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Research article/Araştırma makalesi

Morphological and anatomical investigations on endemic *Hyacinthella acutiloba* in Turkey

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Abstract

Morphology and anatomy of *Hyacinthella acutiloba* K.Press. & Wendelbo (Sivri sümbül) an endemic species for Turkey, belong to Asparagaceae have been investigated. The specimens collected from two populations of natural habitat in Sivas province. In morphological studies, biometric measurements of the plant organs such as bulb, scape, leaf and flower have been carried out and pollen morphology has been studied. Pollen shape is prolate and ornamentation is reticulate. For anatomical investigations the hand cross-sections of root, scape and leaf were taken with a razor blade. The adventive root, scape and leaf anatomies of species display the common properties of monocotyledons. The leaves are amphistomatic and mesophyll is isolateral.

Key words: Anatomy, Asparagaceae, Endemic, *Hyacinthella acutiloba*, Morphology

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Türkiye'ye endemik olan *Hyacinthella acutiloba* üzerinde morfolojik ve anatomik araştırmalar

Özet

Türkiye'ye endemik bir tür olan Asparagaceae familyası üyesi *Hyacinthella acutiloba* K.Press. & Wendelbo (Sivri sümbül) türünün morfolojisi ve anatomisi incelenmiştir. Bitki örnekleri Sivas ilindeki iki doğal yayılış ortamından toplanmıştır. Morfolojik çalışmalarda bitkinin soğan, skapus, yaprak ve çiçek gibi organlarının biyometrik ölçümü gerçekleştirilmiştir ve polen morfolojisi incelenmiştir. Polen şekli prolat ve ornamentasyonu retikülattır. Anatomik araştırma için jilet kullanılarak bitkinin kök, skapus ve yaprağından enine kesit alınmıştır. Türün adventif kök, skapus ve yaprak anatomileri monokotil bitkilerin ortak özelliklerini göstermektedir. Yapraklar amfistomatik ve mezofil izolateraldir.

Anahtar kelimeler: Anatomi, Asparagaceae, Endemik, *Hyacinthella acutiloba*, Morfoloji

1. Introduction

Asparagaceae family has 143 genera and 3632 species which are distribute naturally in temperate, sub-tropical and tropical, and contains ornamental, vegetable, aromatic and medicinal plants (The Plant List, 2010). The family is represented in 19 genera and 182 species in the Flora of Turkey (Güner et al., 2012). *Hyacinthella* Schur is a genus of 17 species distributed in mainly Mediterranean regions (The Plant List, 2010). Genus is represented 12 species, which 10 of them are endemic, in Turkey (Güner et al., 2012). *H. acutiloba* K.Perss. & Wendelbo is an endemic species distributed in Kayseri, Sivas, Malatya and Erzincan province within B6 and B7 square in Turkey. The chromosome number is known as 2n=18 (Persson and Wendelbo, 1984). According to Red Data Book of Turkish Plants, threat category of species is lower risk/conservation dependent (LR/cd) (Ekim et al., 2000). *Hyacinthella* genus is constantly changing place between families (Liliaceae, Hyacinthaceae, recently Asparagaceae). Therefore, determination of morphological and anatomical characteristics of all species in detail will contribute to state of systematically place of the genus.

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There are some studies on the morphology and anatomy of this genus. *Hyacinthella micrantha* (Boiss.) Chouard (Kandemir et al., 2000), *H. lineata* (Steudel) Chouard (Selvi et al., 2008), *H. lazulina* K. Persson et J. Persson, *H. heldreichii* (Boiss.) Chouard and *H. campanulata* K.Press. & Wendelbo (Atayeter, 2007) and *H. glabrescens* (Yetişen et al., 2012) endemic species for Turkey, have been investigated morphologically and anatomically. *H. acutiloba* is closely related to *H. lineata* by morphological characters (Persson and Wendelbo, 1984). There is no report on anatomical characters of a Turkish endemic *H. acutiloba.* In this study, the morphological and anatomical characters of the species have been reported here in detail.

2. Materials and methods

Examined specimens were collected from natural habitats in Sivas (Turkey). Locality 1: Sivas province, from Sivas to Gürün, near Böğrüdelik village, (N 38° 57’ 26” ; E 37° 16’ 15”, 1966 m), 16.04.2011, M. Tekin 1064. Localite 2: Sivas province, from Sivas to Ulaş, near Ziyarettepe, (N 39° 33’ 08.9” ; E 37° 01’ 12.1”, 1400 m), 12.06.2012, M. Tekin 1246. Apart the seed, for state of morphological and anatomical properties were used the specimes which collected from both locality. For state of seed morphology properties were used the specimens which collected from locality 2. Some plants were prepared as herbarium materials and voucher specimens were deposited in the Cumhuriyet University Herbarium, Faculty of Science, Department of Biology (CUFH). Others were fixed in 70% ethyl alcohol for anatomical studies. Taxonomic descriptions of the plants were carried out according to Persson and Wendelbo (1984). Seeds were observed an Olympus SZ61 Stereomicroscope and images were taken with a ProgRes C12 Plus digital camera. For pollen morphology, slides prepared were as described by Wodehouse (1935). Measurements were taken from 50 pollen grains derived from the herbarium materials. For anatomical studies, hand sections from fixed samples were taken with a razor blade and some sections were stained with Alcian blue (Sigma) for pectic substances, Safranin (Sigma) for lignin (20 µl Alcian Blue + 20 µl Safranin in 10 ml 25% glycerin for four hours) and Sudan III (Sigma) for suberin (Jensen, 1962). The stained and unstained sections were mounted in glycerin-gelatin to make permanent preparations (Jensen, 1962). Pollen slides and anatomical sections were examined using an Olympus BH2 microscope fitted with a digital camera. Images were taken with a ProgRes C12 Plus digital camera and selected images were processed in PhotoShop 7.0. The anatomical drawings were performed with the aid a camera lucida system coupled light microscope.

3. Results

3.1 Morphological Properties

Hyacinthella acutiloba K.Perss. & Wendelbo in Candollea 36:524

Perennial, 8-15 cm. Adventive roots pale brown and 1-7.5 cm. Bulbs subglobose to ovoid, 1.2-3.2x1.5-2.7 cm. Tunics greyish-brown and thickened membranous. Leaves 3, oblong-elliptic, 5-9.5x0.4-2.3 cm and generally shorter than scape. Lamina surface glabrous, margin ciliolate-scabrid particulary at base. Scape single, occasionally paired, 6-13 cm, erect, glabrous and greenish, tinged or spotted purplish. Raceme cylindrical with 8-35 flowers, rachis green- purplish. Bracts 0.8-1.1 mm, membranous, faintly bilobed, medium violet. Pedicels 1.5-6 mm at anthesis. Perigon 4-6 mm, violet blue, tubular-campanulate; tubes 2.5-3.6 mm, lobes triangular, 1.5-2.5 mm and lobes apex subacute to obtus. Stamens attached to perigon tube. Filaments 0.7-1 mm, hairless, slender and shorter than anthers. Anthers 1.3-1.8 mm, dorsifixed, longitudinal dehiscent and deep violet. Pollen grains are monosulcate, 27-32 x 37-42 µm (E-P), prolate (P/E ratio = 1.34). Exine sculpture is reticulate (Figure 2 A,B). Style 1.8-2.4 mm. Ovary 1.5-2x1.7-2.4 mm, ovoid to subglobose and light green. Fruit 3.2-4 x 4.2-5 mm and subglobose and usually bearing 6 seeds. Seeds 1.4-2 x 2.1-2.7 mm and black colored (Figure 2 C). Table 1 shows the morphological measurements obtained from *H. acutiloba* and literature knowledge.

Figure 1A. *H. acutiloba* in natural habitat
Şekil 1A. Doğal yaşam ortamında *H. acutiloba*

Figure 1B. Raceme of *H. acutiloba*
Şekil 1B. *H. acutiloba* rasemusu

Figure 2. Pollen and seed properties of *H. acutiloba.* A. Pollen grain in optical section (Prolate). B. Exine ornamentation (Reticulate). Scale bar= 10µm. C. Seed morphology. Scale bar= 1 mm
Şekil 2. *H. acutiloba*'nın polen ve tohum özellikleri. A. Optik kesitte polen tanesi (Prolat). B. Ekzin ornamentasyonu (Retikülat). C. Tohum morfolojisi

Habitat: Quercus scrub, rocky limestone slopes, gypsaceous hills, 1550-2100 m.
Flowering period: April-May.
Phytogeographic region: Irano-Turanian element.
Distribution in the World: Turkey (Endemic).
Distribution in Turkey: Central Anatolia. Sivas, Kayseri, Malatya, Erzincan.

3.2 Anatomical Properties

3.2.1 Root: The root of *H. acutiloba* displays common features of monocotyledons (Figure 3 A,B,C,D). Epidermis is composed of oval or spherical shaped, single layered cells. The cell walls of epidermal cells are suberised and its outer wall is thicker than the other walls. Exodermis is made up of big suberised, single layered cells. They do not contain intercellular space (Figure 4). Cortex is 8-10 layered and consists of parenchymatous cells with intercellular space and thin walls. Some cells contain calcium oxalate (CaOx) raphide crystals (Figure 3 C,D). The single layered endodermis circularly is arranged central cylinder which follows the cortex. Endodermal cells have not secondary wall thickness and Casparian strip is distinct (Figure 5). Pericycle is single layered and thin walled. Protoxylem ridges are 7 or 8 and these are alternate with the phloem. The centre of the vascular cylinder is occupied by parenchyma cells (Figure 3 C,D).

Table 1. Morphological mesurements of *H. acutiloba* and their comparisons with literature knowledges
Tablo 1. *H. acutiloba*'nın morfolojik ölçümleri ve bu ölçümlerin literatür kayıtları ile karşılaştırılmaları

	Tekin and Meric (present study)	Persson and Wendelbo, 1984 (*H. acutiloba*)	Selvi et al., 2008 (*H. lineata*)
Plant length(cm)	8-15	Not recorded	10-18
Bulb (width x length) (cm)	1.2-3.2x1.5-2.7	Not recorded	1.2-2.5x2-2.5
Root length (cm)	1-7.5	Not recorded	1.5-5
Leaf width (mm)	4-23	(5-)10-20(-35)	8-15
Leaf length (cm)	5-9.5	Not recorded	5.5-6.5
Scape length (cm)	6-13	Not recorded	8-15
Bract length (mm)	0.8-1.1	Not recorded	Not recorded
Pedicel length (at anthesis) (mm)	1.5-6	Not recorded	2-7
Perigon length(mm)	4-6	Not recorded	4.5-6
Perigon tube length (mm)	2.5-3.6	Not recorded	Not recorded
Perigon lobe length (mm)	1.5-2.5	Not recorded	1.5-2.1
Filament length(mm)	0.7-1	Not recorded	0.5-0.8
Anther length (mm)	1.3-1.8	Not recorded	1.5-2
Style length (mm)	1.8-2.4	Not recorded	2.2-2.5
Ovary (width x length) (mm)	1.5-2x1.7-2.4	Not recorded	1-1.5x1-2
Fruit (width x length) (mm)	3.2-4 x 4.2-5	Not recorded	Not recorded
Seed (width x length) (mm)	1.4-2 x 2.1-2.7	Not recorded	Not recorded
Pollen (E x P) (μm)	27-32 x 37-42	Not recorded	Not recorded

3.2.2 Scape: Transverse sections taken from the stem are observed as follows (Figure 6 A,B,C,D): Epidermis with cuticle is composed of single layered, ovoid or spheroid cells. The outer tangential wall of epidermal cells is thicker than radial and inner tangential walls. Cortex is 5-6 layered and consists of parenchymatous cells with intercellular space and thin walls. Some cells of the cortex contain CaOx raphide crystals. The 6-7 layered sclerenchyma tissue is on the inner side of the cortex (Figure 6 C,D). Scape contains about 23-25 vascular bundles of different sizes in vascular cylinder. Vascular bundles consist of xylem and phloem are collateral type and they begin under sclerenchyma tissue. In inner circles, the bundles are bigger than outer ones (Figure 6 C,D). The pith consists of parenchymatous cells. Epidermis of scape has a few anomocytic type stomata. The stomata cells are located at the same level with the other epidermal cells.

3.2.3 Leaf: There is a single layered epidermis on both surface of leaf. Both epidermises are covered with a thin cuticle. The outer walls of epidermal cells are undulate and thickened. The leaf is amphistomatic. Stoma cells are equally present on the surfaces of both sides. They located on the same level with the other epidermal cells (mesomorphic). Stoma type is anomocytic. The mesophyll is isolateral and differentiated as palisade and spongy parenchyma. Under upper epidermis, the mesophyll contains 1-2 layers of palisade which is comprised of oval-shaped cells with intercellular spaces. Under lower epidermis, palisade parenchyma is composed of two layers of elongated cells. Lacunae are absent in the mesophyll. Mesophyll has a few raphide crystal idioblast. Vascular bundles of different sizes are arranged in one row and located in the spongy parenchyma cells. The xylem faces towards the upper surface while the phloem faces the lower epidermis. The big bundles have sclerenchyma fibers cap which is situated over phloem and xylem (Figure 7 A,B,C). Both epidermises do not contain trichomes.

4. Conclusions

In this study, morphological and anatomical features of *H. acutiloba* have been investigated, and the morphological features of species have been compared with Persson and Wendelbo (1984). There are very limited data with respect to the morphological features of species in Flora of Turkey and the East Aegean Islands (Persson and Wendelbo, 1984; vol: 8, pp. 278). The morphological characters and biometric measurement of most organs have been reported in this study. Persson and Wendelbo (1984) have been reported that *H. acutiloba* is like *H. lineata* for morphological properties. Our morphological results are also compared with *H. lineata* (Selvi et al., 2008) in table 1. There are a few differences related to morphological characters between both species.

Anatomical features of the root are similar with studied *Hyacinthella* species (Kandemir et al., 2000; Atayeter, 2007; Selvi et al., 2008; Yetişen et al., 2012) and *Bellevalia mathewii* Özhatay & Koçak (Asparagaceae) (Doğu et al, 2011). In root cortex of *H. lineata,* sand crystals have been reported by Selvi et al. (2008). In root cortex cells of *H. glabrescens* have been observed raphide and sand crystals by Yetişen et al., 2012. *H. acutiloba* have calcium oxalate (CaOx) raphide crystals in root cortex.

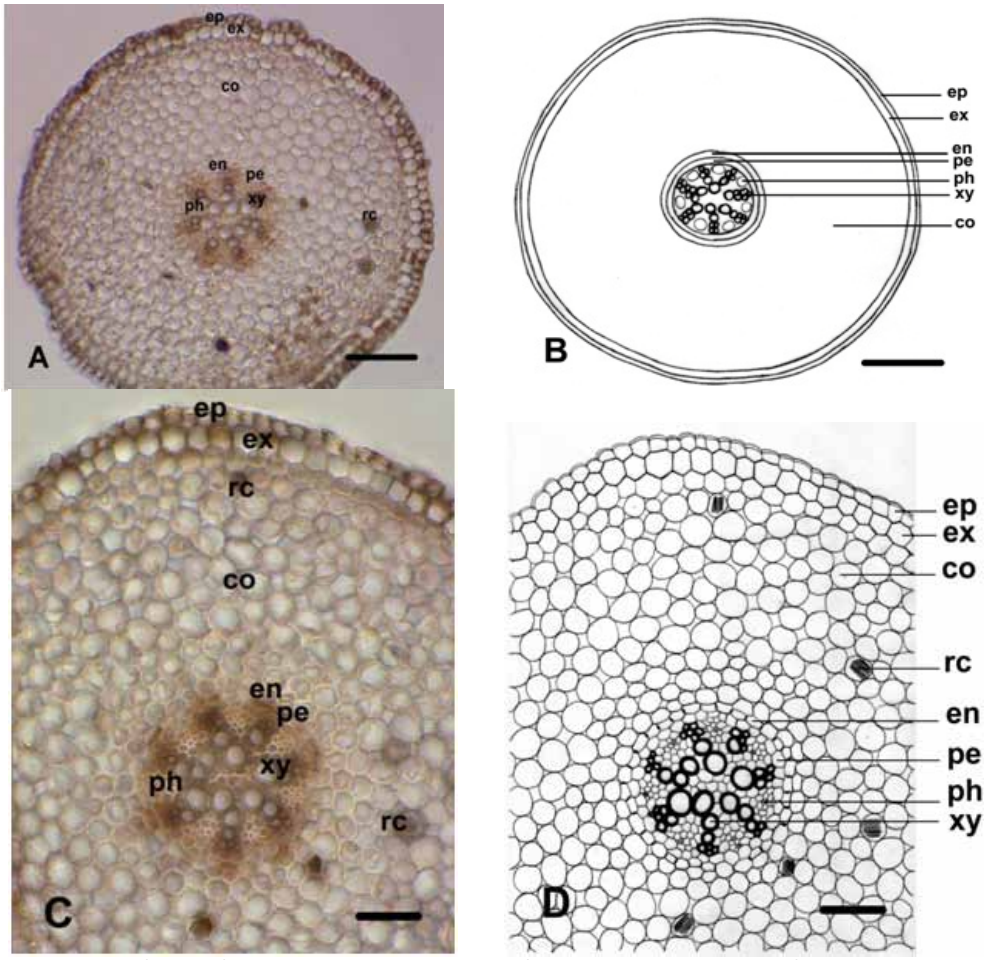

Figure 3. The cross-section of root of *H. acutiloba*. A. Low magnification microphotograph of root section. B. Diagram of root section. Scale bar= 100 µm. C. The microphotograph of root cross-section. D. Camera lucida drawing of root cross-section. co, cortex; en, endodermis; ep, epidermis; ex, exodermis; pe, pericycle; ph, phloem; rc, raphide crystals; xy, xylem. Scale bar= 50 µm

Şekil 3. *H. acutiloba*'nın kökünden enine kesit. A. Kök kesitinin düşük büyütmedeki mikrofotoğrafı. B. Kök kesiti diyagramı. C. Kök enine kesiti mikrofotoğrafı. D. Kök enine kesitinin Camera lucida çizimi. co, korteks; en, endodermis; ep, epidermis; ex, ekzodermis; pe, perisikl; ph, floem; rc, rafit kristalleri; xy, ksilem

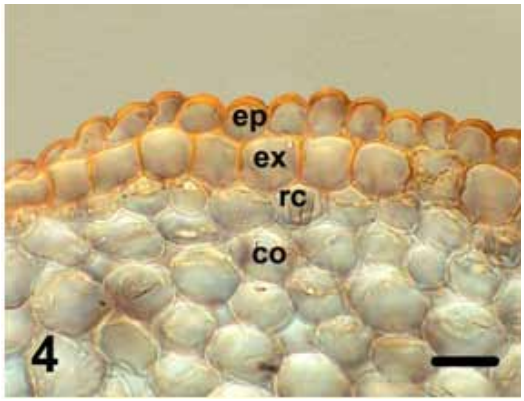

Figure 4. Epidermis and exodermis in root (stained with Sudan III). co, cortex; ep, epidermis; ex, exodermis; rc, raphide crystals. Scale bar= 20µm

Şekil 4. Kökte epidermis ve ekzodermis (Sudan III ile boyalı). co, korteks; ep, epidermis; ex, ekzodermis; rc, rafit kristalleri

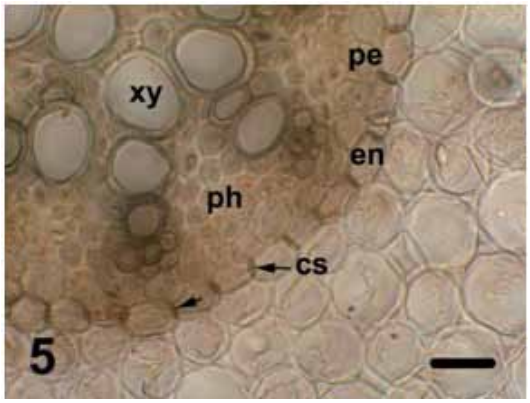

Figure 5. Endodermis with Casparian strip in root. cs, Casparian strip (arrows); en, endodermis; pe, pericycle; ph, phloem; xy, xylem. Scale bar= 20µm

Şekil 5. Kökte kaspari şeritli endodermis. cs, Kaspari şeridi; en, endodermis; pe, perisikl; ph, floem; xy, ksilem

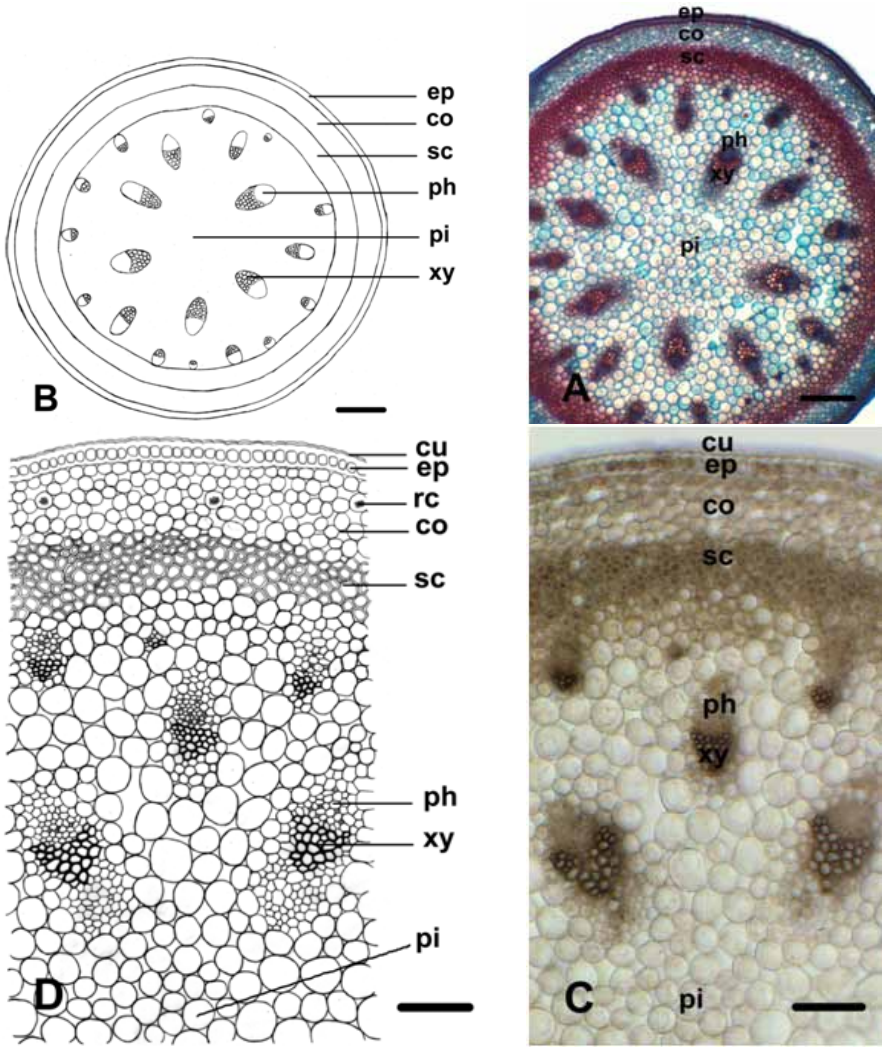

Figure 6. The cross-section of scape of *H. acutiloba.* A. Low magnification microphotograph of scape section (Stained with Alcian blue and Safranin). B. Diagram of scape section. Scale bar= 200. C. The microphotograph of scape cross-section. D. Camera lucida drawing scape cross-section. co, cortex; cu, cuticle; ep, epidermis; ph, phloem; pi, parenchymatic pith; rc, raphide crystals; sc, sclerenchyma; xy, xylem. Scale bar=100 µm.
Şekil 6. *H. acutiloba*'nın skapus enine kesiti. A. Skapus kesitinin düşük büyütmedeki mikrofotoğrafı (Alcian blue ve Safranin ile boyalı). B. Skapus kesitinin diyagramı. C. Skapus enine kesiti mikrofotoğrafı. D. Skapus enine kesitinin Camera lucida çizimi. co, korteks; cu, kutikula; ep, epidermis; ph, floem; pi, parenkimatik öz; rc, rafit kristalleri; sc, sklerenkima; xy, ksilem

In *H. micrantha*, *H. lazulina*, *H. heldreichii* and *H. campanulata* have not crystals in their roots (Kandemir et al., 2000; Atayeter, 2007). It has been reported that some endodermal cells have suberized thickness in roots of *H. micrantha* (Kandemir et al., 2000), *H. lazulina*, *H. heldreichii*, *H. campanulata* (Atayeter, 2007) and *H. lineata* (Selvi et al., 2008), and Casparian strip is not distinct in these species. In *H. acutiloba*, endodermal cells have not suberised wall thickness, and Casparian strip is distinct.

The scape of *H. acutiloba* contains monolayer epidermis, 5-6 layered parenchymatic cortex, multilayer sclerenchyma tissues, various sized vascular bundles and parenchymatic pith. These features of stem are similar with *H. lineata*, *H. lazulina*, *H. heldreichii*, *H. campanulata* and *H. glabrescens* (Atayeter, 2007; Selvi et al., 2008; Yetişen et al., 2012). However, *H. micrantha* is not sclerenchyma tissues in its stem (Kandemir et al., 2000). Although *H. acutiloba* have calcium oxalate raphide crystals in scape cortex, *H. glabrescens* have sand crystals it's in the cortex cell (Yetişen et al., 2012). The other studied *Hyacinthella* species have not crystals in their stems (Kandemir et al., 2000; Atayeter, 2007; Selvi et al., 2008).

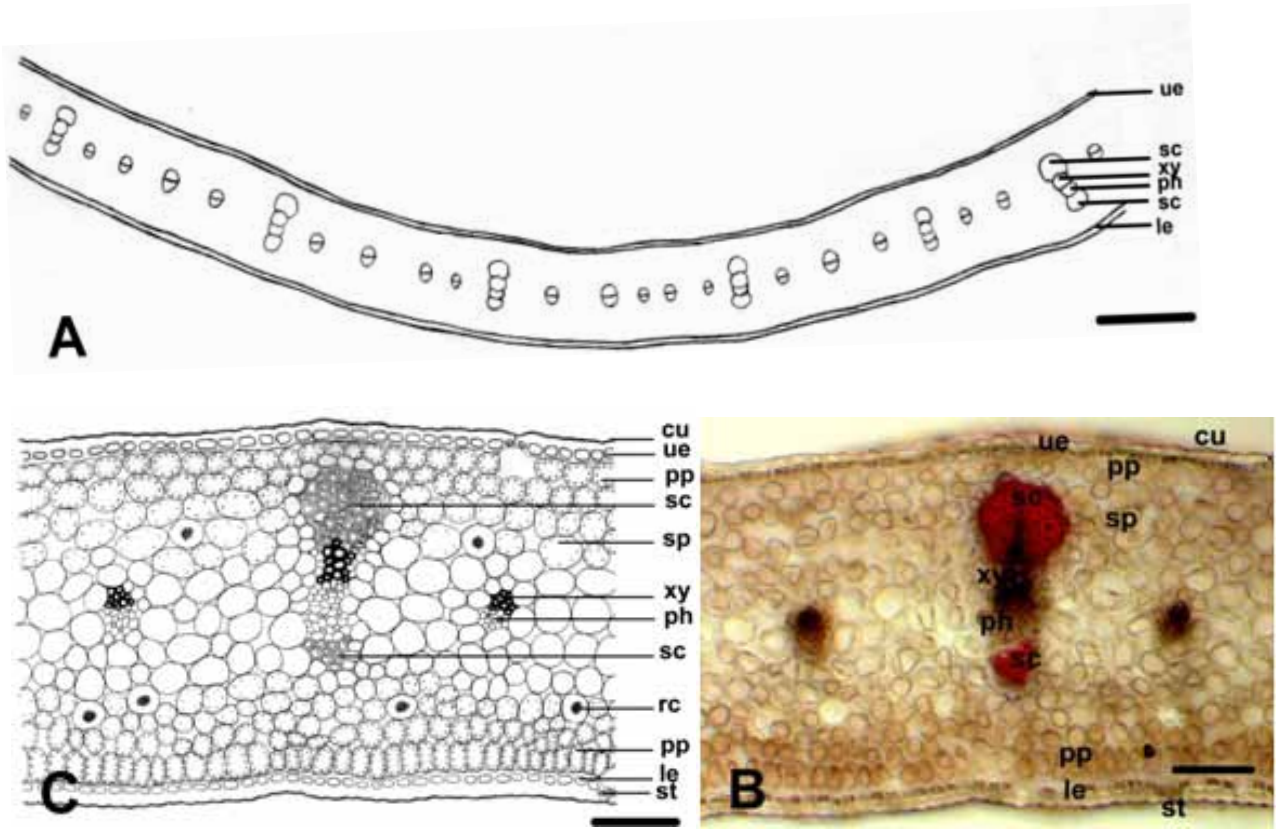

Figure 7. The cross-section of leaf of *H. acutiloba.* A. Diagram of leaf section. Scale bar= 500 µm. B. The microphotograph of leaf cross-section (Stained with Safranin). C. Camera lucida drawing of leaf cross-section. cu, cuticle; le, lower epidermis; ph, phloem; pp, palisade parenchyma; rc, raphide crystals; sc, sclerenchymatic cap; sp, spongy parenchyma; st, stoma; ue, upper epidermis; xy, xylem. Scale bar= 100µm
Şekil 7. *H. acutiloba*'nın yaprak enine kesiti. A. Yaprak kesiti diyagramı B. Yaprak enine kesiti mikrofotoğrafi (Safranin ile boyalı). C. Yaprak enine kesitinin Camera lucida çizimi. cu, kutikula; le, lower epidermis; ph, floem; pp, palizat parenkiması; rc, rafit kristalleri; sc, sklerenkimatik lifler; sp, sünger parenkiması; st, stoma; ue, üst epidermis xy, ksilem

Anatomical properties of the leaf in *H. acutiloba* are observed similarly with studied *Hyacinthella* species (Kandemir et al., 2000; Atayeter, 2007; Selvi et al., 2008). Mesophyll of *H. acutiloba* is isolateral and has got calcium oxalate raphide crystals. The raphide crystals are also present in *H. campanulata,* while *H. lazulina, H. heldreichii, H. micrantha* and *H. lineata* has not raphide crystals in their leaf mesophyll (Kandemir et al., 2000; Atayeter, 2007; Selvi et al., 2008).

The results are show that studied *Hyacinthella* species are almost similar with regard to their general anatomical features. However, the present/absent, location and morphology of the CaOx crystals is different in these species.

The functional significance of CaOx crystals within the plants remains unclear, although various functions have been them such as protection against foraging animals (Molano-Flores, 2001), detoxification of heavy metals or toxic oxalate (Franceschi and Nakata, 2005), calcium regulation in plant cells (Franceschi, 1989; Kostman and Franceschi, 2000; Volk et al., 2002), light gathering and reflection (Kuo-Huang et al., 2007), tissue support and plant rigidity (Franceschi and Horner, 1980). Specially, the raphide crystals are very important to defence against herbivores, because the sharp ends of them irritate the mucous membrane of animals' mouth (Vogel, 2004). However, the location, shape and present or absent of calcium oxalate crystals are controlled genetically and they can be used as an anatomical feature together with morphological characters.

References

Atayeter, E. 2007. The morphological and anatomical characteristics of some endemic *Hyacinthella* Schur (Liliaceae) taxa. M.Sc. Thesis, Selçuk University, Konya.

Doğu, S., Dinç, M. and Ünal, A. 2011. Anatomical characteristics of *Bellevalia mathewii* Özhatay & Koçak (Liliaceae). Biological Diversity and Conservation 4/3: 14-18.

Ekim, T., Koyuncu, M., Vural, M., Duman, H., Aytaç, Z., and Adıguzel, N. 2000. Red Data Book of Turkish Plants. Turkish Association for the Conservation of Nature, Ankara.

Franceschi, VR. 1989. Calcium oxalate formation is a rapid and reversible process in *Lemna minor*. Protoplasma 148: 130-137.

Franceschi, VR. and Horner, HT. 1980. Calcium oxalate crystals in plants. Bot. Rev., 46: 361-427.

Franceschi, VR. and Nakata, PA. 2005. Calcium oxalate in plants: formation and function. Annual Review of Plant Biology 56: 41-71.

Güner, A., Özhatay, N., Ekim, T. and Başer, KHC. 2000. Flora of Turkey and the East Aegean Islands. Vol. 11 (Supp. 2), Edinburgh University Press, Edinburgh.

Jensen, WA. 1962. Botanical Histochemistry: principles and practice. W.H. Freeman and Company, London.

Kandemir, N., Akçin, ÖE. and Cansaran, A. 2000. A morphological and anatomical investigation on some geophytes distributed in the vicinity of Amasya. Ot Sistematik Botanik Dergisi 7: 127-147.

Kostman, TA. and Franceschi, VR. 2000. Cell and calcium oxalate crystals growth is coordinated to achieve high-capacity calcium regulation in plants. Protoplasma 214: 166-179.

Kuo-Huang, LL., Ku, MSB. And Franceschi, VR. 2007. Correlations between calcium oxalate crystals and photosynthetic activities in palisade cells of shade-adapted *Peperomia glabella*. Bot. Stud. 48: 155-164.

Molano-Flores, B. 2001. Herbivory and calcium concentrations affect calcium oxalate crystal formation in leaves of *Sida* (Malvaceae). Ann. Bot. 88: 387-391.

Persson, K. and Wendelbo, P. 1988. *Hyacinthella* Schur. In: Davis, PH. (eds), Flora of Turkey and the East Aegean Islands, Vol. 8. Edinburgh Univ. Press, Edinburgh, 274-279.

Selvi, S., Erdoğan, E. and Daşkın, R. 2008. Morphological, Anatomical and Ecological Studies on *Hyacinthella lineata* (Liliaceae). Ekoloji Vol.17, No. 68: 24-32.

The Plant List, 2010. http://www.theplantlist.org/browse/A/Asparagaceae.

Wodehouse, RP. 1935. Pollen grains. Mc. Grew Hill, New York.

Yetişen, K., Özdemir, C., Küçüködük, M., Akyol, Y. 2012. A morphological and anatomical study of *Hyacinthella glabrescens* (Liliaceae). Phytologia Balcanica 18: 319-322.

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