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ORIGINAL PAPER

ANATOMICAL AND PALYNOLOGICAL STUDIES ON ENDEMIC *Verbascum weidemannianum* Fisch. & Mey. (SCROPHULARIACEAE) IN TURKEY

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Abstract

In this study, the anatomical, palynological and karyological properties of *Verbascum weidemannium* Fisch. & Mey. growing around Sivas in Anatolian part of Turkey were investigated for the first time. As a result of root and stem anatomy studies showed that both organs are in secondary structure. Cambium consists of 2-3 cell layer in root and 2-4 cell layer in stem. Leaves have dendroid hair on both surfaces. According to mesophyll layer leaf is bifacial. Stomata are anomositic and located on both surface. Sepals have dendroid hairs on both surface and have 7-11 cell layered chlorenchyma. Petals are covered single layered papillose cubic and rectangular epidermis cells in upper surface and nonpapillose cells in lower surface. The filament is approximately triangular shaped in crooss-section and vascular bundle is concentric-hadrocentric. Pollen grains of V. wiedemannianum are tricolpate, oblate-spheroidal; P/E ratio is 0.98 and exine ornamentation is reticulate.

Keywords: Anatomy, endemic, palynology, Verbascum weidemannianum

INTRODUCTION

The genus *Verbascum* L. (*Scrophulariaceae*) comprises some 360 species across the world (Heywood, 1993). In Turkey, with addition of 129 hybrids, the genus is represented by 243 species, which are divided into 13 partly artificial groups. The endemism ratio of the genus is very high, with 193 endemic species (80 %) (Huber-Morath, 1978; Davis et al. 1988; Ekim, 2000). Subsequently, eight species and six hybrids were described (Vural and Aydogdu, 1993; Sutory, 2001, 2004; Karavelioğulları et al., 2004, 2005, 2008, 2009a; Ozhatay, 2006; Kaynak et al., 2006; Parolly and Tan, 2007; Parolly and Eren, 2008; Dane and Yılmaz, 2009) and eight new species were recorded (Dane and Yılmaz, 2005; Yılmaz and Dane, 2008; Bani et al., 2010; Karavelioğulları et al., 2006; 2009b; 2011). Anatomical investigation has been carried out by Koktay (1974), Lersten and Curtis (1997), Yılmaz and Dane (2011), (Al-Hadeethy et al., 2014). Pollen morphology of the family *Scrophulariaceae* has been examined by a number of researchers, including Erdtman (1952), Moore and Webb (1978), Inceoglu (1982), Vargehese (1986), Karim and El-Oqlah (1989), Minki and Eshbaugh (1989), Karavelioğulları et al. (2005), Juan et al. (1999, 2000), Vujicic et al. (1993).

A comprehensive review of *Verbascum* biological activities and using in folk medicine of Turkey has recently been reported by Kahraman et al. (2012). The flowers of *V. phlomoides* L., *V. densiflorum* Bertol., and *V. thapsus* L. are consumed as tea for expectorant and mucolytic purposes. The leaf infusion is used as sudorific, sedative, diuretic and constipate remedy (Baytop, 1984). It is traditionally consumed as a tea to relieve abdominal pains. In traditional Turkish medicine mullein is used for treatment of respiratory disorders such as bronchitis, dry coughs, tuberculosis and asthma. Also, *Verbascum* species are used to treat hemorrhoids, rheumatic pain, superficial fungal infections, wounds and diarrhea. The oil made from *Verbascum* flowers is used to help soothe earache and can be applied externally for eczema and other types of inflammatory skin conditions. The leaves, roots and the flowers are also antiseptic, antispasmodic, astringent, emollient, nervine, vulnerary, analgesic, antihistaminic, anticancer, antioxidant, antiviral, bactericide, cardiodepressant, estrogenic, fungicide, hypnotic and sedative. In addition to the above mentioned common uses, these species have been used for pruritic conditions in urogenital organs (Kahraman et al., 2012; Baytop, 1999; Turker and Camper, 2002).

Verbascum wiedemannianum Fisch. & C.A.Mey. is an endemic and medicinal plant for Turkey (Ozdener and Kutbay, 2011) and is distributed in North and Inner Anatolia (Huber-Morath, 1978). Antioxidative and antimicrobial activity of leaves, stem and flowers of *V. wiedemannianum* were shown by Tepe et al. (2006). According to Red Data Book of Turkish Plants, treat category of *V. wiedemannianum* is Least Risk (LR) by IUCN criteries (Ekim et al., 2000).

There is no anatomical and palynological record for *Verbascum weidemannianum* which is grown in Turkey. So the aim of this study was to investigate the anatomical and palynological fetaures of *V. weidemannianum.*

MATERIAL AND METHODS

As a plant material flowering aerial and underground parts of *V. wiedemannianum* were collected in 2012-2014 from the following localities. *Locality 1*. **B6** Sivas: Hafik to Sivas, after Durulmuş village, roadside, 1320 m, 39° 49' 47,0″ N, 37° 15' 08,5″ E, M. Tekin 1234, 27.05.2012 ibid. Tekin 1579, 09.06.2014; *Locality 2*. **B6** Sivas: Sivas to Bingöl viilage, after Bingöl viilage, roadside, 1343 m, 39° 42' 25,1″ N, 37° 09' 38,1″ E, M. Tekin 1235, 27.05.2012; *Locality 3*. **B6** Sivas: Zara to Hafik, 1308 m, 39° 52' 04,7″ N, 37° 30' 52,0″ E, M. Tekin 1555, 29.05.2014. Voucher specimens were converted into herbarium material and were deposited at the Herbarium unit of the Biology Department (CUFH), Faculty of Science, Cumhuriyet University, Sivas-Turkey. For anatomical studies, the root, stem, stem leaf and fruit of the some specimens were fixed and conserved in 70% ethyl alcohol. Hand sections were made with a razor blade and sections were stained with Alcian blue (Sigma) for pectic substances, Safranin (Sigma) for lignin in the rate of 3/2. Sections were left in dye about 5 minutes for staining. The stained sections were mounted in glyceringelatine to obtain permanent preparations (Jensen, 1962). Sections were examined using an Olympus light microscope BX51. Images were taken with an Olympus DP 70. For palynological investigations pollen grains supplied from herbarium specimens and were prepared following (Wodehouse, 1935). For measurements of polar axis, equatorial diameter, exine thickness and lumina size of pollen grains, 100x objective of a Olympus light microscope CX21 were used. Measurements were based on 20 pollen grains. For morphological descriptions of pollen grains, the terminology of Punt et al. (2007) was followed. Statistical studies were performed using SPSS 15.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Anatomical properties

Root: Root is at the secondary structure. The outermost, there is multilayered periderm. Periderm cells are very flattened. Under periderm, there is cortex layer. Cortex is 6-10 layered and parencymatous. Parencyma cells are usually irregular or rarely rectangular-ovoid shaped. There is 2-3 layered cambium between seconder phloem and seconder xylem which constitute a major part of the root cross section.

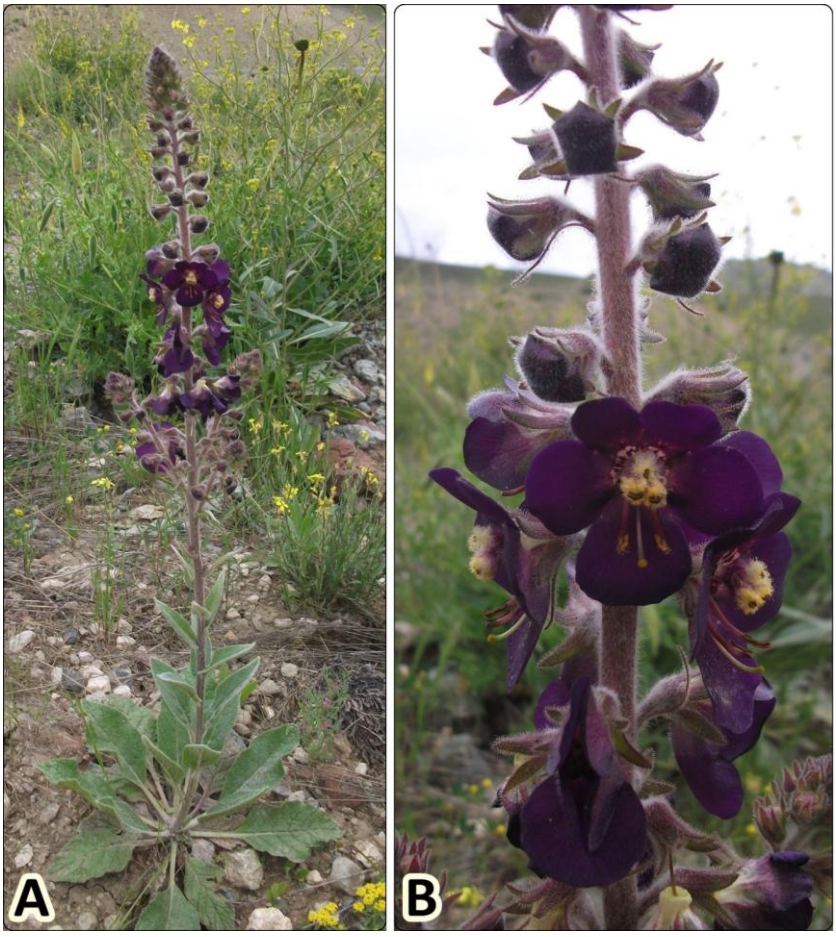

Figure 1. A. *V. wiedemannianum* in natural habitat, **B.** Inflorescence and flowers of *V. wiedemannianum.*

Stem: In stem cross section, the outermost, there is a single row of the epidermis consists of rectangular and oval cells. Cuticle layer is thin on epidermal cells. There are eglandular multicellular hairs on epidermis. Cortex is 7-11 layered and parenchymatous. Cells of cortex are generally oval or occasionally circular shaped. There is a discontinuously sclerenchymatous sheath on the phloem. There is a cambium between phloem and xylem.

Cambium cells are 3-7 layered, and generally rectangular or square, occasionally irregular in shape. Inside, there are xylem parencyma cells and xylem sclerenchyma cells which close to pith cells. The pith is large and consists of parenchymatous cells. There are also pith rays which consists of usually single, occasionally double adjacent rows of small parenchymatous cells.

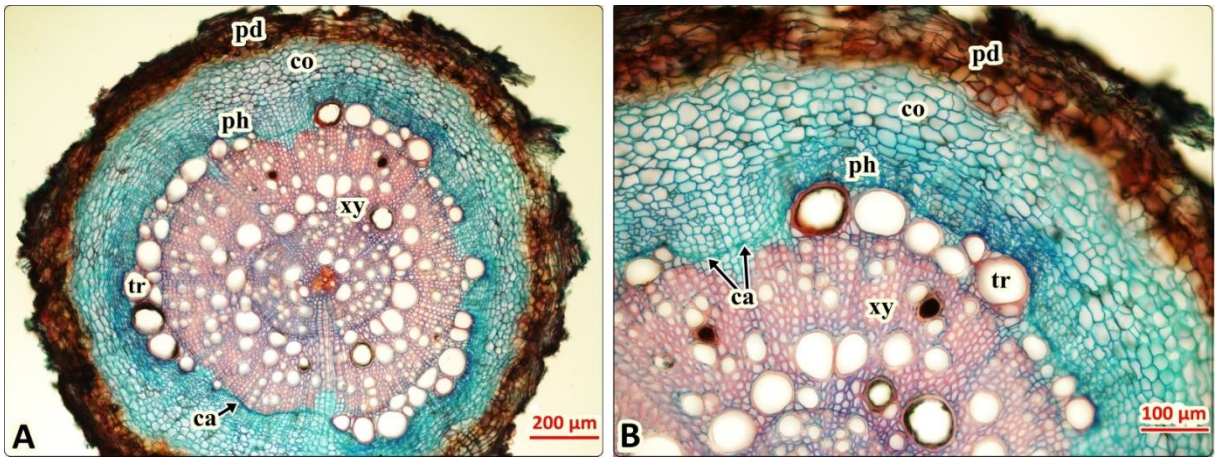

Figure 2. The cross section of root of *V. wiedemannianum:* **A.** 10×; **B.** 20× (ca: cambium; co: cortex; pd: periderm; ph: phloem; tr: trachea; xy: xylem).

Leaf: Cross section of leaf were taken from midrib and the area of between ribs. The adaxial and abaxial epidermis consists of a single row of cells which are almost rectangular-oval shaped. Epidermis cells are covered by a thin cuticular layer on both adaxial and abaxial surfaces. However, the outer wall of the adaxial and abaxial epidermis cells are quite thick and are covered with intensive multicellular eglandular hairs which known as specific to *Verbascum* genus: condlesticks hairs. Leaf is bifacial. Palisade parenchyma cells which located adjacent the adaxial epidermis are 2 or rarely 3 layered and generally rectangular, cylindrical or rarely irregular shaped. Spongy parenchyma is 3-5 layered and is located between palisade parencyma and abaxial epidermis. Spongy parenchyma cells are generally ovoid or irregular shaped. There is a large midrib which have roughly truncated cone shaped in the median region of the leaf. Vascular bundles are surrounded by parenchymatic bundle sheath cells. Parencymatic bundle sheath cells which around midrib are intense, and palisade or spongy parencyma is not included in this area. Stomata type is anomocytic and stomata cells are present on both the adaxial and abaxial epidermis.

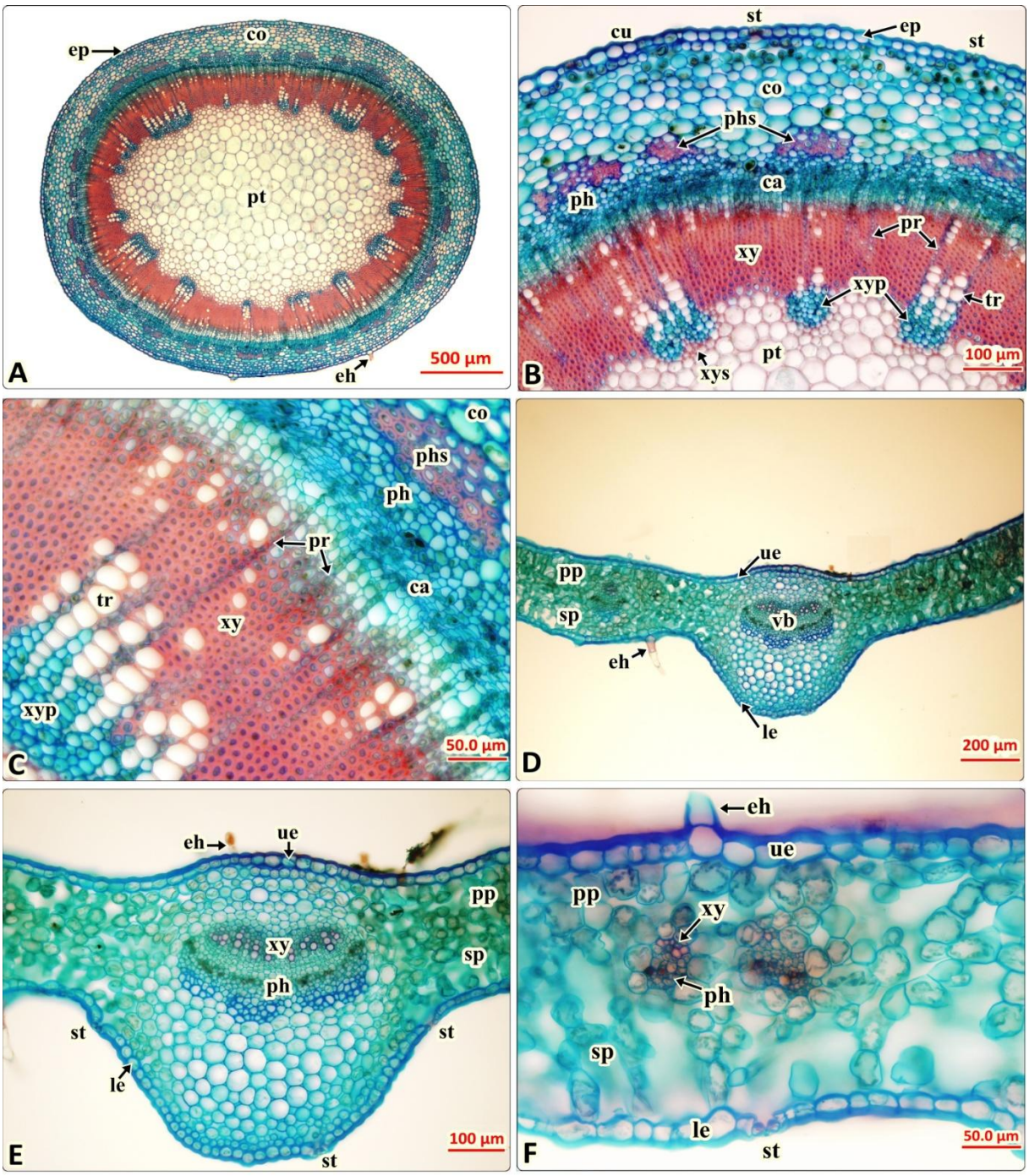

Figure 3. The cross section of stem and leaf of *V. wiedemannianum:* **A-C.** Cross section of stem, **A**: 4×; **B:** 20×; **C:** 40×. **D-F.** Cross section of leaf, **D:** 10×; **E:** 20×; **F:** 40× (ca: cambium; co: cortex; cu: cuticle; eh: eglandular hair; ep: epidermis; le: lower epidermis; ph: phloem; phs: phloem sclerenchyma; pp: palisade parenchyma; pr: pith ray; pt: pith; sp: spongy parenchyma; st: stoma; tr: trachea; ue: upper epidermis; vb: vascular bundle; xy: xylem; xyp: xylem parenchyma; xys: xylem sclerenchyma).

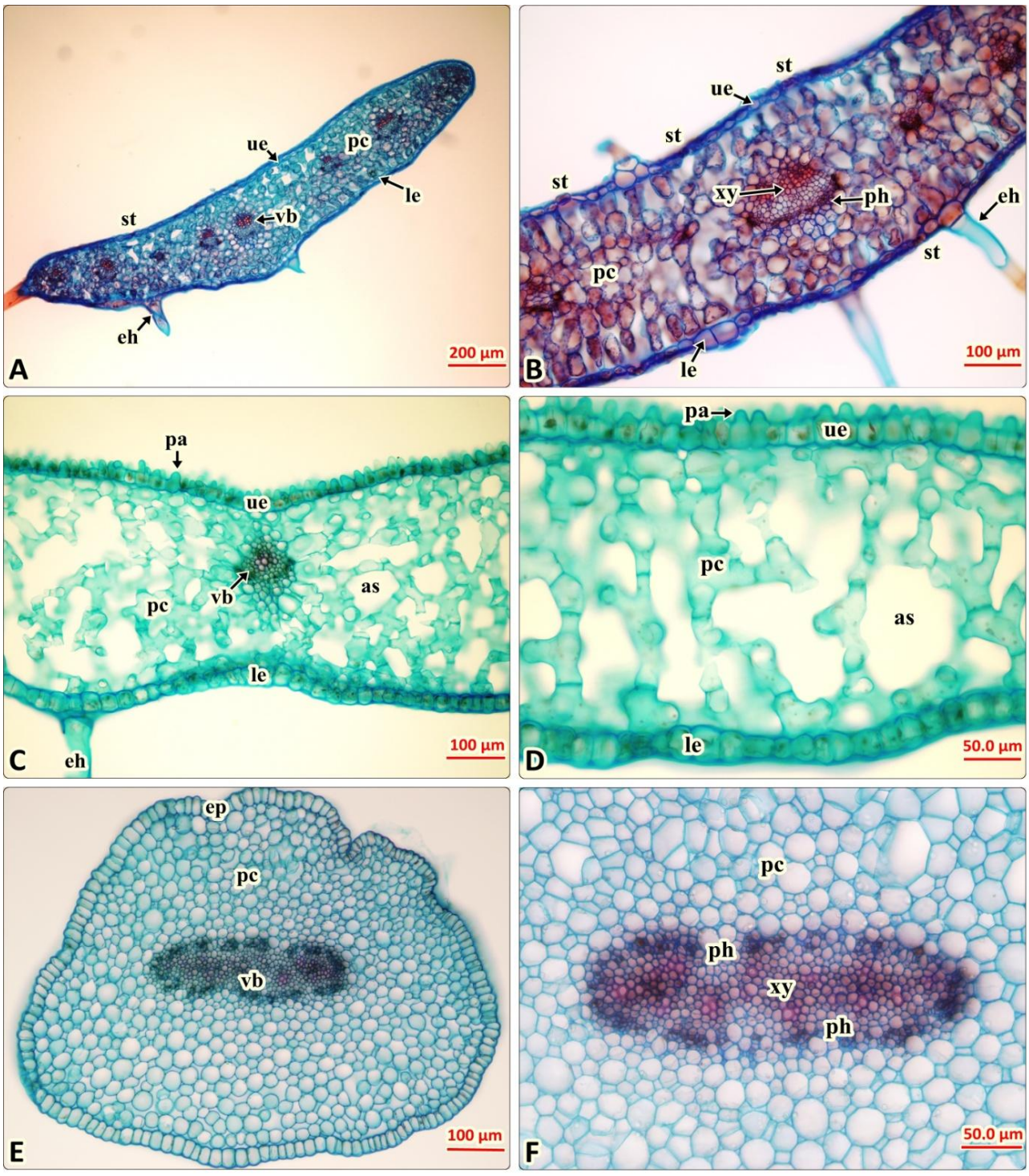

Figure 4. The cross section of some generative organs of *V. wiedemannianum:* **A-B.** Cross section of sepal, **A:** 10×; **B:** 20×. **C-D.** Cross section of petal, **C:** 20×; **D:** 40×. **E-F.** Cross section of filament, **E:** 20×; **F:** 40× (as: air space; eh: eglandular hair; le: lower epidermis; pa: papillae; pc: parenchymatous cell; ph: phloem; st: stoma; ue: upper epidermis; vb: vascular bundle; xy: xylem).

Table 1. The anatomical mesurements of *V. wiedemannianum*.

		Length		Width	
		Min - Max	Mean ±SD	Min-Max	Mean ±SD
ROOT	Cortex cell	13,56 - 52,64	27,82 ±10,42	7,28 - 34,53	19,03 ±7,96
	Trachea	-	-	21,70 -97,19	49,80 ±24,09
STEM	Cuticle	-	-	0,81 - 1,32	1 ± 0,12
	Epidermis cell	16,78 - 42,35	25,81 ±6,71	12,43 - 31,84	22,30 ±5,8
	Cortex cell	1,32 - 57,63	28,11 ±14,03	7,83 - 38,11	20,04 ±8,53
	Cambium cell	4,66 - 11,18	8,34 ±1,61	1,47 - 4,37	2,99 ± 0,78
	Trachea	-	-	11,19 - 34,90	20,06 ±6,26
	Pith cell	34,00 - 150,98	92,82 ±34,67	9,45 - 118,04	76,90 ±30,37
LEAF	Upper cuticle	-	-	0,64 - 1,14	0,92 ±0,11
	Lower cuticle	-	-	0,64 - 1,03	0,84 ±0,11
	Upper epidermis	18,05 - 38,60	28,67 ±4,08	15,83 - 32,78	25,31 ±4,12
	Upper epidermis, upper wall thickness			5,86 - 10,38	8,03 ±1,16
	Lower epidermis	18,43 - 41,74	26,21 ±5,75	11,13 - 32,52	21,13 ±4,66
	Lower epidermis, upper wall thickness	-	-	5,81 - 11,98	7,6 ±1,47
	Palisade parenchyma cell	32,76 - 71,42	47,07 ±7,88	18,71 - 34,54	25,64 ±4,14
	Palisade parenchyma thickness	-	-	63,78 - 137,99	101,84 ±20,63
	Spongy parenchyma cell	26,32 - 56,08	39,78 ±7,96	21,15 - 37,79	26,79 ±4,67
	Spongy parenchyma thickness	-	-	76,06 - 162,21	120,41 ±18,77
	Mesophylle thickness	-	-	175,26 - 254,16	225,88 ±21,18
	Leaf thickness	-	-	218,68 - 311,76	274,01 ±25,28
SEPAL	Upper cuticle			0,46 - 1,06	0,68 ±0,14
	Lower cuticle			0,42 - 1,39	0,82 ±0,23
	Upper epidermis cell	18,16 - 39,82	24,30 ±5,19	10,58 - 29,44	20,70 ±4,82
	Upper epidermis, upper wall thickness			6,18 - 10,57	7,95 ±1,13
	Lower epidermis cell	17,43 - 39,40	26,9 ±5,59	12,67 - 31,20	21,68 ±4,54
	Lower epidermis, upper wall thickness			7,47 - 11,21	8,84 ±1,11
	Parencyma cell	16,40 - 52,85	29,01 ±9,30	11,46 - 38,74	22,66 ±8,23
PETAL	Upper epidermis cell	22,21 - 40,28	32,62 ±4,71	15,36 - 26,19	21,74 ±2,97
	Upper epidermis, upper wall thickness			1,49 - 2,56	2,04 ±0,26
	Lower epidermis cell	22,22 - 34,47	29,63 ±3,06	6,77 - 32,94	22,13 ±6,01
	Lower epidermis, upper wall thickness			2,32 - 4,97	3,33 ±0,76
Filament	Epidermis cell	19,81 - 34,82	26,59 ±3,94	5,31 - 26,66	14,94 ±5,33
	Epidermis cell, upper wall thickness			2,16 - 4,58	2,87 ±0,54
	Parenchyma cell	11,25 - 44,84	24,52 ±10,64	10,28 - 37,28	19,71 ±8,49

Sepal: Cross sections of sepal were taken mibrid and the area of between ribs. The adaxial and abaxial epidermis consists of a single layered cells which are generally oval or rarely rectangular shaped. Cuticle on the adaxial and abaxial epidermis and the outer wall of the adaxial and abaxial epidermis cells are thinner than that on the leaf epidermis cells. There are eglandular dendroid hairs on both surface of the epidermis. There is 7-11 layered photosynthetic parenchyma between adaxial and abaxial epidermis. Photosynthetic parencyma cells are ovoid, rectangular or irregular shaped. There is a main vascular bundle which roughly circular shaped in the median region of the sepal. There are stomata on both epidermis.

Petal: Petals have a uniseriate papillose adaxial epidermis and uniseriate nonpapillose abaxial epidermis cells which are cuboid or rectangular shaped. Cuticle is very thin. There are eglandular hairs and rarely stomata on abaxial epidermis. The mesophyll is homogeneous and is constituted of spongy parenchyma. Spongy parencyma cells are branched and have very large air cavities between them. The vascular bundles are collateral and are disposed in the middle area of the mesophyll.

Filament: At the cross section, filament is roughly triangular shaped. Single layered epidermis cells are generally rectangular shaped. There is large vascular bundle in midle of the cross section. Type of vascular bundle is concentric-hadrocentric. There are multilayeredparencymatous cells which are generally circular or ovoid shaped, between vascular bundle and epidermis.

Palynological properties

Pollen grains oblate-spheroidal, polar axis 23,82-(27,34) 29,90 µm and equatorial diameter 26,04-(28,00)-29,96 µm. (P/E ratio: 0,98), outline at the polar view circular to subtriangle, outline at the equatorial view mostly oblate circular. Exine thickness 0,77-(1.11)-1,43. Tectum reticulate (Table 2).

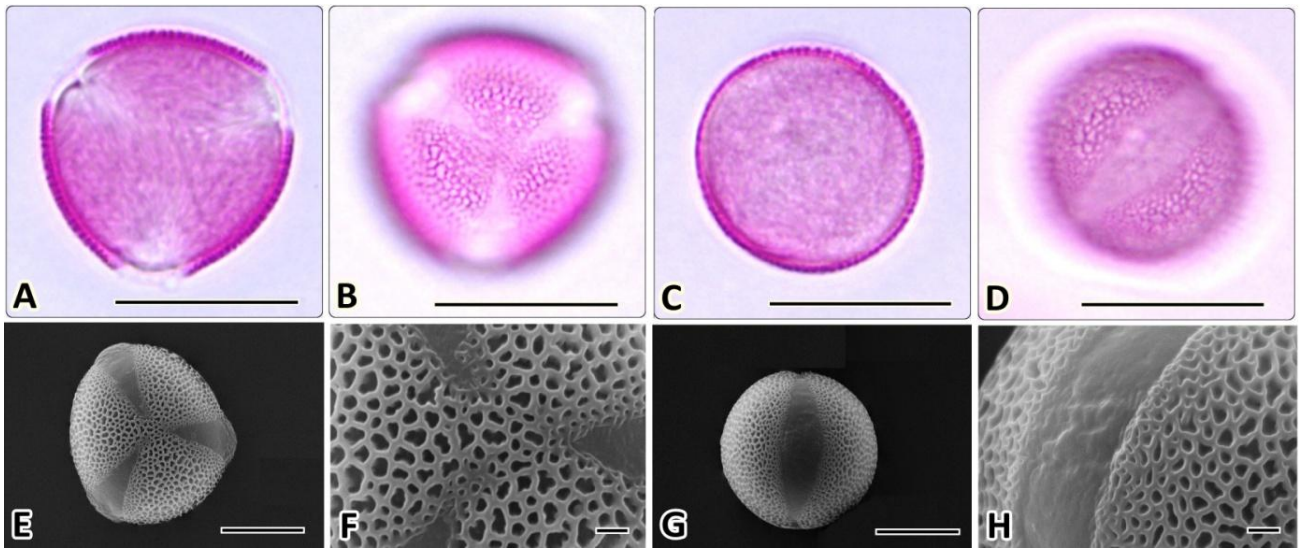

Fig. 5. Optical microscope (A-D) and Scanning Electron Microscope (E-H) images of *V. wiedemannianum* pollen grains: A. polar view (high focus); B. apertures and exine ornamentation in polar view (low focus); C. equatorial view (high focus); D. apertures and exine ornamentation in equatorial view (low focus); E. polar view; F. apertures and exine ornamentation in polar area; G. Equatorial view; H. aperture and exine ornamentation in equatorial area (Scale bar indicate 20 µm in A-D; 10 µm in E, G; 1 µm in F, H).

Table 2. The palynological mesurements and observations of *V. wiedemannianum*.

Polar axis (µm)				Equatorial axis (µm)				P/E ratio	Shape	Size	Exine thickness (µm)			
min	mean	max	std	min	mean	max	std				min	mean	max	Std
23,82	27,34	29,90	±1,69	26,04	28,00	29,96	±0,96	0,98	Oblate-spheroidal	medium	0,77	1,11	1,43	±0,14

DISCUSSION

The present study is the first study on the anatomy and palynology of *V. weidemannianum* in Turkey. *V. weidemannianum* is endemic and taxon has been placed under EN of the IUCN threat category.

In the anatomical part of this study, palisade parenchyma cells of the leaves which located adjacent the adaxial epidermis are 2 or rarely 3 layered and generally rectangular, cylindrical or rarely irregular shaped. Spongy parenchyma of the leaves is 3-5 layered and is located bettween palisade parencyma and abaxial epidermis. Spongy parenchyma cells are generally ovoid or irregular shaped. In the study of Yılmaz and Dane (2011) the morphological, anatomical, palynological and karyological properties of two subspecies of *Verbascum ovalifolium* (subsp. *ovalifolium* and subsp. *thracicum*), as well as of *V. purpureum* were investigated. In the anatomical study, epidermal cells of the cauline leaves belonging to *V. ovalifolium* subsp. *thracicum* were pentagonally or hexagonally shaped, while the epidermal cells of the other taxa were undulating in shape. There were raphid crystals in the upper epidermis cells of the leaves belonging to *V. ovalifolium* subsp. *ovalifolium*, which do not exist in other taxa. Idioblasts were observed in the epiderma of the leaves of *V. purpureum*, but could not be seen in *V. ovalifolium*. Koktay (1974) provided information on the general anatomical characteristics of 11 *Verbascum* species. In the study of (Koktay, 1974) epiderma of *V. pinnatifidum, V. degenii, V. sinuatum* covered with a thick layer of cuticle, epidermis cells flat-shaped and approximately of equal size; palisade parenchyma 3–5 layered in *V. pinnatifidum* and *V. degenii* growing in sandy and salty soils is 2–3 layered in the other studied species and spongy parenchyma is 9–10 layered in *V. pinnatifidum* and *V. degenii* .

In the study of (Yılmaz and Dane, 2011) all examined taxa had tricolpate pollen type, prolate pollen shape and reticulate exine ornamentation.

CONCLUSION

In conclusion, *V. weidemannianum* has been investigated in terms of anatomy and palynology for the first time with this study.

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