





Article

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MORPHOLOGY, ANATOMY, PALYNOLOGY AND SEED MICROMORPHOLOGY OF TURKISH ENDEMIC *Verbascum exuberans* HUB.-MOR. (SCROPHULARIACEAE)

*Morfologia, Anatomia, Palinologia e Micromorfologia de Sementes da Espécie Endêmica Turca **Verbascum exuberans** Hub.-Mor. (Scrophulariaceae)*

ABSTRACT - *Verbascum* L. is the largest genus of the family Scrophulariaceae and includes several species of medicinal importance. The high morphological diversity among the species of the genus *Verbascum* causes problems in the delimitation of the species. In this study, morphological, anatomical, palynological and seed properties of *Verbascum exuberans* were investigated. It is a local endemic restricted to the provinces of Manisa and Izmir in West Anatolia. In the root cross-section, the xylem elements occupy a considerable area. The upper part of the epidermis cells in the stem cross-section is covered a distinct cuticle layer. The main vein of the leaf is shaped as bicollateral bundle. Idioblasts were not observed in the epidermis of the leaves of *V. exuberans*. Seeds of *V. exuberans* are dark brown and oblong to prismatic and alveolate. Hilum is short and orbicular. The seed coat ornamentation is reticulate-rugose. Pollen grains usually radially symmetrical, isopolar, prolate, tricolpate. Tectum is reticulate

Keywords: leaf, pollen, root, stem, mullein.

RESUMO - *Verbascum* L. é o maior gênero da família Scrophulariaceae e inclui várias espécies de importância medicinal. A alta diversidade morfológica entre as espécies do gênero *Verbascum* causa problemas na delimitação das espécies. Neste estudo, as propriedades morfológicas, anatômicas, palinológicas e de sementes de *Verbascum exuberans* foram investigadas. É uma planta endêmica local restrita às províncias de Manisa e Izmir, na Anatólia Ocidental. Na seção transversal da raiz, os elementos do xilema ocupam uma área considerável. A parte superior das células da epiderme na seção transversal da haste é coberta por uma camada de cutícula distinta. A veia principal da folha é moldada como feixe bicollateral. Idioblastos não foram observados na epiderme das folhas de *V. exuberans*. Sementes de *V. exuberans* são marrom-escuras e oblongas a prismáticas e alveoladas. O hilo é curto e orbicular. A ornamentação do revestimento da semente é reticuladourugosa. Grãos de pólen geralmente radialmente simétricos, isopolares, prolatos, tricolpados. Tectum é padrão reticular.

Palavras-chave: folha, pólen, raiz, haste, verbasco.

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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). Within the last decade several new taxa have been described (Karaveliogullari et al., 2014). Anatomical investigations on some *Verbascum* species have been carried out by Koktay (1974), Lersten and Curtis (1997), Bagci and Çakir (2005), Çakir and Bagci (2006), Özdemir and Altan (2007), Kheiri et al. (2009), Yilmaz and Dane (2011), Alan and Gökman (2015) and Küçük (2017). There are many SEM studies based on seed micromorphology of the some *Verbascum* species taxa (Juan et al., 1997; Petković et al., 1997; Karaveliogullari et al., 2006, 2011, 2014; Attar et al., 2007; Kheiri et al., 2009; Cabi et al., 2011; Aytaç and Duman, 2012; Duman et al., 2017) Pollen morphology of the genus *Verbascum* has been examined by several researchers, including Lobin and Pormbski (1994), Karaveliogullari et al. (2005, 2011, 2014), Bagci and Çakir, (2005), Bukhari and Alfarhan (2006), Çakir and Bagci (2006), Kheriri et al. (2006), Pehlivan et al. (2008), Yilmaz and Dane (2011), Asmat et al. (2011), Aytaç and Duman (2012), Al-Hadeethy et al. (2014), Ranjbar and Nouri (2015), Duman et al. (2017) and Öztürk et al. (2018). There is no anatomical and palynological record for *Verbascum exuberans* Hub.-Mor. The objectives of this study are to give a detailed account of the morphological, anatomical, pollen and seed micromorphological features of *V. exuberans* from Manisa, Turkey.

MATERIAL AND METHODS

The specimen of *V. exuberans* was collected from natural population (Campus of MCBU, road side, 18.06.2012, 110 m, Yunusemre-Manisa; 38°40'37.89" N 27°18'26.64" E). Some of these specimens were used for anatomical, morphological and palynological studies, while others were prepared as herbarium material. Voucher specimens were deposited in the Herbarium of the MCBU. Description of species followed Huber-Morath (1978) in The Flora of Turkey and The East Aegean Islands. The holotype of *V. exuberans* (at Kew Herbarium), from Manisa, was also confirmed. Measurements, colours and other details given in the description are based on living material, alcohol-preserved specimens, data derived from field notes and The flora of Turkey and The East Aegean Islands.

For anatomical studies, living material was kept in 70% ethyl alcohol. Freehand sections were taken and investigated in cross-sections from the root, stem and leaf. Hand cut sections were stained with sartur reactive. Anatomical measurements were based on 30 cells. Measurements and photos were taken using a Leica DM3000 motorize binocular light microscope. For palynological investigations, pollen samples were obtained from herbarium samples. The pollen slides were prepared using the standard method of acetolysis (Erdtman, 1960). Measurements and observations were made using a Leica DM3000 motorize binocular light microscope and scanning electron microscope (SEM) observations. The polar length (P), the equatorial length (E), the colpus length (CLG), colpus width (CLT), the exine and the intine thickness for 30 pollen grains were measured under the light microscope (1000x) and P/E ratios were calculated. For scanning electron microscopy (SEM), unacetolyzed pollen grains were first mounted on double sided carbon tape affixed to aluminum stubs, covered with gold with an Polaron SC7620 sputter coater and photographed at a magnification of 2000x and 10000x with a JEOL JSM-6060 SEM to determine exine sculpturing of the pollen. Slides and plant samples are deposited at Manisa Celal Bayar University, Faculty of Art and Science, Department of Biology. The terminology used is in accordance with Faegri and Iversen (1964), Kremp (1965), Erdtman (1969) and Moore and Web (1978). Seeds were first examined using a Olympus SZ51 stereomicroscope to ensure that they were of normal size and were mature. In order to determine the average seed sizes, 30 mature seeds were measured. For SEM, the mature seeds were first mounted on double sided carbon tape affixed to aluminum stubs, covered with gold with an Polaron SC7620 sputter coater and photographed at a magnification of 150x and 800x with a JEOL JSM-6060 SEM to determine coat sculpturing of seed. The terminology used for describing the seed coat features follows Juan et al. (1997), Attar et al. (2007) and Cabi et al. (2011).

RESULTS AND DISCUSSION

Expanded morphological characteristics

Biennial herb, 200-250 cm tall, with hard, rough, densely eglandular white or yellowish white adpressed stellate-tomentose indumentum. Stem robust, angular, to 2 cm thick, many-branched. Basal leaves lanceolate, acute, 50-60 x 8-10 cm, entire, petiole broadly winged, indistinct; cauline smaller, sessile, auriculate, semi-amplexicaule, upper ovate to cordate triangular, acuminate, \pm undulate. Inflorescence with numerous branches, forming very dense, pyramidal panicle, with clusters of 3-5 flowers. Bracts ovate to orbicular, shortly acuminate. Pedicels to 7 mm; bracteoles lanceolate. Calyx 7 mm, cleft to 2/3 into ovate-lanceolate, acute lobes. Corolla rotate, yellow, unequally 5-lobed, 20-25 mm diam., without pellucid glands, tomentose outside. Stamens 5; filaments free, 7-8 mm long, orange-yellow, with dense whitish-yellow wool up to anthers; anthers reniform, 1-2 mm, orange. Style filiform 5 mm long; stigma clavate, orange-yellow. Capsule oblong-cylindrical, 2-3 x 3.5-4 mm glabrous. Seed numerous, oblong to prismatic, 0.6-1 x 1-1.1 mm, dark brown, alveolate and reticulate-rugose (Figure 1, Table 1).



Figure 1 - General appearance of *Verbascum exuberans*.

usually larger than the widths and consisting of regular shaped cells. The xylem occupies a wider area and filled the center of the root. The trachea cells settled irregularly, and bigger than the tracheid cells and their length is more than their width. The phloem occupies a narrow area than the xylem.

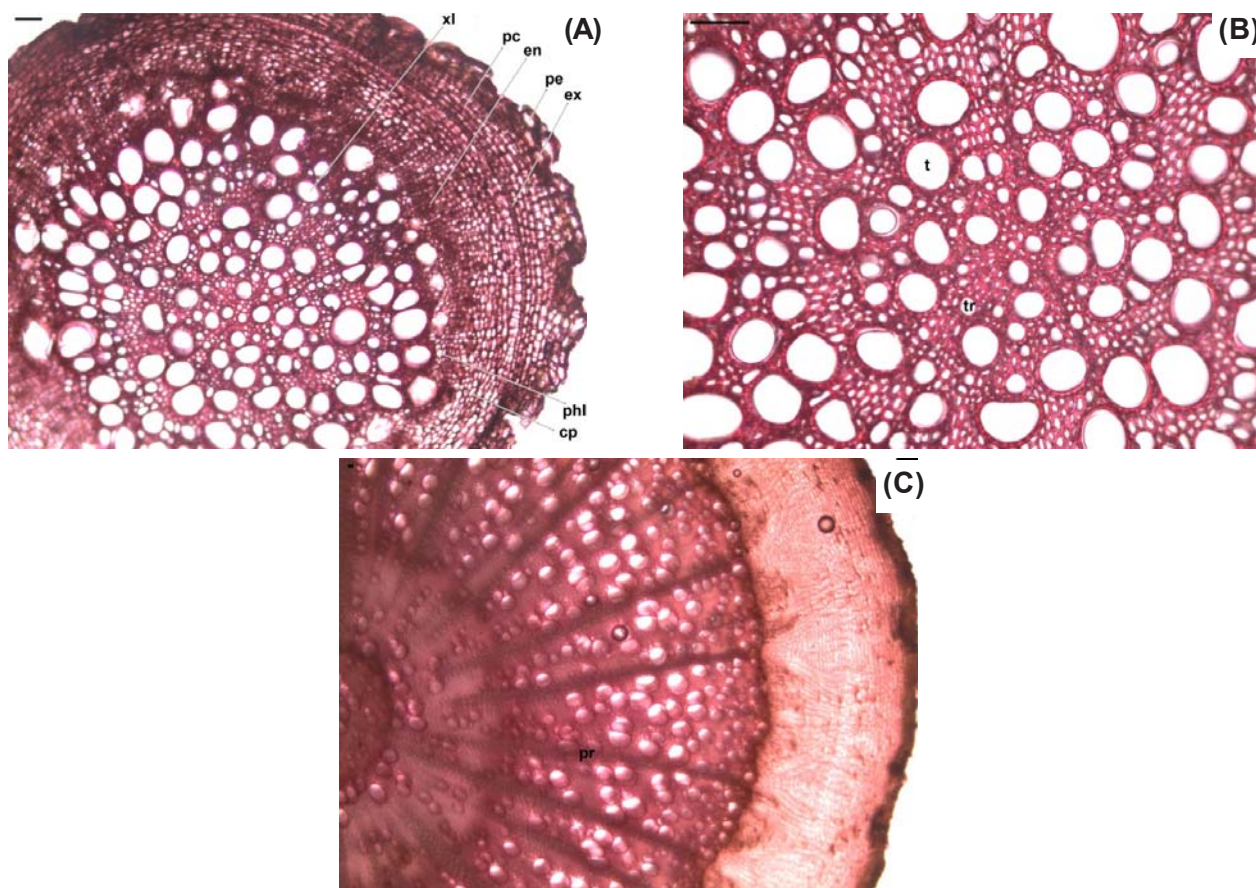
Stem (Figure 3A-C, Table 2): When the cross-sections of the stem are examined, the outer, peridermis is covered with the cuticle layer at the outermost. Epidermis is formed of cells arranged in a single layer and properly ordered cells. Just under epidermis it lies collenchyma cells, formed 2-3 layers cells. After the collenchyma layer, there is usually parenchyma that is composed

Table 1 - Some morphological characters of *Verbascum exuberans* based on the present study and Flora of Turkey (1978)

Character	Present study	Huber-Morath (1978)
Stem (cm)	180-200	-
Basal leaf (cm)	4-9x17-36	8-10x50-60
Cauline leaf (cm)	2.5x3.5-20	-
Lower bract (cm)	1-3.7x1-4.5	-
Upper bract (cm)	0.4-1x0.5-1.5	-
Pedicel (mm)	0.2-0.6	-
Calyx (mm)	2-4	7
Corolla diam (mm)	20-30	20-25
Stamen length (mm)	9-11	-
Anther length (mm)	1-2	-
Pistil length (mm)	5-6	-
Seed (mm)	0.6-1x1-1.1	-

Anatomy

Root (Figure 2A-C, Table 2): In the cross sections of the root, peridermis, cortex, endodermis, floem and xylem are arranged from the outside to the inside. Peridermis is usually formed in two layer cells. The primary cortex located under the peridermis is trapped in a narrow area. Parenchymatic cortex, located between exodermis and endodermis, is



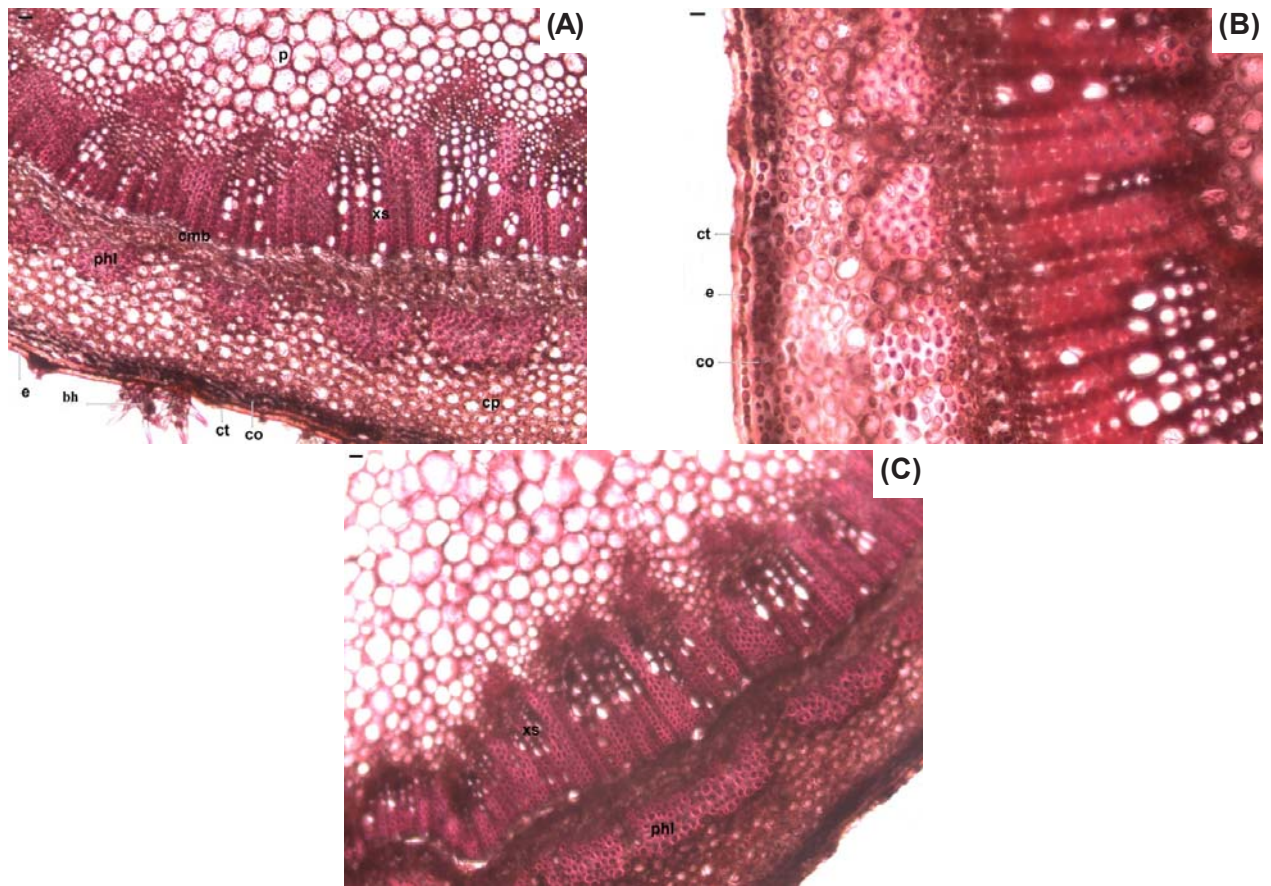
pe: periderm, pc: primer cortex, ex: exodermis, en: endodermis, cp: Cortex parenchyma, xl: Xylem, phi: Phloem, t: trachea, tr: tracheid, pr: pith ray.

Figure 2 - Cross-sections of the root of *Verbascum exuberans* (Scale Bar: a, b: 100 μ m; c:125 μ m).

Table 2 - Anatomical measurements of various tissues of *Verbascum exuberans*

	Width (μ m)		Length (μ m)	
	Min.- Max.	Mean \pm SD	Min.- Max.	Mean \pm SD
Root				
Peridermis cell	20-60	45.55 \pm 13.56	10-40	21.66 \pm 09.35
Cortex cell	15-60	36.50 \pm 14.15	10-20	16.50 \pm 03.37
Trachea cell	40-100	71.66 \pm 20.37		
Tracheid cell	30-50	36.00 \pm 08.43		
Stem				
Cuticle	5-15	07.85 \pm 03.65		
Epidermis cell	15-40	28.50 \pm 09.73	10-20	16.00 \pm 4.59
Cortex region	145-180	165.35 \pm 13.41		
Cortex cell	10-40	29.00 \pm 09.06		
Trachea cell	40-70	51.00 \pm 08.75		
Tracheid cell	20-35	26.40 \pm 06.48		
Pith cell	40-130	63.52 \pm 23.70		
Leaf				
Cuticle	10-20	12.75 \pm 03.98		
Adaxial epidermis	20-110	67.00 \pm 33.01	40-80	55.00 \pm 12.69
Abaxial epidermis	50-100	71.20 \pm 20.25	40-70	55.00 \pm 09.71
Palisade parenchyma	30-50	34.00 \pm 06.99	90-40	114.00 \pm 16.46
Spongy parenchyma	60-90	72.27 \pm 09.04		

Abbreviations: SD:Standard deviation.



e: epidermis, bh: brached hair, ct: cuticula, co: collenchyma, cp: cortex parenchyma, p: parenchymatic cells, xs: Xylem, phl: Phloem, cmb: cambium.

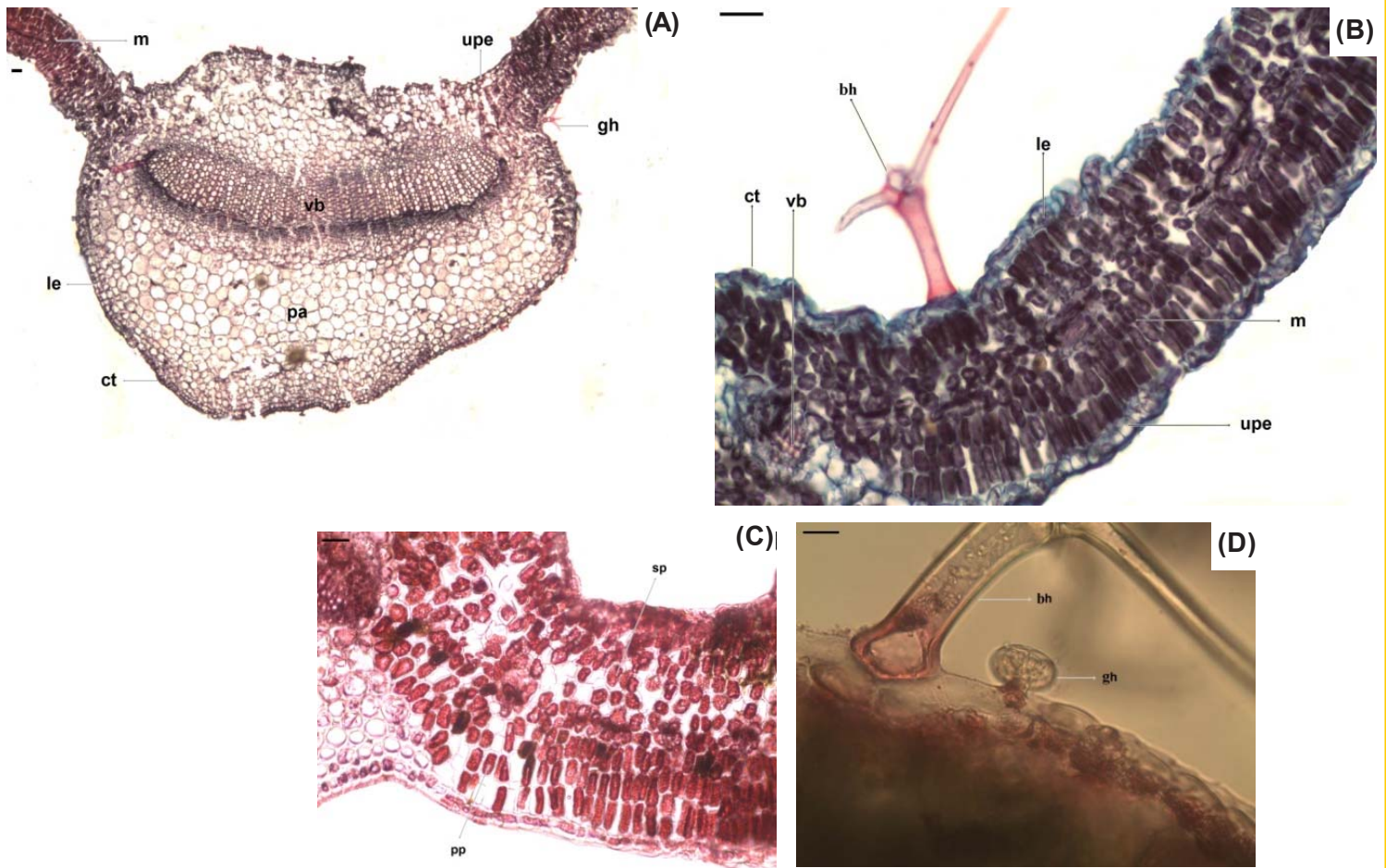
Figure 3 - Cross-sections of the stem of *Verbascum exuberans* (Scale Bar: a: 12.5 μ m; b: 7 μ m; c: 10 μ m).

of cells larger and smaller than the width. Parenchymal tissue in the cortex covered more area than other tissues. Vascular bundles are regularly arranged around the cambium and are open collateral type. The xylem occupies a wider area than the phloem. Usually, the length of tracheas are bigger than their width. The pith of the plant stem is formed of parenchymatous cells, which large and small.

Leaf (Figure 4A-D, Table 2): In the leaves, outer surface of the adaxial and abaxial epidermis are covered by cuticle. The adaxial and abaxial epidermis are single-layer and they have amaryllis-type stomata mostly surrounded by 3-4 epidermis cells. Mesophyll tissue is differentiated as palisade and sponge parenchyma in mesophyll, there are 3 layers under the adaxial epidermis and 2 layers of palisade parenchyma on the abaxial epidermis. The cells of the palisade parenchyma are long, cylindrical or rectangular in shape and are regularly aligned. There are sponge parenchyma cells, formed 3-4 layered cells between the lower and upper palisade parenchyma cells, The cells of the sponge parenchyma are oval or polygonal, there is more space between cells. There are dense candle hairs (multicellular branched hairs) and glandular hairs on adaxial and abaxial epidermis surfaces. Vascular bundle is located solely in the parenchymal tissue in the middle vessel region. The xylem elements are arranged in a straight line and are in a single layer. The midrib has developed fairly well with respect to the sides. The parenchyma tissue, located around the vascular bundle covers a large area. The cells of the parenchyma tissue are polygonal shape and frequently arranged.

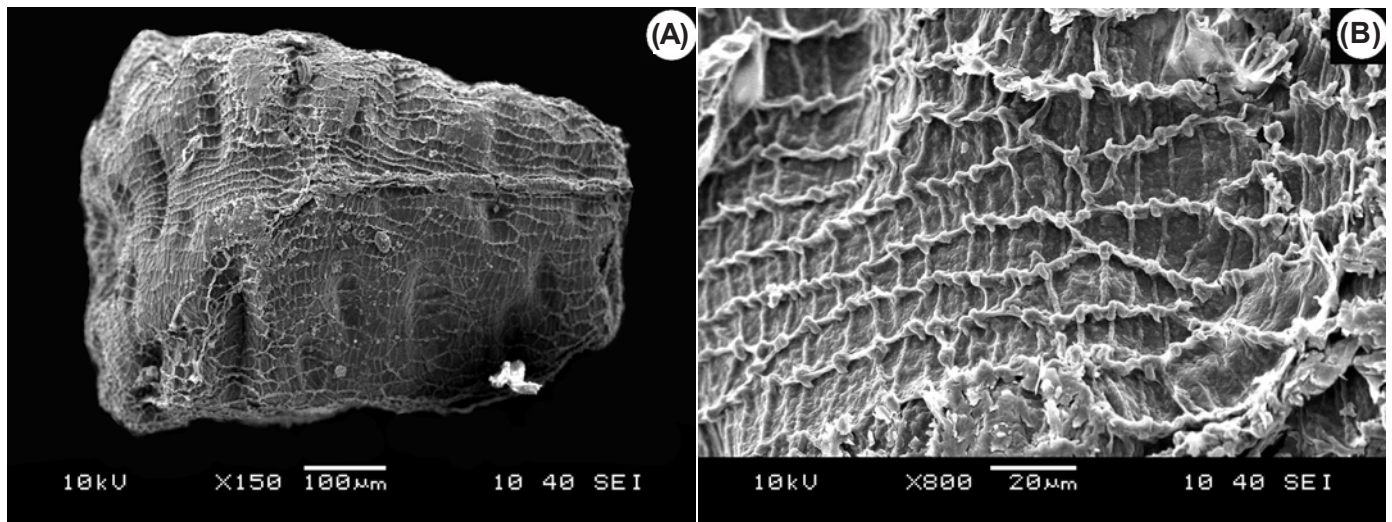
Seed micromorphology

Seeds are dark brown and oblong to prismatic and alveolate. The size of seeds is 0.6-0.8 x 0.4-0.5 mm. Hilum is short and orbicular. The seed coat ornamentation is reticulate-rugose (Figure 5A,B).



m: mesophyl, upc: upper epidermis, le: lower epidermis, ct: cuticula, vb: vascular bundle, pa: parenchyma, pp: palisade parenchyma, sp: spongy parenchyma, sh: stomata, nc: neighbor cell, ec: epidermis cell, bh: branched hair, gh: galandular hair.

Figure 4 - Cross-sections of the leaves of *Verbascum exuberans* (Scale Bar: a: 110 μm ; b: 100 μm ; c: 140 μm ; d: 3 μm).

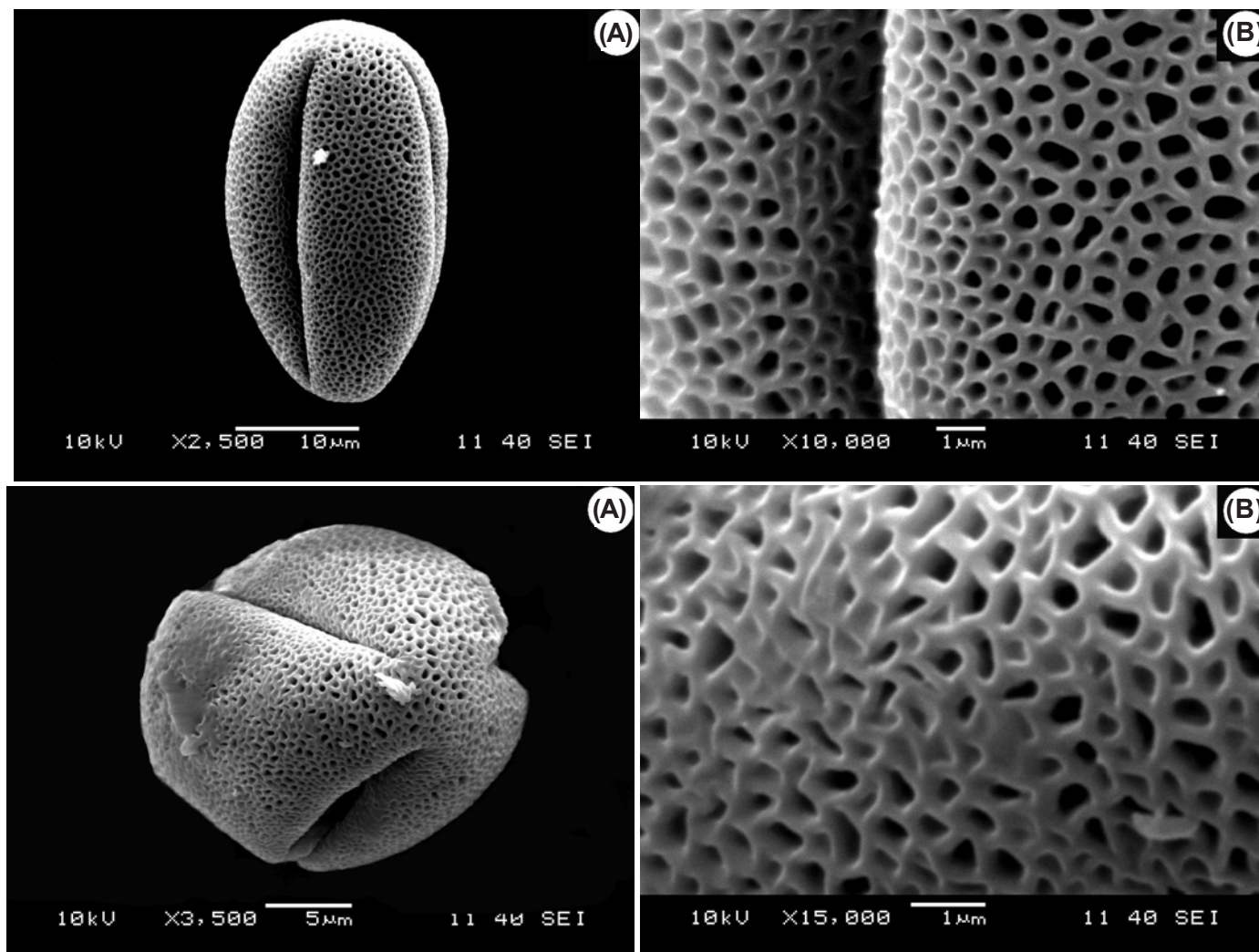


(A) General appearance. (B) Surface ornamentation.

Figure 5 - SEM micrographs of the seed of *V. exuberans*.

Pollen morphology

Pollen shape of *V. exuberans* is prolate. The shape of aperture is tricolpate, sculpture is tectate, and exine ornamentation is reticulate (Figure 6A-D, Table 3).



(A) Equatorial view. (C) Polar view. (B-D) Exine sculpturing.

Figure 6 - SEM micrographs of the pollen of *V. exuberans*.

Table 3 - Pollen characteristics of *V. exuberans*

Feature	<i>V. exuberans</i>	Feature	<i>V. exuberans</i>
Pollen Type	Tricolpate	Sculpture	Reticulate
Pollen Shape (A)	Prolate. P/E=1.57	P (µm)	31.05 ±1.28
Exine thickness (µm)	1.08 ± 0.10	E (µm)	19.70 ±1.85
Apertures	Colpus	Clg (µm)	25.78 ±2.50
Structure	Tectate. ect/end=3/1	Clt (µm)	1.31 ±0.57

Abbreviations: A: Asetoliz; P: Polar axis; E: Equatorial axis; Clg: Colpus length; Clt: Colpus width; ect: ectexine; end: endexine.

The anatomical analysis given in this work provides the first detailed anatomy features of *V. exuberans* is comparable with some other investigated *Verbascum* members, with reference to root, stem and leaf anatomy. We found that the results were similar as for the anatomical structure

of root, stem and leaf other *Verbascum* species in literature. In our study, it was observed that *V. exuberans* has a very large xylem field at the root and a cuticle layer at the stem. Also, the same features were remarked by the researchers (Özdemir and Altan, 2007; Alan and Gökman, 2015; Küçük, 2017).

The vascular cambium was indistinguishable as reported in root of *Verbascum orgyale* Boiss. & Heldr. (Alan and Gökman, 2015). We found that the result was similar as for the root while cambium can be clearly seen in the stem of *V. exuberans*. The analysis of the leaf of cross-section *V. exuberans* showed that there are dense candle hairs and glandular hairs on epidermal cells. Similar results were reported for the other investigated *Verbascum* species (Alan and Gökman, 2015; Küçük, 2017).

The pollen of *V. exuberans* is radially symmetrical, isopolar, prolate, tricolpate, the exine exhibits a tectate structure and reticulate ornamentation. Öztürk et al. (2018) reported that the tricolporate aperture type were *V. cariense* Hub.-Mor., *V. nudatum* Murbeck var. *nudatum*, *V. bithynicum* Boiss., *V. lasianthum* Boiss ex Benth, *V. pycnostachum* whereas *V. georgicum* Benth. was tricolpate. Al-Hadeethy et al. (2014) reported that the aperture types of *Verbascum* species (20 species) were tricolporate. Çakir and Bağcı (2006) noted that the tricolpate aperture types are *V. euphraticum* Benth and *V. melitense* Boiss. Aperture type of *V. thapsus* reported to have tricolporate aperture type (Asmat et al., 2011). Kheiri et al. (2006) reported that the tricolporate aperture type were *V. szovitsianum* Boiss., *V. agrimonifolium* (C. Koch) Hub.-Mor. *V. mucronatum* Lam., *V. sinuatum* L., *V. macrocarpum* Boiss., *V. oreophilum* C. Koch var. *oreophilum* (Bordz) Hub.-Mor. and *V. cheirantifolium* Boiss. The pollen shape of *V. exuberans* was prolate. The average polar axis (P) was measured 31.05 µm and the equatorial axis (E) was measured 19.70 µm. Previous studies on *Verbascum* pollen reported that the pollen shapes of *V. cariense*, *V. nudatum* var. *nudatum*, *V. bithynicum*, *V. lasianthum*, *V. pycnostachum* and *V. georgicum* were observed as prolate, subprolate, prolate-spheroidal and oblate-spheroidal. The average polar axis (P) was measured between 23.1–26.3 µm and the equatorial axis (E) was measured between 16.7–27.8 µm (Öztürk et al., 2018). Kheyri et al. (2006) reported that *V. mucronatum* pollen has prolate, and prolate-spheroidal shapes, with polar axis ranging between 25.08–40.12 µm, and equatorial axis between 15.48–28.84 µm. Al-Hadeethy et al. (2014) reported that some *Verbascum* pollen has prolate shape. Pollen shape of *V. thapsus* were oblate-spheroidal (Asmat et al., 2011).

In conclusion, we aimed at providing a comprehensive morphological, anatomical, palynological and seed micromorphological description of *V. exuberans*, endemic to Turkey. It is reached from this study that, these data could contribute to the taxonomy of the genus *Verbascum*.

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