

# Leaf anatomical traits of *Crataegus orientalis* Pall. ex M.Bieb. (Rosaceae) from Turkey

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**ABSTRACT:** *Crataegus orientalis* Pall. ex M.Bieb., is a widespread species of the genus *Crataegus* L. (Rosaceae), which has an important place in traditional folk medicine. In this study, *Crataegus orientalis* subsp. *orientalis* and *Crataegus orientalis* subsp. *szovitsii* (Pojark.) K.I.Chr. from Turkey were investigated with regard to leaf anatomy. For this reason, transverse sections and surficial sections were taken from samples. Leaves of the both taxa was found as dorsiventral, hypostomatic and mesomorphic. Cyclocytic stomata, simple single non-glandular trichomes and calcium oxalate crystals were defined as significant anatomical features of these taxa. All results were compared with leaf anatomical features of the Rosaceae family and the characteristics of the genus *Crataegus* were elucidated by detecting similarities and differences.

**KEYWORDS:** *Crataegus orientalis*; Rosaceae; leaf anatomy; Turkey.

## 1. INTRODUCTION

The genus *Crataegus* L. is placed in the tribe Maleae Small, subfamily Maloideae C. Weber of the Rosaceae family based on the latest nomenclatural changes [1,2], and is represented by over 200 species widely distributed in Western Asia, North America and Europe. In addition, some of them are widely cultivated as ornamental plants [3,4]. In Turkey, this genus comprises 28 taxa corresponding to 24 different species, 10 of which are endemic [5]. The most significant taxonomic characters of *Crataegus* are indumentum, teeth and shape of leaves. Additionally, members of this genus exhibit yellow-red fruits and greenish calyx [6,7]. According to Dönmez [7], the taxonomy of *Crataegus* is a difficult issue because of the polymorphic characters, hybridisation, inadequate collections and deficiency of field observations. *Crataegus orientalis* Pall. ex M.Bieb. is one of the most widely used species of this genus traditionally. It is a shrub or small tree usually 3 - 5 m tall. Leaves are obovate-oblong and deeply 3 - 7 lobed with adpressed grey hairs. Flowers are arranged in 4 - 10 flowered corymbs; sepals are 4 - 5 mm long, triangular-cuspidate, reflexed in fruit. Reddish-orange fruits are about 2 cm in diameter [6,8]. This species is represented by two subspecies in Turkey: *Crataegus orientalis* subsp. *orientalis* and *Crataegus orientalis* subsp. *szovitsii* (Pojark.) K.I.Chr. Defined differences between the two subspecies [6,8,9] are:

1. Fruit reddish-orange, pyrenes 4 (- 5); sepals reflexed in fruit ..... *C. orientalis* subsp. *orientalis*
2. Fruit red, pyrenes (2 -) 3 - 4; sepals spreading in fruit ..... *C. orientalis* subsp. *szovitsii* (Figure 1)

*C. orientalis* subsp. *orientalis* is widespread in Turkey, especially in the North and in the northern part of Central Anatolia, where it grows in rocky areas, open areas and forests at 450 - 2240 m of elevation. *C. orientalis* subsp. *szovitsii* naturally grows within steppes and *Quercus* sp. forests at 700 - 2150 m of elevation in northern, central and eastern Anatolia [6,8] (Figure 2).

As far as the ethnobotanical uses are concerned, *C. orientalis* subsp. *orientalis* is commonly known as "Alıç, Kırmızı Alıç", while *C. orientalis* subsp. *szovitsii* is known as "Koyun Alıcı, Deli Alıç" in Anatolia [5,8,10,11]. It is reported that various parts (flowers, leaves, roots, fruits) of these taxa have traditionally been used in folk medicine. In East Anatolia, roots of *C. orientalis* subsp. *orientalis* are boiled and bathed in their water for the treatment of rheumatic pains and against swelling [12]; decoction of fruits are used as vasodilator and cardiotoxic in the treatment of cardiac disorders [13]. It is also known that drinking infusion of flowers of subsp. *szovitsii* every morning on an empty stomach can treat cardiac disorders and asthma [14]. Other studies

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revealed that these taxa have antithrombotic, antinociceptive, antiinflammatory, and antioxidant activities [15-17] owing to their content of phenolic acids, flavonoids and organic acids [19-21].



Figure 1. Fruit details of *Crataegus orientalis* subsp. *szovitsii*.

Though taxonomic problems related to the genus remain, few anatomical studies were fulfilled to provide further valuable diagnostic features. Hence, the main purpose of this study is to define the leaf anatomy of *C. orientalis* subsp. *orientalis* and *C. orientalis* subsp. *szovitsii* and detect distinctive anatomical features of these taxa.

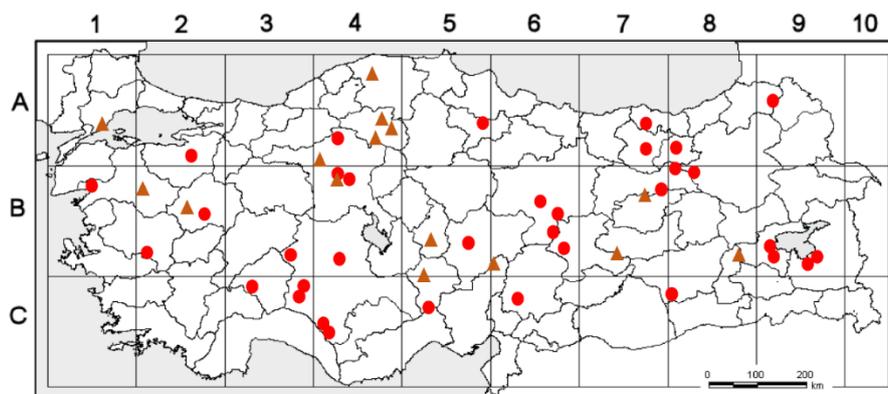


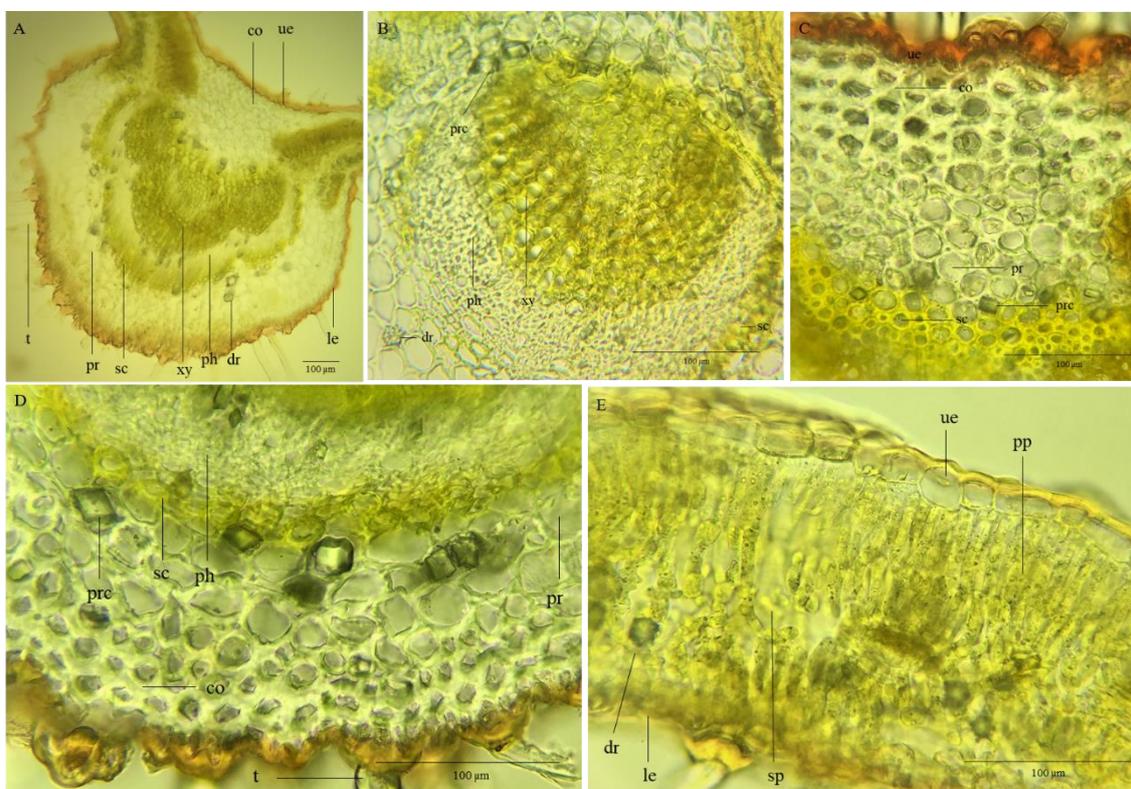
Figure 2. Distribution map of *Crataegus orientalis* subsp. *orientalis* (●) and *Crataegus orientalis* subsp. *szovitsii* (▲) in Turkey.

## 2. RESULTS

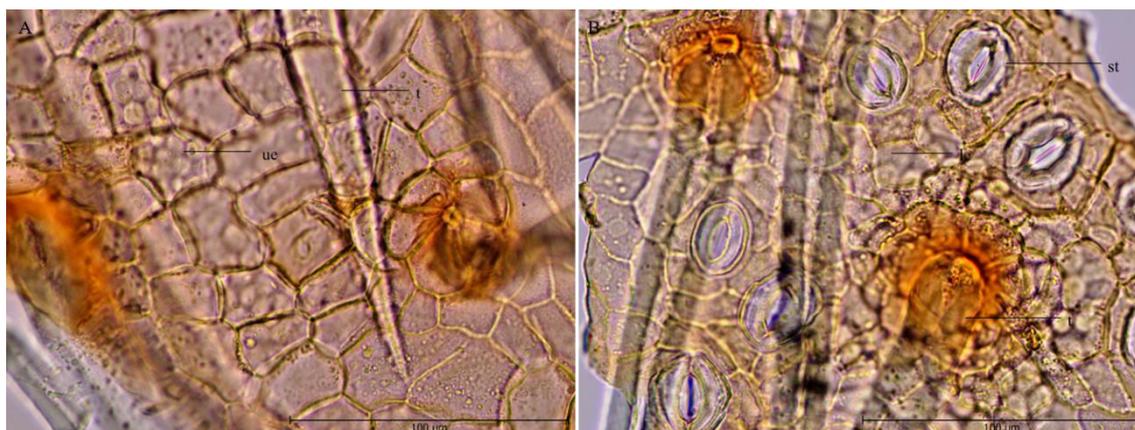
The anatomical investigation of *C. orientalis* subsp. *orientalis* revealed a dorsiventral, hypostomatic and mesomorphic leaf. In the transverse sections, both upper and lower epidermis consist of single-layered cells with a thin cuticle layer. The upper epidermal cells are bigger than the lower ones. Single simple non-glandular thick-walled trichomes, varying in length, have been observed on both leaf surfaces. The mesophyll is composed of 1 – 2 layers of palisade cells under the upper epidermis and spongy cells with wide intercellular spaces. Leaf blade thickness ranges from 267.73 to 290.80  $\mu\text{m}$ , with a mean value of 271.92  $\mu\text{m}$  (Figure 3).

In the midrib, which is on average 616.300  $\mu\text{m}$  thick, 4 – 5 layers of collenchyma develop under both lower and upper epidermis. Parenchyma cells are located between the collenchyma layers and the collateral vascular bundles. Moreover, several druses and prismatic crystals of calcium oxalate were detected in the midrib and the mesophyll of the leaf.

On the surface sections, epidermis has polygonal cells with usually straight anticlinal walls. Cyclocytic stomata, which are found only on the lower surface, are oval shaped and varying sized (Table 1). Each stoma is surrounded by 5 – 7 subsidiary cells (Figure 4). The stomatal index for the lower surface of the lamina was calculated as 10.38.



**Figure 3.** The transverse sections of the leaf of *Crataegus orientalis* subsp. *orientalis*; **A**, the midrib, **B**, vascular bundle, **C**, detail of midrib at upper surface, **D**, detail of midrib at lower surface, **E** intervacular part of the leaf blade; co collenchyma, dr druse, le lower epidermis, ph phloem, pp palisade parenchyma, pr parenchyma, prc prismatic crystal, sc schleranchyma, sp spongy parenchyma, st stomata, t non-glandular trichome, ue upper epidermis, xy xylem.

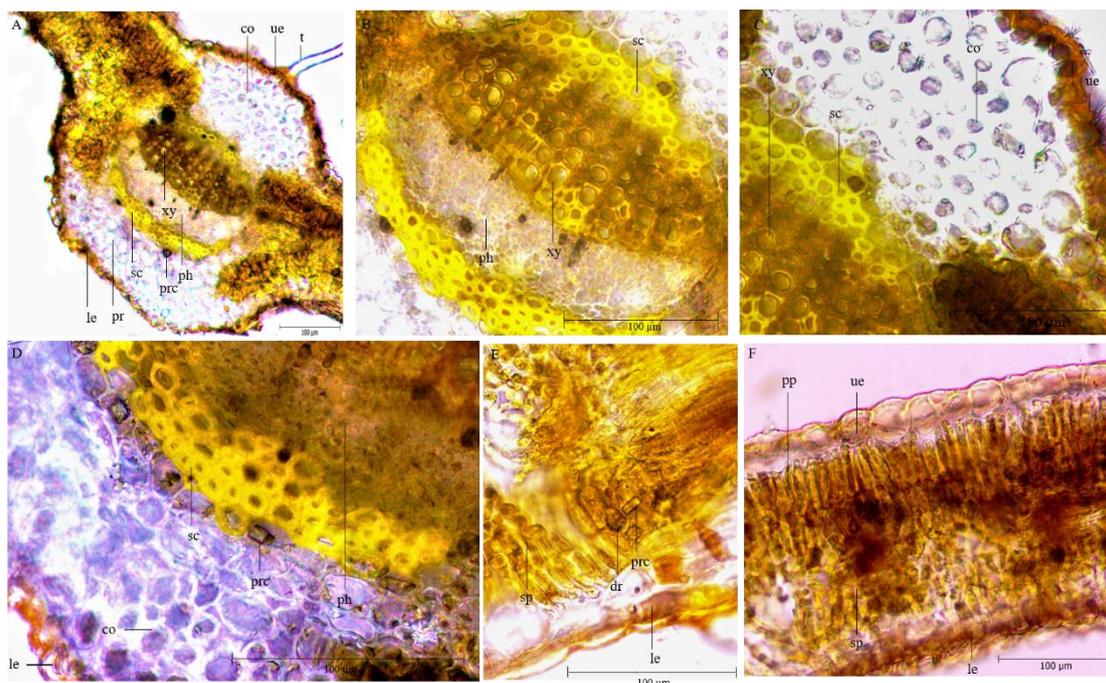


**Figure 4.** The surface sections of the leaf of *Crataegus orientalis* subsp. *orientalis*; **A**, upper surface of the leaf, **B**, lower surface of the leaf; le lower epidermis, st stoma, t non-glandular trichome, ue upper epidermis.

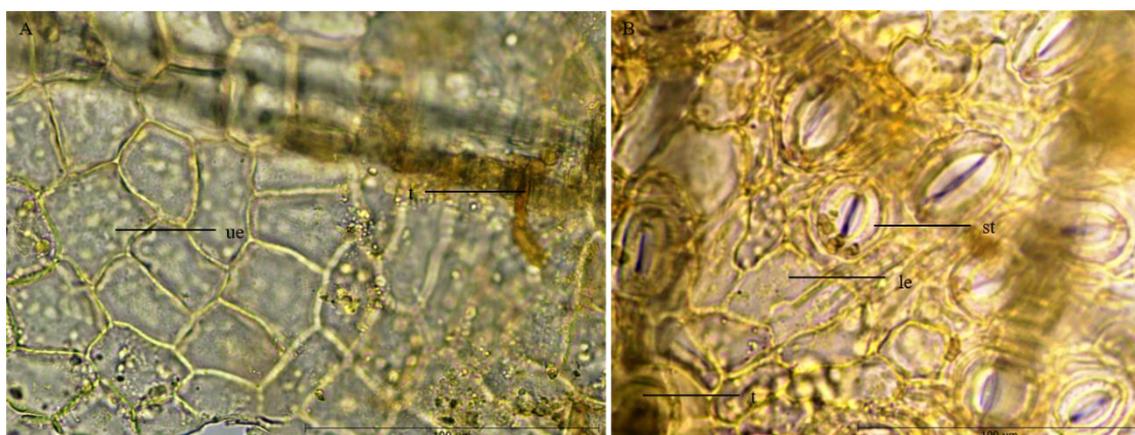
The leaf of *C. orientalis* subsp. *szovitsii* was also found as dorsiventral, hypostomatic and mesomorphic like that one of *C. orientalis* subsp. *orientalis*. In the transverse sections, both upper and lower epidermis are one-layered and covered by a thin cuticle layer. The upper epidermal cells are bigger in size than the lower ones. The trichomes are simple single thick-walled non-glandular, varying in size and distributed on both sides of the leaf surfaces. The mesophyll comprises 1 – 2 layers of palisade cells under the upper epidermis

and spongy cells with wide intercellular spaces. The leaf blade thickness is 207.49 – 232.64  $\mu\text{m}$  with a mean value of 221.13  $\mu\text{m}$  (Figure 5).

Regarding the midrib region, 4 – 6 layers of collenchyma are present under the upper and lower epidermis. Between the collenchyma layers and the collateral vascular bundles, parenchyma cells were observed. The leaf thickness at the midrib is on average  $478.90 \pm \mu\text{m}$ . Many druses and prismatic crystals of calcium oxalate were also noticed in both leaf midrib and mesophyll. On the surface sections, the epidermal cells appear as polygonal in shape like those ones of *C. orientalis* subsp. *orientalis*. Similarly, stomata are oval shaped, varying sized and located only on the lower surface (Table 1). The stomatal type is also cyclocytic, with each stoma is surrounded by 5 – 7 subsidiary cells (Figure 6). The stomatal index for the lower surface of the lamina was calculated as 12.33.



**Figure 5.** The transverse sections of the leaf of *Crataegus orientalis* subsp. *szovitsii*; **A**, the midrib, **B** vascular bundle, **C**, detail of midrib at upper surface, **D**, detail of midrib at lower surface, **E**, **F**, intervacular part of the leaf blade; co collenchyma, dr druse, le lower epidermis, ph phloem, pp palisade parenchyma, pr prismatic crystal, sc scleranchyma, sp spongy parenchyma, st stomata, t non-glandular trichome, ue upper epidermis, xy xylem.



**Figure 6.** The surface sections of the leaf of *Crataegus orientalis* subsp. *szovitsii*; **A** upper surface of the leaf, **B** lower surface of the leaf; le lower epidermis, st stoma, t non-glandular trichome, ue upper epidermis.

**Table 1.** Measurements from leaf anatomical traits of *C. orientalis* subsp. *orientalis* and *C. orientalis* subsp. *szovitsii*.

	<i>C. orientalis</i> subsp. <i>orientalis</i>						<i>C. orientalis</i> subsp. <i>szovitsii</i>					
	Length (µm)			Width (µm)			Length (µm)			Width (µm)		
	Min	Max	Av	Min	Max	Av	Min	Max	Av	Min	Max	Av
UEC	17.28	37.75	28.19	13.77	18.26	16.03	20.78	37.23	27.90	18.31	35.11	27.76
LEC	12.37	19.05	16.20	7.82	17.43	12.22	12.34	18.34	15.98	12.73	21.14	16.32
LS	23.59	27.72	26.32	16.83	26.41	21.94	27.42	40.77	31.63	17.53	24.53	21.32
PPL				72.40	90.68	80.48				63.50	72.48	68.40
SPL				135.97	144.28	142.64				92.35	101.52	95.38

Abbreviations: Min Minimum, Max Maximum, Av Average, UEC Upper epidermis cell, LEC Lower epidermis cell, LS Lower stoma, PPL Palisade parenchyma layer, SPL Spongy parenchyma layer.

### 3. DISCUSSION

In the Flora of Turkey and the East Aegean Islands [6], *C. orientalis* was classified into two varieties: var. *orientalis* and var. *obtusata* Browicz. As a result of recent revisions, *C. orientalis* var. *obtusata* is reduced to a synonym; consequently, *C. orientalis* is represented by two subspecies in Turkey, *C. orientalis* subsp. *orientalis* and *C. orientalis* subsp. *szovitsii* [5,8]. However, discussions on this taxonomic classification continue, and various data obtained from taxonomic studies including anatomical investigations could help to clarify this issue. Thus, in this study significant anatomical features of the leaves (e.g. types of trichomes, stomata, mesophyll, crystals) of these taxa were investigated.

Shared anatomical features observed in both taxa are: dorsiventral, hypostomatic and mesomorphic leaves, cyclocytic stomata, which are surrounded by 5 – 7 subsidiary cells, and presence of calcium oxalate crystals (druses and prismatic crystals). On the other hand, some differences in measurements were found regarding size of epidermal cells and stomata, width of mesophyll and stomatal index. While stomatal index is found to be fairly constant at species level, ecological factors are quite effective on the stoma size and density [22-24]. Although according to Meltcafe & Chalk [25], simple trichomes with variation in structure and anomocytic stomatal type are found in Rosaceae family, cyclocytic stomatal type was also reported in previous studies for several genera, such as *Crataegus*, *Pyracantha* Roemer, *Cydonia* Mill. [26-29]. Nevertheless, anomocytic stomatal type was found in some *Malus* Mill. and *Sibbaldia* L. species [30,31]. Simple single non-glandular trichomes are characteristic for some species of *Crataegus*, *Malus* and *Sorbaria* A. Braun, as well as for *Cydonia oblonga* Mill. [28-30,32]. Similarly to our results, distribution of stomata in some *Crataegus* species was only found on lower epidermis [28,33].

Previous anatomical investigations on *Crataegus* species from Turkey [33] highlighted the occurrence of druse crystals and prismatic crystals in the midrib of *Crataegus monogyna* Jacq. subsp. *monogyna* and in the mesophyll and epidermis of *Crataegus pentagyna* Waldst. & Kit. ex Willd., while both tetragonal and prismatic crystals were reported for *C. orientalis* [34]. Furthermore, calcium oxalate crystals were also found in different parts of the leaf lamina in the genus *Rosa* L. [35] and Rosaceae family [25,36-38]. Regarding the mesophyll structure, many species of *Rosa*, *Cotoneaster* Medik., *Pyrus* L. and *Potentilla* L. have dorsiventral leaves, with palisade tissue in the upper surface and spongy parenchyma in the lower surface [35,38-41]. Conversely, isobilateral mesophyll was described for some species of the Rosaceae family [39,40,42], while Baldemir & Güvenç [33] reported isobilateral mesophyll in *C. pentagyna* and dorsiventral mesophyll in *C. monogyna* subsp. *monogyna*.

### 4. CONCLUSION

Conducted studies revealed that cyclocytic and anomocytic stomata type, simple non-glandular trichomes, calcium oxalate crystals, dorsiventral or isobilateral mesophyll are the most prominent leaf features for Rosaceae family. Regarding the genus *Crataegus*, cyclocytic stomata type, simple single non-glandular trichomes and presence of calcium oxalate crystals can be considered as demonstrative anatomical properties which could help to clarify taxonomy of this genus. Nevertheless, to confirm the diagnostic value of these characters within the genus and among Rosaceae, further comparative anatomical studies with the other species of this genus should be fulfilled.

## 5. MATERIALS AND METHODS

The anatomical investigation was carried out on leaf samples obtained from herbarium materials. *C. orientalis* subsp. *orientalis* collected from Arslanköy (İçel) (ISTE 98194) and *C. orientalis* subsp. *szovitsii* collected from Kepsut (Balıkesir) (ISTE 109913) were preserved in the Herbarium of Istanbul University Faculty of Pharmacy. Leaves were pretreated with immersed in warm water. Transverse sections and surficial sections were hand-cut with a razor, stained with Sartur reagent (lactic acid, Sudan III, aniline, iodine, potassium iodide, alcohol and water) [43,44] and observed under light microscope. For the anatomical investigations and measurements, at least 10 individual specimens and 15 leaves per individual specimens were used. Detailed photographs were taken by Canon Power Shot A640 and required measurements (Table 1) were made by KAMERAM© software. The stomatal index (SI) was calculated according to the following formula [45]:  $SI = (S/S + E) \times 100$ , where 'S' refers to the number of stomata per unit area and 'E' refers to the number of epidermal cells in the same unit area.

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