

INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
 ISSN Print: 1560-8530; ISSN Online: 1814-9596
 16-229/2016/18-5-957-964
 DOI: 10.17957/IJAB/15.0191
<http://www.fspublishers.org>



Full Length Article

Comparative Leaf and Scape Anatomy of Some *Scilla* Taxa in Turkey

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Abstract

Comparative anatomical study on eight *Scilla* taxa (*S. bifolia*, *S. melaina*, *S. siberica* subsp. *armena*, *S. leepii*, *S. ingridae*, *S. mesopotamica*, *S. autumnalis* and *S. cilicica*) growing in Turkey was made using light microscopy techniques. Leaf and scape anatomical properties of the taxa were compared and relationships among taxa were determined. In anatomical studies, paraffin method was used. The cross-sections of the scape, leaves and surface-sections the leaves of these taxa were taken and photographed. Length and width measurements of stomata in the upper and lower surfaces of leaves were made and the mean and standard deviation values of stomata were calculated. Raphida crystals were found in the mesophyll of all taxa. On both surfaces of leaves, anomocytic type stomata were observed. The different and similar anatomical characters in the leaf and scape were determined and the taxa were classified into three groups; such as (1) *S. ingridae*, *S. mesopotamica*, *S. melaina*, and *S. leepii* are independent species with close relationships; (2) *S. bifolia*, *S. siberica* subsp. *armena* and *S. cilicica* are very close taxa; (3) *S. autumnalis* is different from other investigated taxa. Also, these taxa can be distinguished as mesophyll type; isolateral (*S. bifolia*, *S. cilicica* and *S. siberica* subsp. *armena*) and unifacial (*S. melaina*, *S. leepii*, *S. ingridae*, *S. mesopotamica* and *S. autumnalis*). According to our data, *S. bifolia* was considered as a complex species of *Scilla* genus in Turkey. © 2016 Friends Science Publishers

Keywords: *Scilla* taxa; Comparative anatomy; Leaf; Scape; Turkey

Introduction

Anatolia is one of the richest regions for natural plants and geophytes create an important part of this richness. Most of the geophytes species grow in Anatolia. While some geophytes are used as ornamental plants, others are used as medicine and food. Because of excessive collecting some geophytes, their populations have been damaged. *Scilla* genus was transferred to Asparagaceae family from Hyacinthaceae family (Güner *et al.*, 2012). It is represented by 18 species in Turkey and the rate of endemism is about 33.3%. Whereas this genus comprises about 100 species distributed in South Europe, the Mediterranean region and central and western Asia. The genus is important among geophyta plants, since some *Scilla* taxa (*S. autumnalis* L.) are used as decorative ornamental plants (Perry, 1974; Bangani *et al.*, 1999). Moreover, polyphenolic compounds have in the bubs and leaves of *S. autumnalis* (Özay *et al.*, 2013).

The life form of all investigated taxa is perennial. The *S. leepii* and *S. mesopotamica* distribute only in the vicinity of Elazığ, Diyarbakır and Şanlıurfa-Karaca Mountain, respectively (Mordak, 1984; Satıl and Akan, 2006; Eker and Akan, 2010). Both taxa have extremely limited distribution and are endemic to Turkey. So, *S. mesopotamica* and *S.*

leepii were placed in CR (critically endangered) and in LR (nt) (near threatened) categories, respectively (Ekim *et al.*, 2000; Anonymous, 2001). Because of various reasons (dam construction, excessive collection, tourism, agricultural fight, forest fires), *S. melaina*, *S. leepii*, *S. ingridae*, *S. mesopotamica*, *S. siberica* subsp. *armena* and *S. cilicica* are under threat of extinction. These taxa may be included among the rare species of Turkey in the future. *S. autumnalis* has widespread distribution in Turkey, Mediterranean, South-Western England, Portugal, Libya and North Africa. Vaughan *et al.* (1997) reported it as complex or cryptic species. However, it was critically endangered in some countries (specially, Romania) and was taken into protection in Romania (Banciu *et al.*, 2010). *S. autumnalis* is also different from other investigated taxa in terms of morphological characters. These differences may be caused from different flowering time (in autumn) and distribution in widespread areas in Turkey and in the world.

Since *S. bifolia* has widespread distribution, it has lots of problems in the morphological characters. Namely, Speta (1991) reported as two new taxa *S. dedea* and *S. pruinoso* from the South of Turkey. Later, the two species were described as synonymy of *S. bifolia* by Özhatay (2000). Moreover, Yıldırım (2014) reported that *Puschkinia bilgineri* is similar *S. bifolia* regarding to flower and seed

characters. On the other hand, *S. melaina*, *S. leepii*, *S. ingridae*, *S. mesopotamica*, *S. siberica* subsp. *armena* and *S. cilicica* are closely related to each other morphologically and sometimes these taxa are mixed with each other. So, there are some taxonomic problems in the morphological structures of investigated taxa. In this paper, to minimize above mentioned problems, it is aimed to determine their taxonomic places and relationship degrees according to their leaf and scape anatomical characters. Therefore, it is believed that it contributes to other studies solving some of the problems of these taxa.

Materials and Methods

The fresh samples of the eight taxa were collected from different locations of Turkey and the distribution areas of *Scilla* taxa were listed in Table 1. The distribution areas of each taxa were marked on the map (Fig. 1). Taxonomic description of the samples was identified according to Mordak (1984) and Güner et al. (2012). All of the taxa were known as perennial plants. While the sample materials of *S. autumnalis* were taken in November, the sample materials of other taxa were taken in March and April. The leaf materials were selected from mature leaves on the plants. Fresh scape and leaf samples were fixed in 70% alcohol solution and anatomical investigations were carried out on samples preserved in 70% alcohol solution. Paraffin method was used for preparing cross-sections of the scape and leaf parts (Algan, 1981). The cross-sections of the scape and leaf parts were taken with a microtom and photographed. The prepared permanent slides were used to compare different anatomical characters of scape and leaves. In the scape cross-sections, only scape photographs of the different taxa were given. 9 anatomical characters of the leaves and scape (micropapillae in cuticle, papillae in upper and lower epiderma, margin extensions, mesophyll structure, vascular bundle types, cavities, crystal types, vascular bundles in the periphery of scape and vascular bundles at the middle of scape) were used for distinguishing of investigated taxa (Table 2). For investigations stomata, surface-sections from the upper and lower surfaces of leaves were taken with the help a razor and were prepared permanent slides. Appropriate stomata samples were photographed with 40-magnification lens (X40) of Olympus light microscope. Length and width measurements of stomata in the upper and lower surfaces of leaves were recorded. Ten different observations were made for each taxa and the mean and standard deviation values of stomata were calculated according to Seçer (2013). The results of analysis were given in Table 3.

Results

The Scape Anatomic Properties of the *Scilla* Taxa

***S. autumnalis*:** Epiderma is single layered, small and square

shaped. The cuticle is thick. Papillae and micropapillae are seen the epiderma and cuticle, respectively. Cortex composed of oval shaped and large parenchyma cells (Fig. 2a). In the cortex, sclerenchymatic cylinder is 3–5 layered. Vascular bundles are 4 large and 4 small (Table 2). The pith is composed of large, thin walled parenchymatic cells.

***S. bifolia*:** Epidermis is single layered, small and square shaped. The thick cuticle is with micropapillae and the on the epiderma is seen papillae. Cortex is composed of oval shaped and small parenchyma cells. In the cortex, there are not cavities and sclerenchymatic cylinder. Vascular bundles are 8 large and 2 small. The pith is composed of large, thin walled parenchymatic cells (Fig. 2b).

***S. cilicica*:** The cuticle is thick. Epiderma is single layered, small and square shaped. Epiderma and cuticle are with papillae and micropapillae, respectively. Cortex composed of oval shaped and small parenchyma cells. There are not cavities and sclerenchymatic cylinder in the cortex. Vascular bundles are 5 large and 5 small. The pith has large, thin walled parenchymatic cells.

***S. ingridae*:** Epiderma is single layered, small and square shaped and cuticle is thin. Papillae and micropapillae are seen on the epiderma and cuticle, respectively. Cortex is oval or hexagon shaped and large parenchyma cells. There are rare raphida crystals in the cortex. Vascular bundles are 6–9 large and single row. There are large, thin walled parenchymatic cells in the pith.

***S. leepii*:** The cuticle is thin and epiderma is single layered, small and square shaped. There are rare papillae and micropapillae on the epiderma and cuticle, respectively. Cortex is composed of oval or hexagon shaped and large parenchyma cells. In the cortex, dense raphida crystals are found. Vascular bundles have two layers. The number of vascular bundles on the first ring is 8–10 and 5–7 on the second ring (Table 2). The pith is composed of large, thin walled parenchymatic cells.

***S. melaina*:** Epiderma is single layered, small and square shaped. The cuticle is thin. There are rare papillae and micropapillae on the epiderma and cuticle, respectively. Cortex is oval or hexagon shaped and large parenchyma cells (Fig. 2c). There are dense raphida crystals in the cortex. Vascular bundles are in two rows. The number of vascular bundles on the first ring is 10–12 and 6–7 on the second ring. The pith is composed of large, thin walled parenchymatic cells.

***S. mesopotamica*:** Epiderma is single layered, small and square shaped and the cuticle is thin. Papillae and micropapillae are seen on the epiderma and cuticle, respectively. Cortex is composed of oval or hexagon shaped and large parenchyma cells. In the cortex, there are rare raphida crystals. Vascular bundles are in single row and their number is 7–10 (Table 2). The pith has large, thin walled parenchymatic cells.

***S. siberica* subsp. *armena*:** The cuticle is thin and epiderma is single layered, small and square shaped. Rare papillae and micropapillae are seen on epiderma and cuticle, respectively.

Table 1: The distribution areas of *Scilla* taxa in Turkey “(E)” indicates endemic

Taxa	Localities
<i>S. autumnalis</i>	Aydın: Kuşadası, rocky areas Muğla: city cemetery, open areas Muğla: Göktepe, open areas
<i>S. bifolia</i>	Samsun: Çetirli Pınar Village, open areas Denizli: Honaz Mountain, shrub areas Muğla: the between Fethiye- Söğüt, open areas Edirne: the between Havsa Uzunköprü, rocky areas
<i>S. cilicica</i>	Samsun: Çetirli Pınar Village, open areas İçel: Yukarı Fındık Fountain, open steppe Kayseri: Pınarbaşı, Tersakan Village, open steppe
<i>S. ingridae</i>	Nevşehir: Göreme, open steppe Gaziantep: Nurdağı Passage, steppe areas İçel: Anamur-Akpınar Village, step areas
<i>S. leepii</i> (E)	Niğde: Ala Mountain, rocky areas Elazığ: the between Ergani Maden, open areas
<i>S. melaina</i>	Erzincan: Cevizli Village, steppe and metamorphic areas Adana: Düldül Mountain, shrub areas Gaziantep: Sof Mountain, Işıklı Village rocky areas
<i>S. mesopotamica</i> (E)	Adana: Tekir Mountain, steppe volcanic rocks Urfa: Siverek, Karaca Mountain, Rame Creek, rocky areas
<i>S. siberica</i> subsp. <i>armena</i>	Urfa: Halfeti, Fırat edge, rocky areas Sivas: Yıldızeli vicinity, open areas Sivas: Zara vicinity, open areas

Table 2: Distinctive scape and leaf anatomical characters for distinguishing of investigated *Scilla* taxa

Taxa	Micropapillae in cuticle	Papillae in upper and lower epiderma	Margin extensions	Mesophyll	Palisade layer	Spongy layer	Vascular bundles of leaves	Cavities	Crystal types of leaves	Vascular bundles in the periphery of scape	Vascular bundles at the middle of scape
<i>S. autumnalis</i>	Rare conspicuous	Present	Globose	Homogeneous	Absent	2-3 layered, oval shaped, chloroplast and 7-8 layered without chloroplast	Middle and collateral type	Present and small	Rare raphida crystals	4 large and 4 small	Absent
<i>S. bifolia</i>	Conspicuous	Present	18 cells	Heterogeneous	1-2 layered, short rectangular, chloroplast	4-6 layered, oval shaped, without chloroplast	Middle and collateral type	Present and small	Raphida crystals	8 large and 2 small	Absent
<i>S. cilicica</i>	Rare conspicuous	Present	6-7 cells	Heterogeneous	2-(-3) layered, short rectangular, chloroplast	4-5 layered, oval shaped, without chloroplast	Middle and collateral type	Present and large	Raphida crystals	5 large and 5 small	Absent
<i>S. ingridae</i>	Rare conspicuous	Present	6-8 cells	Homogeneous	Absent	2 layered, oval shaped, chloroplast, 7-8 layered, oval-elliptical, without chloroplast	Middle and collateral type	Present and very large	Raphida crystals	6-9 large	Absent
<i>S. leepii</i>	Conspicuous	Present	6-8 cells	Homogeneous	Absent	2 layered oval shaped, chloroplast, 4-5 layered oval shaped, without chloroplast	Middle and collateral type	Present and very large	Raphida crystals	8-10 small	5-7 large
<i>S. melaina</i>	Extremely conspicuous	Present	6 cells	Homogeneous	Absent	1-2 layered chloroplast, 5-6 layered oval shaped, without chloroplast	Middle and collateral type	Present and large	Rare raphida crystals	10-12 small	6-7 large
<i>S. mesopotamica</i>	Extremely conspicuous	Absent	Absent	Homogeneous	Absent	2-layered chloroplast, 5-6 layered oval shaped, without chloroplast	Middle and collateral type	Present and very large	Dense raphida crystals	7-10 large	Absent
<i>S. siberica</i> subsp. <i>armena</i>	Extremely conspicuous	Present	4-6 cells	Heterogeneous	2 layered, short rectangular, chloroplast	5-7 layered, oval shaped, without chloroplast	Middle and collateral type	Present and large	Dense raphida crystals	7-8 small	4-5 large

Cortex has oval shape and small parenchyma cells. In the cortex, there are cavities and they are 2–3 layered with chloroplast parenchyma. Vascular bundles are in two rows (Fig. 2d). The number of vascular bundles on the first ring is 7–8 and 4–5 on the second ring. There are large, thin walled parenchymatic cells in the pith.

The Leaves Anatomic Properties of the *Scilla* Taxa

***S. autumnalis*:** Leaf is in unifacial type (homogeneous structure) (Fig. 3a). Both epiderma are square shaped and small celled. Cuticle is very thick and with rare micropapillae. Leaf margin extension is in globose structure. General cross-section of leaf is semi-circle shaped. Stomata are dense and large in the upper and lower epiderma. They are in anomocytic type and have two epidermis cells (Fig. 4a). Mesophyll contains 1–3 layers in the upper epiderma and 2–3 layers in the lower epiderma with dense chloroplast parenchymatic cells (Fig. 3a). Parenchyma in the middle of mesophyll is 7–8 layered, oval shaped and with less chloroplast. Raphida crystals and cavities are rarely observed in the mesophyll. Vascular bundles are arranged in one row and were with sclerenchyma at phloem pole only. Bundle sheaths are one layered, thin walled parenchymatic cells.

***S. bifolia*:** Leaf is in isolateral type (heterogeneous structure) (Fig. 3b). Upper and lower epidermis cells are square shaped, small celled and with konic papillae. The cuticle is thick on both epiderm and it is with micropapillae. Margin extensions of leaf are composed of 18 cells. Stomata in the upper and lower epiderma are large, dense and without correspondence cells. Stoma is in anomocytic type (Fig. 4b). Palisade parenchyma are 1–2 layered in the lower epiderma and 2 layered in the upper epiderma, large celled, cylindrical shaped and with dense chloroplast. Spongy parenchyma is 4–6 layered, large celled, oval shaped and with less chloroplast. Raphida crystals and cavities are in the spongy parenchyma (Fig. 3b). Vascular bundles are lined in rows and parenchymatic bundle sheath is around of vascular bundles. Trachea are chain shaped. Sclerenchyma cells are only at the phloem poles.

***S. cilicica*:** Leaf is in isolateral type. Both epidermis cells are square shaped, small and single layered. Cuticle layer is thin and rarely micropapillae. The leaf margin extensions have 6–8 cells. Stoma is large, dense and without correspondence cells in both epiderma. They are in anomocytic type and have two epidermis cells (Fig. 4c). Stomata are lower than epidermis cells. Mesophyll is consisted of palisade and spongy parenchyma (Fig. 3c). The palisade parenchyma is 2 layered in the upper epiderma, 2-(3) layered in the lower epiderma. These cells are large and with dense chloroplast. The spongy parenchyma is 4–5 layered oval shaped and with less chloroplast. The cavities are in the centre of mesophyll. Raphida crystals are less in number in the spongy parenchyma. Vascular bundles are typically one row. Sclerenchyma is generally at phloem poles.

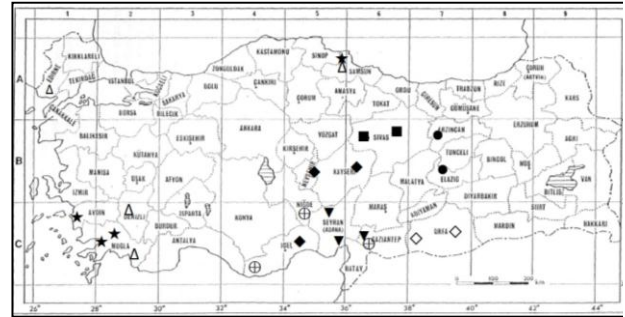


Fig. 1: The map of the distribution areas of *Scilla* taxa in Turkey; ★, *S. autumnalis*; △, *S. bifolia*; ◆, *S. cilicica*; ⊕, *S. ingridae*; ●, *S. leepii*; ▼, *S. melaina*; ◇, *S. mesopotamica*; ■, *S. siberica* subsp. *armena*

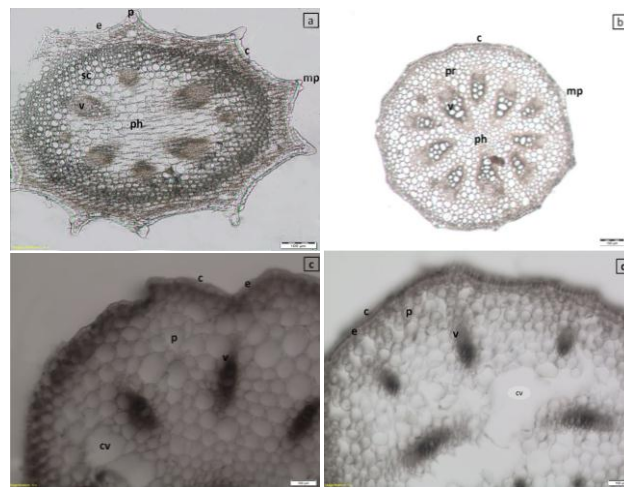


Fig. 2: The scape cross-sections of *Scilla* taxa. a, *S. autumnalis*; b, *S. bifolia*; c, *S. melaina*; d, *S. siberica* subsp. *armena*. c, cuticle; e, epiderma; pr, parenchyma; sc, sclerenchyma, v, vascular bundle; p, papillae; mp, micropapillae; ph, pith; cv, cavities

***S. ingridae*:** Leaf is in unifacial type (Fig. 3d). Epiderma is square shaped and large celled. Thin cuticle is rarely micropapillae. Stomata in the lower and upper epiderma are rare and very large. Stomata are in anomocytic type and have two epidermis cells (Fig. 4d). Margin extensions of leaf are consisted of 6–8 cells. Mesophyll is consisted of 2–layers with dense chloroplast and 7–8 layers without chloroplast parenchyma (Fig. 3d). There are cavities and dense raphida crystals in the parenchyma. Vascular bundles contain of bundle sheath one layered, thin walled and without chloroplast parenchymatous cells. Vascular bundles have sclerenchyma only at phloem pole. They are typically one row and large.

***S. leepii*:** Leaf is in unifacial type. Upper and lower epidermis cells are square shaped, small celled. The cuticle is thick on both epiderma. There are micropapillae and papillae on the cuticle and epiderma layers.

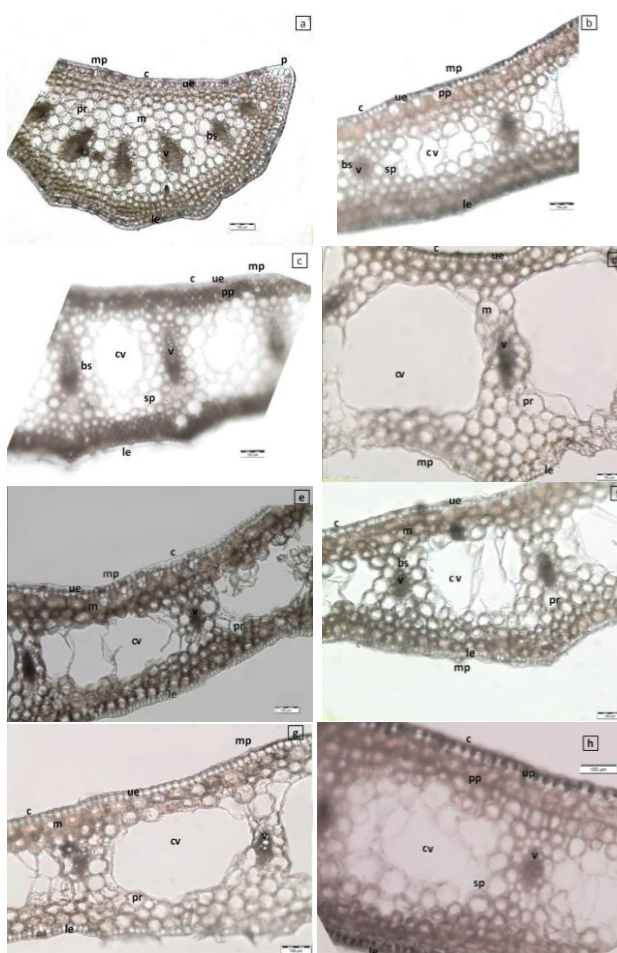


Fig. 3: The leaf cross-sections of *Scilla* taxa. a, *S. autumnalis*; b, *S. bifolia*; c, *S. cilicica*; d, *S. ingriddae*; e, *S. leepii*; f, *S. melaina*; g, *S. mesopotamica*; h. *S. siberica* subsp. *armena* c, cuticle; ue, upper epidermis; m, mesophyll; pr, parenchyma; cl, chloroplast; pp, palisade parenchyma; sp, spongy parenchyma, v, vascular bundles; bs, bundle sheath; le, lower epiderma; p, papillae; mp, micropapillae; cv, cavities

Leaf margin extensions are 6–8 cells. Parenchyma of mesophyll is 2–3 layered, large celled, cylindrical shaped, with dense chloroplast. Parenchyma at the center of mesophyll are 3–5 layered, large celled, oval shaped and with less chloroplast. There are cavities and raphida crystals in the mesophyll (Fig. 3e). Stomata in the upper and lower epiderma are large, dense and have two epidermis cells. They are in anomocytic type (Fig. 4e). Vascular bundles are lined in rows. Sclerenchyma cells are only at the phloem poles of vascular bundles.

***S. melaina*:** Leaf is in unifacial type. Both epidermis cells are square shaped, small and single layered. Thin cuticle is with dense micropapillae. Small papillae are dense in the upper and lower epiderma. Upper epiderma are larger than lower epiderma. Margin extension has 6 cells.

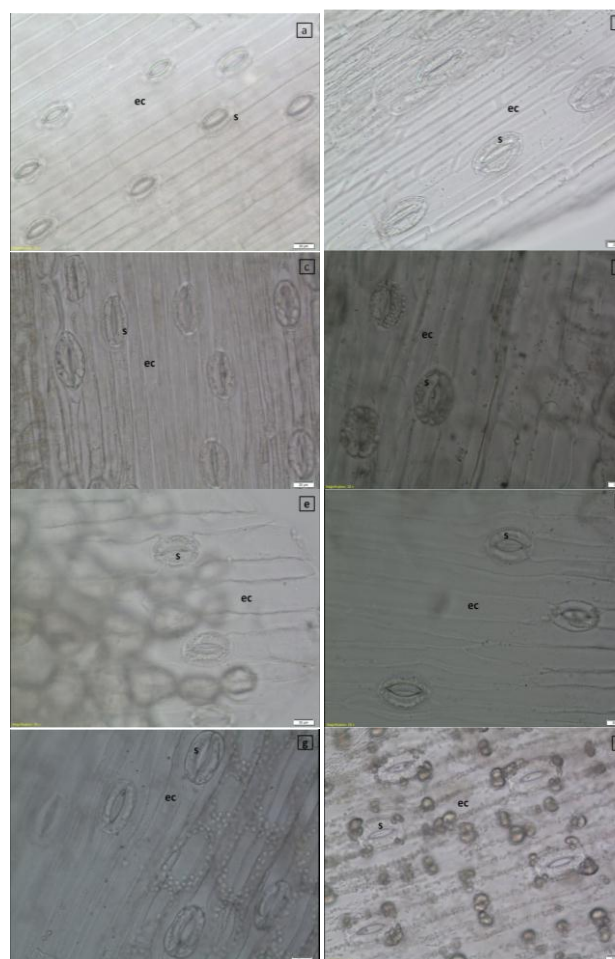


Fig. 4: The leaf surface-sections of *Scilla* taxa. a, *S. autumnalis*; b, *S. bifolia*; c, *S. cilicica*; d, *S. ingriddae*; e, *S. leepii*; f, *S. melaina*; g, *S. mesopotamica*; h. *S. siberica* subsp. *armena*. ec, epidermis cell; s, stomata

Anomocytic stoma is large, dense and two epidermis cells in both epiderma (Fig. 4f). A 1–2 layered parenchyma in the upper epiderma and 2 layered parenchyma in the lower epiderma are large and with dense chloroplast. The parenchyma at the centre of mesophyll is 5–6 layered oval shaped and with less chloroplast (Fig. 3f). The mesophyll has cavities and rare raphida crystals. Vascular bundles in the mesophyll layer are in single row. There are sclerenchyma cells only at phloem poles and parenchymatic bundle sheath in the vascular bundles.

***S. mesopotamica*:** Leaf is in unifacial type. Epiderma is square shaped and large celled. Cuticle is thin and dense micropapillae. Stomata in the lower and upper epiderma are rare and very large. Stoma is in anomocytic type and has two epidermis cells (Fig. 4g). The margin extension has not cells. Mesophyll is consisted of 2-layers in the upper epiderma, 1–2 layers in the lower epiderma with chloroplast parenchyma and 5–6 layers in the center without chloroplast parenchyma (Fig. 3g). Parenchymatic cells are large and

oval shaped. There are cavities and dense raphida crystals in the mesophyll. Vascular bundles contain bundle sheath thin wall and without chloroplast parenchymatous cells. Sclerenchyma cells are only at the phloem poles.

***S. siberica* subsp. *armena*:** Leaf is in isolateral type (Fig. 3h). Upper and lower epidermis cells are square shaped, with dense papillae and large celled. Thick cuticle is with dense micropapillae. While stomata in the upper epiderma are very large and in less number, stomata in the lower epiderma are dense. Stoma is in anomocytic type (Fig. 4h). The leaf margin extensions have 4–6 cells. Mesophyll is consisted of 2 layers in the upper epiderma and 2–3 layers in the lower epiderma, dense chloroplast palisade parenchyma. Spongy parenchyma is 5–7 layered, large celled and with less chloroplast (Fig. 3h). Dense raphida crystals and cavities are observed in the mesophyll. Vascular bundles are with sclerenchyma at phloem pole only. There is bundle sheath around the vascular bundles. It contains one layer, thin wall parenchymatic cells. Vascular bundles are typically in one row and large. The trachea is chain shaped.

Discussion

In this study, the leaf and scape anatomical properties of *Scilla* taxa growing naturally in Turkey were compared. Some problems of these taxa has been tried to solve. Stace (1984) and Charlton (1988) reported that anatomical data can be used to solve some monocotyledoneous taxonomic problems. Although similar anatomical characters were obtained among investigated taxa, the different anatomical characters (the presence micropapillae and papillae, heterogeneous or homogeneous structure of mesophyll, the presence large or small cavities in the mesophyll, epidermal extensions of the leaves margin, and the number of vascular bundles in the periphery and in the middle of scape) were obtained among investigated taxa.

Since the epidermis cell extensions of the leaf margins of these taxa contain differences regarding to the shape and number of cells, these properties may have taxonomic value among taxa. The same state was seen in anatomical studies on the *Scilla beirana* Samp. and *S. verna* complex (related species: *S. verna* Hudson, *S. ramburei* Boiss., *S. odorata* Link, *S. monophyllos* Link, *S. pau* Lacaita and *S. merinoi* Ortiz et al., unrelated species: *S. peruviana* L., *S. hyacinthoides* L., *S. lili-hyacinthus* L. and *S. obtusifolia* Poiré) by Almeida et al. (1998), Almeida and Rossello (1999). Also, these researchers reported that the members of *S. verna* complex have very similar in the leaf and scape anatomical characters. The margins extensions of leaves have 18 cells in *S. bifolia*, 6–7 cells in *S. cilicica*, 6 cells in *S. melaina*, 4–6 cells in *S. siberica* subsp. *armena*, 6–8 cells in *S. leepii* and *S. ingridae*. The epidermal extensions are absent in *S. mesopotamica*, while the epidermal extensions are in globose shape in *S. autumnalis*.

Table 3: Stomatal measurements in the upper and lower epiderma of the leaves (mean values \pm standard deviation)

Taxa	Leaf surface	Length (μm)	Width (μm)
<i>S. autumnalis</i>	Upper epiderma	27.4 \pm 1.10	23.03 \pm 1.29
	Lower epiderma	24.1 \pm 1.85	22.6 \pm 1.31
<i>S. bifolia</i>	Upper epiderma	41.7 \pm 1.25	30.3 \pm 1.06
	Lower epiderma	40.8 \pm 0.92	29.9 \pm 1.10
<i>S. cilicica</i>	Upper epiderma	41.5 \pm 1.35	28.7 \pm 1.15
	Lower epiderma	39.6 \pm 1.50	27.2 \pm 1.23
<i>S. ingridae</i>	Upper epiderma	46.8 \pm 1.39	31.5 \pm 3.06
	Lower epiderma	48.4 \pm 1.26	30.7 \pm 1.63
<i>S. leepii</i>	Upper epiderma	40.1 \pm 1.52	30.4 \pm 1.95
	Lower epiderma	35.2 \pm 2.04	28.4 \pm 1.43
<i>S. melaina</i>	Upper epiderma	41.2 \pm 2.84	30.6 \pm 1.59
	Lower epiderma	39.7 \pm 2.21	29.2 \pm 1.87
<i>S. mesopotamica</i>	Upper epiderma	47.3 \pm 2.04	32.5 \pm 2.01
	Lower epiderma	48.4 \pm 2.06	33.6 \pm 1.34
<i>S. siberica</i> subsp. <i>armena</i>	Upper epiderma	48.8 \pm 1.14	23.2 \pm 1.98
	Lower epiderma	46.7 \pm 1.33	22.7 \pm 1.16

Mesophyll is in homogeneous structure in *S. autumnalis*, *S. ingridae*, *S. leepii*, *S. melaina*, and *S. mesopotamica* (Fig. 3a, d, e, f, g). But, mesophyll is in heterogeneous structure in *S. bifolia*, *S. siberica* subsp. *armena* and *S. cilicica* (Table 2, Fig. 3b, c, h). Homogeneous mesophyll is composed of only parenchymatic cells. Heterogeneous mesophyll comprised of palisade and spongy parenchyma. The above mentioned state was found in *S. beirana*, the member of *S. verna* complex and some *Scilla* species by Almeida et al. (1998), Almeida and Rossello (1999), Satıl and Akan (2006), respectively. Homogeneous and heterogeneous mesophyll structures were reported as important characters in *Scilla* taxa by these researchers. Moreover, the importance of mesophyll types were reported in leaf anatomical studies of endemic *Iris* taxa in Turkey by Kandemir (2015). The investigated taxa can be distinguished as mesophyll type; isolateral (*S. bifolia*, *S. cilicica* and *S. siberica* subsp. *armena*) and unifacial (*S. melaina*, *S. leepii*, *S. ingridae*, *S. mesopotamica* and *S. autumnalis*).

Franceschi and Nakata (2005), Kandemir (2011) and Kandemir et al. (2012) reported that crystal shape and distribution are used as a taxonomic character. Uysal (1992) suggested that crystals are important taxonomically in anatomic studies. Raphida crystals and cavities are observed in the mesophyll in all taxa. There are the large cavities in *S. mesopotamica*, *S. melaina*, *S. leepii*, *S. ingridae*, *S. siberica* subsp. *armena* and *S. cilicica*. But, the cavities are small in *S. autumnalis* and *S. bifolia*. While *S. autumnalis* and *S. melaina* have rare raphida crystals, *S. mesopotamica*, *S. melaina*, *S. leepii*, *S. ingridae*, *S. siberica* subsp. *armena*, *S. bifolia* and *S. cilicica* have dense raphida crystals. The above mentioned the two properties may be not significant leaf anatomical characters in discrimination of the investigated taxa, since the two properties are similar among taxa.

Upper and lower epiderma occurred small and square shaped cells in *S. bifolia*, *S. melaina*, *S. autumnalis*, *S. leepii*

and *S. cilicica*, while upper and lower epiderma occurred large and square shaped cells in *S. mesopotamica*, *S. ingridae* and *S. siberica* subsp. *armena*. In the lower and upper epiderma of *S. bifolia*, *S. autumnalis*, *S. cilicica*, *S. ingridae*, *S. leepii*, *S. melaina*, *S. siberica* subsp. *armena*, papillae are seen on both epiderma. In *S. mesopotamica*, papillae are not seen on the lower and upper epiderma. Micropapillae are observed on the cuticle in all taxa. Micropapillae are extremely conspicuous in *S. melaina*, *S. mesopotamica* and *S. siberica* subsp. *armena*. Rudall and Mathew (1990), Kandemir (2011) and Kandemir *et al.* (2012) suggested that micropapillae on the cuticle layer and papillae in epidermis cells have some taxonomic significance. For investigated taxa may not important micropapillae on the cuticle layer and papillae in epidermis cells, since these taxa show great similarity with regard to micropapillae and papillae. However, papillae are not seen on the lower and upper epiderma of *S. mesopotamica*. Perhaps these taxa can be distinguished from other taxa with this character.

For investigated taxa mean and standard deviation values of the stomatal guard cells length and width are shown in Table 3. The analysis results show that some of the taxa (*S. leepii*, *S. melaina*, *S. autumnalis*, *S. cilicica* and *S. siberica* subsp. *armena*) indicated significant differences between stomatal length values in the upper and lower epiderma (Table 3). These properties of stomata from both surfaces may be used discrimination the five taxa (*S. leepii*, *S. melaina*, *S. autumnalis*, *S. cilicica* and *S. siberica* subsp. *armena*). Whereas, differences between stomatal length in the upper and lower epiderma of some taxa (*S. bifolia*, *S. mesopotamica*, *S. ingridae*). On the other hand, relationship between the habitats of the investigated taxa and this property of stomata are not conclusive. Stomata are dense and large in *S. bifolia*, *S. cilicica*, *S. leepii* (Fig. 4b, c, e). But, stomata are rare and very large in *S. ingridae*, *S. mesopotamica*, *S. siberica* subsp. *armena* (Fig. 4d, g, h). In *S. autumnalis* and *S. melaina*, stomata are small and dense (Fig. 3a, f). The largest stomata are found in *S. ingridae*, *S. mesopotamica* and *S. siberica* subsp. *armena* and the smallest stomata are found in *S. autumnalis* among taxa (Table 3). Vascular bundles of leaves have single row and parenchymatic bundle sheath in all taxa. Trachea are chain shaped in *S. bifolia*, *S. siberica* subsp. *armena*, *S. cilicica*. This property of trachea may be used distinguishing of the three taxa.

In investigated taxa, anatomic characters of the scape are very similar. This status was also observed in the scape anatomic characteristics of *S. beirana* and *S. verna* complex (Almeida *et al.*, 1998; Almeida and Rossello, 1999). Epiderma layer of scapes is composed of small and square shaped cells in all taxa. The cuticle layer was thick in *S. autumnalis*, *S. bifolia* and *S. cilicica*. Whereas the cuticle was thin in *S. leepii*, *S. ingridae*, *S. melaina*, *S. mesopotamica* and *S. siberica* subsp. *armena*. Micropapillae and papillae

were seen on the cuticle and epiderma of the scapes of the investigated taxa. Raphida crystals were observed only in *S. leepii*, *S. melaina*, *S. ingridae* and *S. mesopotamica* scapes. The three taxa (*S. leepii*, *S. melaina*, *S. ingridae* and *S. mesopotamica*) with this property of scape may discern from other investigated taxa. The cortex of all taxa comprised of thin walled parenchymatic cells. Also, in the cortex of only *S. autumnalis*, sclerenchyma cylinder with 3–5 layers was seen. The vascular bundles in the periphery of *S. autumnalis* scape formed inside the sclerenchyma cylinder. This property of *S. autumnalis* can be considered as distinguishing taxonomic character. In the these taxa, two different scape anatomy including vascular bundles in the periphery and at the middle of scape or vascular bundles only in the periphery of scape (*S. leepii*, *S. siberica* subsp. *armena* and *S. melaina*) were observed (Fig. 2b, c, d). There are vascular bundles in the middle of scape only of *S. melaina*, *S. siberica* subsp. *armena* and *S. leepii*. These vascular bundles were 5–7 large in *S. leepii*, 6–7 large in *S. melaina*, 4–5 large in *S. siberica* subsp. *armena*. There are not vascular bundles in the middle of scape in other taxa. Generally, vascular bundles are in the periphery of scape and are scattered (Fig. 2). These vascular bundles were 4 large and 4 small in *S. autumnalis*, 8 large and 2 small in *S. bifolia*, 5 large and 5 small in *S. cilicica*, 8–10 small in *S. leepii*, 6–9 large in *S. ingridae*, 10–12 small in *S. melaina*, 7–10 large in *S. mesopotamica* and 7–8 small in *S. siberica* subsp. *armena* (Table 2).

Vaughan *et al.* (1997) reported that the taxonomic status and relationships of *S. autumnalis* were no clear, because of widespread (the Mediterranean, South-Western England, Portugal, Libya and North Africa). So, he called it as *S. autumnalis* species complex. Also, this species is a different species of *Scilla* genus. According to our findings, *S. autumnalis* can be distinguished from other investigated taxa in terms of the leaf and scape anatomical characters such as; mesophyll is heterogeneous or homogeneous structure, stomata are smaller than other taxa, the general structure of the leaf is semi-circle shaped, it has 3–4 layers sclerenchymatic sheath in the scape and leaf margin extension is in globose structure. According to our results, we think that it is appropriate to call as a complex species of *S. autumnalis*. Additionally, we think *S. bifolia* to be a complex species because of the widespread distribution and the taxonomic problems in Turkey. Although they show some differences, the investigated taxa show great similarities regarding to anatomical characters. Therefore, it is possible that these taxa can be related to species of *S. bifolia*, *S. cilicica*, *S. siberica* subsp. *armena*, *S. leepii*, *S. melaina*, *S. ingridae* and *S. mesopotamica* and not to species of *S. autumnalis* among *S. bifolia* complex in Turkey. Of course, the chorosome and chloroplast DNA studies are needed to make a more precise determination of the relationship among these taxa

This study showed that these data can be used to distinguish the investigated taxa from each other despite of

anatomic data with limited taxonomic values. Based on the anatomic characters, we suggested that (1) *S. ingridae*, *S. mesopotamica*, *S. melaina* *S. leepii* are independent species with close relationships; (2) *S. bifolia* *S. siberica* subsp. *armena* and *S. cilicica* are very close taxa; (3) *S. autumnalis* is different from other investigated taxa.

Conclusion

The number and the status of vascular bundles in scape, the status of mesophyll layer in leaves were asserted as distinctive taxonomic characters among the investigated taxa. Moreover, *S. bifolia* may be accepted as a complex species and *Scilla* taxa can be members of *S. bifolia* complex. To make a precise definition, molecular studies are required.

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(Received 05 March 2016; Accepted 02 June 2016)