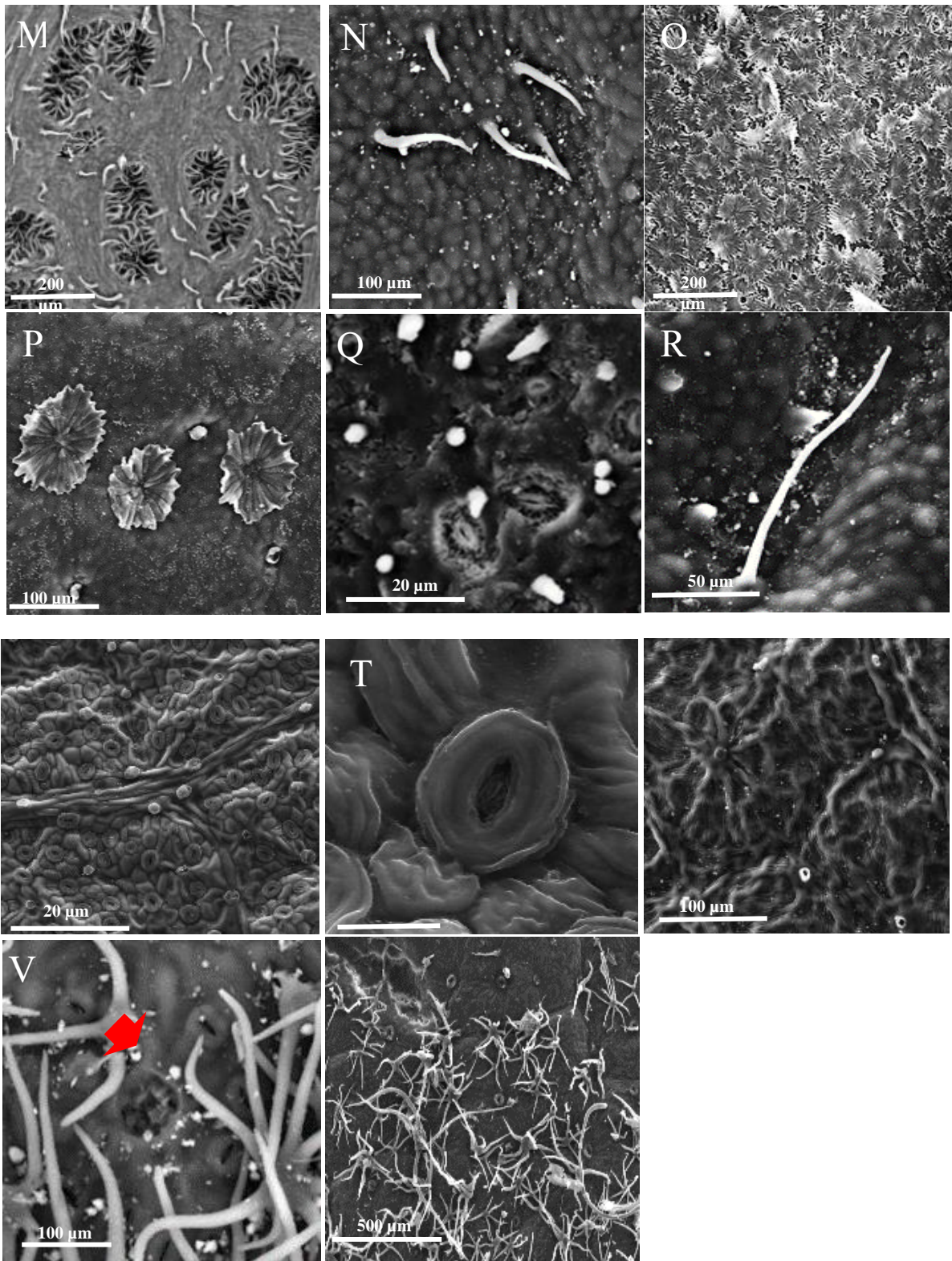


Figure 4. Continued. **M**, more crypts covered by hairs and solitary trichomes with hidden stomata in abaxial and **N**, fine and solitary trichomes in adaxial side of *Nerium oleander*. **O-P**, scutiform non-glandular trichomes in both abaxial and adaxial sides of *Olea europaea*. **Q**, more sunken stomata and short bristle trichomes in abaxial and **R**, frequent numbers of short trichomes along with rare long trichome in adaxial side of *Populus euphratica*. **S**, frequent stomata parallel to the beyond epidermal cells in abaxial, **T**, high magnifications of a stomata with striations around it and **U**, no stomata was observed in adaxial side of *Platanus orientalis*. **V**, highly branched trichomes covered the stomata and trapped fine dust particles between the trichomes (arrow) in abaxial; **W**, numerous different types of trichomes in adaxial side of *Quercus brantii*.

The great density of trichomes is linked to the plant adjusted to the xeric environments (Balok & Hilaire, 2002) and has a direct negative impact (direct defense) on the performance of small and tiny herbivorous. Trichomes, in this sense, are probably functioning more to increase the boundary zone thickness (volume) of the leaf surfaces and, therefore, reduce water loss. Their function in against sap-sucking insects, such as aphids and whitefly is a defence strategy (Dent, 1995; Valverde et al., 2001; Amin et al., 2017). This feature is obviously detected in Persian oak, olive and *Nerium*.

5. Conclusion

This work defined micro-morphological features present on the leaves of 10 woody plant species growing in semi-arid forest, the Zagros Mountains of Iran, and suggested relationships between these features and adaptations to the semi-arid environment of the region. There were various adaptive traits among the examined plants. Most species examined here had trichomes and stomata on both upper (adaxial) and lower (abaxial) sides of the leaves; however, the density and distribution

of these structures were considerably higher on the abaxial side. This trait enables better tolerance of hot weather in summer, reducing water loss through stomata, adsorption of light from the sun and can also lower the negative effects of herbivorous animals attacking the plants. Furthermore, five types of stomata were recognized on the studied plant species, suggesting that variable stomatal types enable these trees to cope with the environment. It can be concluded that leaf micro-morphological features are of high ecological importance within each plant family. These anatomical features of the leaves could be considered as some adaptive strategies to the local climatic conditions. For example, a low stomatal density with an abundance of trichomes, and the restriction of stomata to the abaxial sides of the leaves, are characteristics of xeromorphic plants.

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رابطه بین ریزساختار برگ و سازگاری محیطی در ۱۰ گونه درختی جنگل‌های نیمه خشک

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چکیده

ویژگی‌های ریزریخت شناسی برگ مانند روزنه و کرک شاخص‌های قابل اعتمادی برای پاسخ گیاهان به شرایط محیطی هستند. هدف از مطالعه حاضر تعیین استراتژی‌های سازگاری ۱۰ گونه چوبی در جنگل‌های نیمه خشک زاگرس در غرب ایران بر اساس ریزساختارهای برگ با تاکید بر روزنه‌ها و کرک‌ها و با استفاده از میکروسکوپ نوری و الکترونی بود. انواع مختلف روزنه و کرک شناسایی شدند. روزنه‌ها معمولاً بر سطح زیرین برگ قابل رویت بودند که این ویژگی به کاهش هدر رفت آب از سطح برگ‌های در معرض هوا و نیز تشعشعات خورشیدی کمک می‌کند. کرک‌ها به دو دسته غده‌ای و غیر غده‌ای با زیرگروه‌های منفرد، دو بازو تی شکل تک سلولی، سپرمانند غده‌ای، چند وجهی پایه غده‌ای، حفره‌ای، قلابدار با سیستولیس، دسته‌ای، ستاره‌ای، بلند پیچ‌خورده، چند شعاعی کوتاه، چند شعاعی بلند، ضخیم کوتاه با سیستولیس، سپر مانند و درخت مانند شناسایی شدند. فراوانی کرک‌ها بر روی برگ‌ها نقش آنها را به عنوان عوامل کنترل زیستی نشان می‌دهد. اکالیپتوس تنها گونه مورد مطالعه بود که فاقد کرک در دو طرف برگ بود. همچنین، روزنه‌های نوع آنموسی‌تیک و پاراسیتی‌ک بیشترین نوع را به خود اختصاص دادند. تفاوت‌های چشمگیر بین ویژگی‌های ریزریخت شناسی برگ‌های گونه‌های مختلف بیانگر سازگاری گیاهان با شرایط اکولوژیکی محیطی است.

کلیدواژه‌ها: ریزریخت‌شناسی برگ، کرک‌های غده‌ای و غیر غده‌ای، روزنه، شرایط محیطی، گیاهان نیمه خشک.