

Original article

**EXOMORPHIC SEED CHARACTERS AND ANATOMY OF LEAF
AND STEM OF SOME *VINCETOXICUM*
(ASCLEPIADACEAE/APOCYNACEAE) SPECIES FROM TURKEY**

TÜRKİYE'DE YETİŞEN BAZI *VINCETOXICUM*
(ASCLEPIADACEAE/APOCYNACEAE) TÜRLERİNİN YAPRAK VE DAL
ANATOMİSİ VE EKZOMORFİK TOHUM KARAKTERİSTİĞİ

Ahmet İLÇİM¹, Sevda GÜZEL ÖZAY², Gamze KÖKDİL²

¹ Mustafa Kemal University, Faculty of Arts and Science, Department of Biology, 31000
Antakya-Hatay, TURKEY

² Mersin University, Faculty of Pharmacy, Department of Pharmacognosy, Yenişehir Campus,
33169 Mersin, TURKEY

ÖZET

Vincetoxicum NM Wolf cinsine ait 5 taksonunun ekzomorfik tohum karakteristiği ışık ve taramalı elektron mikroskobu kullanılarak araştırılmıştır. Bazı karakterlerin farklı taksonlarda değişiklik gösterdiği bulunmuştur. Bunlar tohum şekli, tohum yüzeyindeki girinti ve çıkıntılardır. Diğer karakterler ise tohum koma tüyleri, periklinal duvarların konkav/konveks olmaları ve antiklinal duvarların çeşitli derinliklerdeki kanallarıdır.

Bitkilerin gövde ve yapraklarının anatomik karakterleri belirlenmiştir. Çalışılan taksonlar *Asclepiadaceae* familyasının genel anatomik özelliklerine sahiptir.

Anahtar kelimeler: *Vincetoxicum*, Tohum Morfolojisi, Gövde ve Yaprak Anatomisi, SEM

ABSTRACT

Exomorphic seed characters of five Vincetoxicum NM. Wolf. taxa were investigated using light and scanning electron microscopy. It was found that some characters fluctuated through different expressions in

different taxa. These were shape of the seed, and sculpture of the seed coat. The latter characters were the seed coma, the concavity or convexity of the periclinal walls and the channeling of various depths of the anticlinal walls. Anatomical characters of stem and leaf of the plants were described. The studied taxa have general anatomical characteristics of the Asclepiadaceae.

Key words: *Vincetoxicum*, Seed Morphology, Stem and Leaf Anatomy, SEM

INTRODUCTION

The Asclepiadoideae subfamily (formerly named Asclepiadaceae) with 180 genera and 2500-3000 species is distributed mainly in the tropical and subtropical regions of the world (1-3). Most members of the subfamily have milky juice, flowers with five united petals, podlike fruits, and usually, tufted seeds (3, 4). The silky-haired seeds are drawn out of their pods by the wind and are carried off. The genus *Vincetoxicum* NM Wolf has been classified within the subfamily Asclepiadoideae (5). c.100 species are distributed in Asia, especially in mountain ranges, Europe and northeastern United States (6). Some European species have become invasive weeds as far north as Canada (7). The genus *Vincetoxicum* and its allied genera (*Cynanchum* L. and *Tylophora* R.Br.) have been investigated for a long time. Liede (1996) proposed that the genus *Vincetoxicum* is more similar to *Tylophora* than to *Cynanchum* based on cladistic analysis of morphological and chemical characters (6, 8).

Some *Vincetoxicum* species are known for their poisonous effects, as well as their medicinal properties (9). Phenanthroindolizidine alkaloids are characteristic constituents of some genera of Asclepiadaceae including the genus *Vincetoxicum*. Biological activities of these compounds include antibacterial, antifungal, antiinflammatory effects, also have significant cytotoxic activity against various cancer cell lines (10-12).

In Turkey, the genus *Vincetoxicum* is represented by 8 species and 10 taxa (13). *V. parviflorum* Decne., *V. canescens* subsp. *pedunculata* Browicz and *V. fuscatum* subsp. *boissieri* (Kusn) Browicz are taxa endemic to Turkey. The objective of this study is to investigate the exomorphic seed characters and anatomic characters of stems and leaves of five taxa of *Vincetoxicum* and to determine the systematic value of these characters. To the best of our knowledge, this is the first report on exomorphic seed characters and leaf and stem anatomy of *Vincetoxicum* taxa.

MATERIALS AND METHODS

Five taxa were collected from their natural habitats. Voucher specimens were deposited in the Herbarium of the Faculty of Science, Mustafa Kemal University (MKUH) and identified by

Assoc. Prof. Dr. Ahmet İlçim (Department of Biology, Faculty of Arts and Sciences, Mustafa Kemal University, Antakya, Turkey) (Table 1). For every species, five seeds were chosen at random, examined by light microscopy for the seed shape and the seed dimension (length and width); the latter were expressed as the mean of five measurements. For SEM investigation of the spermoderm, a minimum of two seeds were mounted on brass stubs and coated with a thin layer of gold (200 Å) in a sputter coating unit. Scanning was done by a Jeol.- Jsm 5500 - 35C, operated at 40 KV in the Scanning Electron Unit of MKU.

For the stem studies internodes were selected. Tissue was fixed in ethyl alcohol (70 %), dehydrated in ethyl alcohol and xylol series, and embedded in paraffin. Sections (8-20 µm) of all vegetative organs were stained with hematoxylin-eosin.

Table 1. Information on the plant materials used in this study.

Taxa	Locality and Voucher
<i>V. canescens</i> subsp. <i>canescens</i> (Willd.) Decne.	C6: Kahramanmaraş, Engizek Mountain, Fallow fields, 1000 m., 13.VII.2009. A. İLÇİM 1283 MKUH
<i>V. canescens</i> subsp. <i>pedunculata</i> Browicz	A3: Afyon, Dinar; Kumalar Mountain, 1500-1600 m., 20.VII.2009. A. İLÇİM 1284 MKUH
<i>V. fuscatum</i> subsp. <i>fuscatum</i> (Hornem) Reichb.	B6: Kayseri, Pınarbaşı; Hınzır Mountain, 1800 m., 10.VII.2009. A. İLÇİM 1315 MKUH
<i>V. fuscatum</i> subsp. <i>boissieri</i> (Kusn) Browicz	A5: Amasya, Ferhat Mountain, 460 m., 06.VII.2009. A. İLÇİM 1316 MKUH
<i>V. parviflorum</i> Decne.	A7: Trabzon, 1200 m., 28.VI.2009. A. İLÇİM 1334 MKUH

RESULTS

Seed Micromorphology

The present study shows that all seeds of five taxa have narrow wings and pappus. Detailed results of studied *Vincetoxicum* taxa are given below.

V. canescens subsp. *canescens*

The seed of this taxon have ovate shape in outline; it is characterized by a very large hilum and very narrow wings (Fig. 1. a.). The hilum of the seed consists of two different cell types. Outwards of hilum take shape with irregular, pentagonal or hexagonal cells whose periclinal walls are straight and rugulate with irregular projections. (Figs. 1. b., c.). The centre of the hilum consists

of deep concave cells. Hilum region is separated by lengthwise extended cells. The wings cells consist of sparsely granulate pentagonal or hexagonal cells (Figs. 1. d., e.).

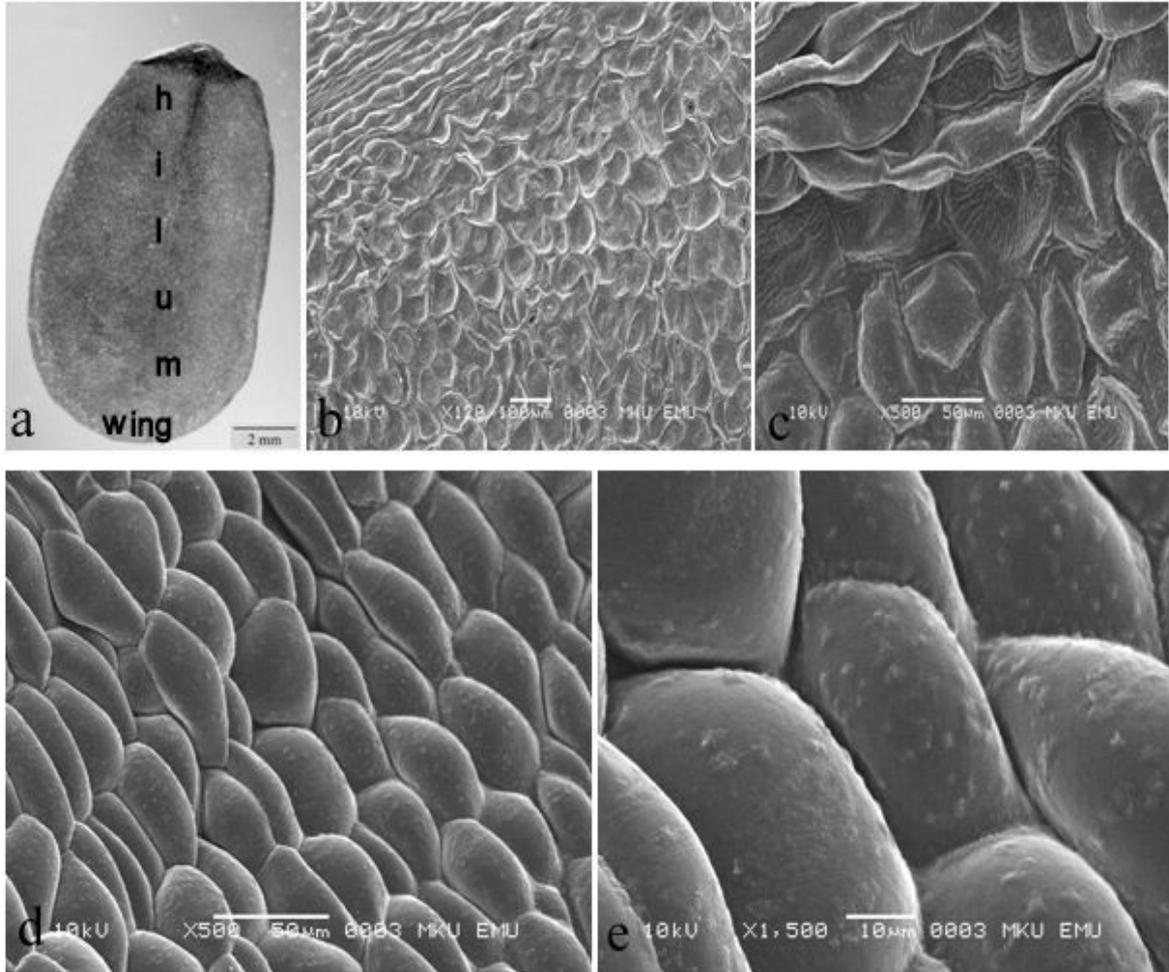


Figure 1. *V. canescens* subsp. *canescens*, **a.** General view of seed; **b, c.** hilum cells; **d, e.** wing cells
V. canescens subsp. *pedunculata*

This subspecies has ovate seeds with narrow wings like in subsp. *canescens* (Fig. 2. a.). But clearly differs from it by homogeneous hilum and wing cells (Figs. 2. b., c.). In this taxa, seed hilum and wing consist of deep concave cells (Figs. 2. d., e.).

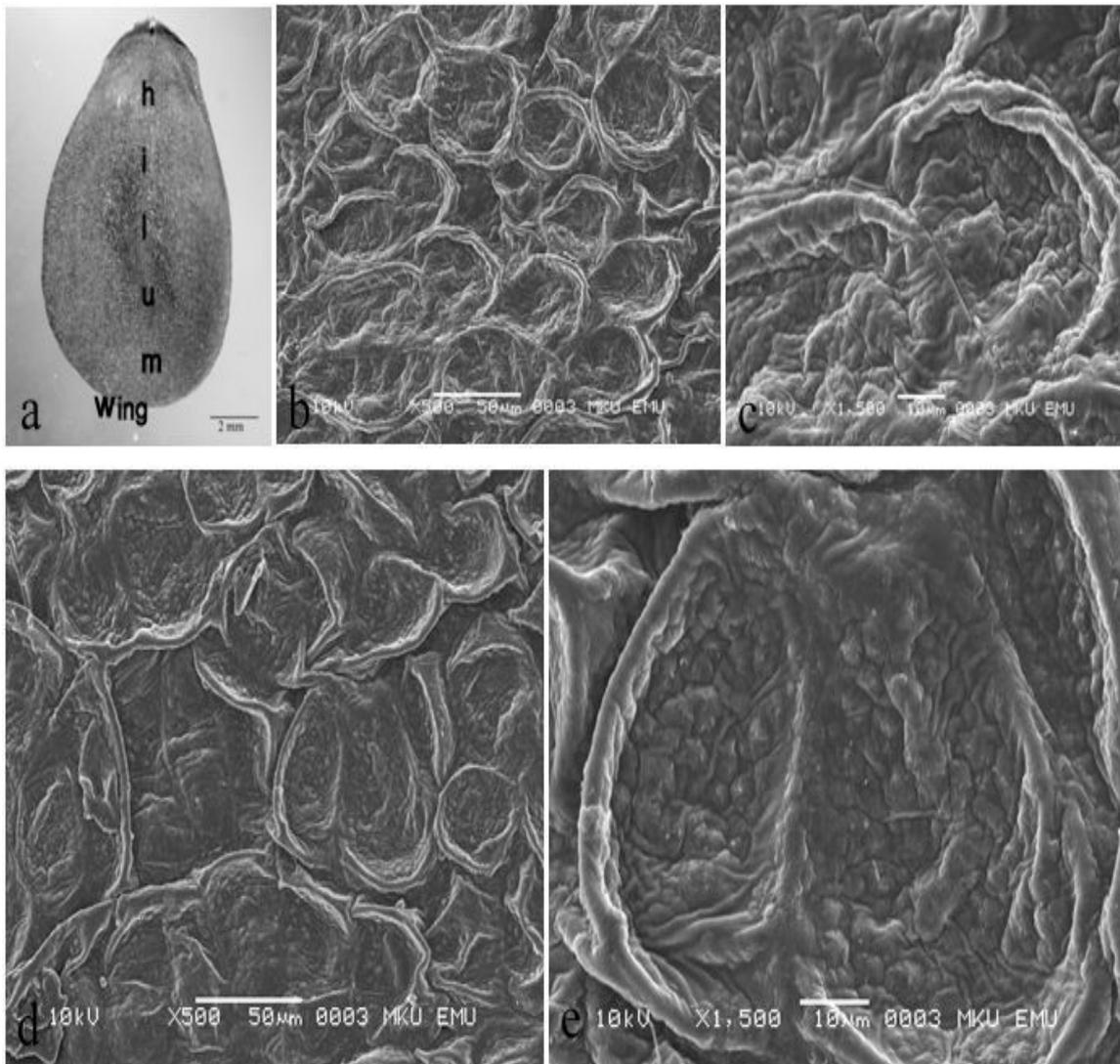


Figure 2. *V. canescens* subsp. *pedunculata* **a.** General view of seed; **b, c.** hilum cells; **d, e.** wing cells

V. fuscatum subsp. *fuscatum*

The seed of the species consists of two regions; hilum and wing (Fig. 3. a.). Hilum region is distinguished by lengthwise extended cells. Hilum and wing cells are differentiated from each other with the cell shapes and ornamentation. Tetragonal hilum cell walls very thin; almost inconspicuously thickened. These cells have very small numerous granules at cell surfaces (Figs. 3. b., c.) Wing cells long, sometimes narrowed at the middle and have grape like secondary structures on outer walls (Figs. 3. d., e.).

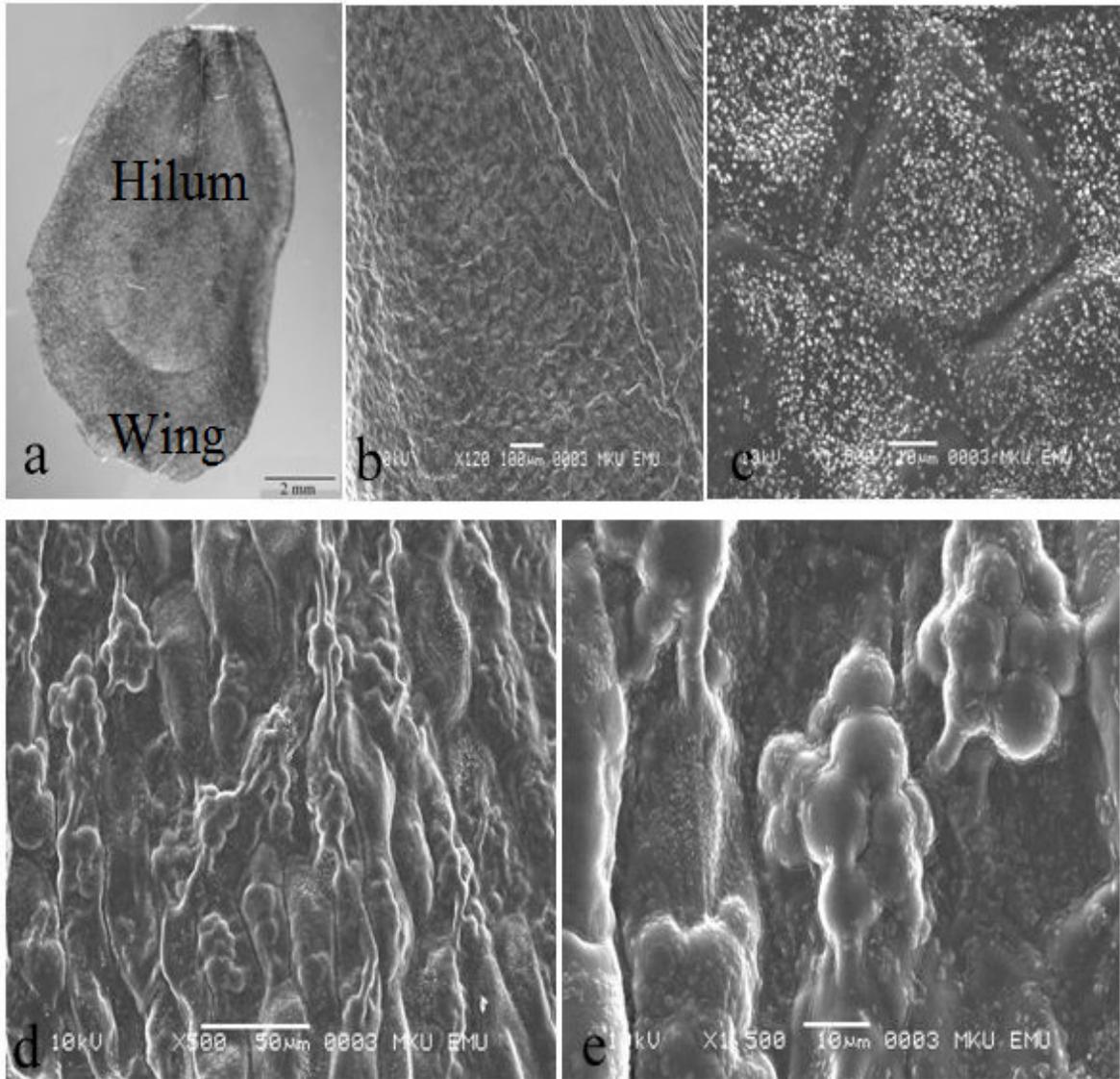


Figure 3. *V. fuscatum* subsp. *fuscatum* **a.** General view of seed; **b, c.** hilum cells; **d, e.** wing cells

V. fuscatum subsp. *boissieri*

This seed is similar to other taxa in macromorphology (Fig. 4. a.). Hilum consists of tetragonal and irregular cells (Figs. 3. b., c.) (Figs. 4. b., c.). Like in *V. fuscatum* subsp. *fuscatum*, hilum cells have very small numerous granulous secondary structures. Some of these cell walls have longitudinal lines and irregular appendages. Wing cells have tetragonal or pentagonal shapes. At this region, longitudinally raised cells observed (Figs. 4. d., e.).

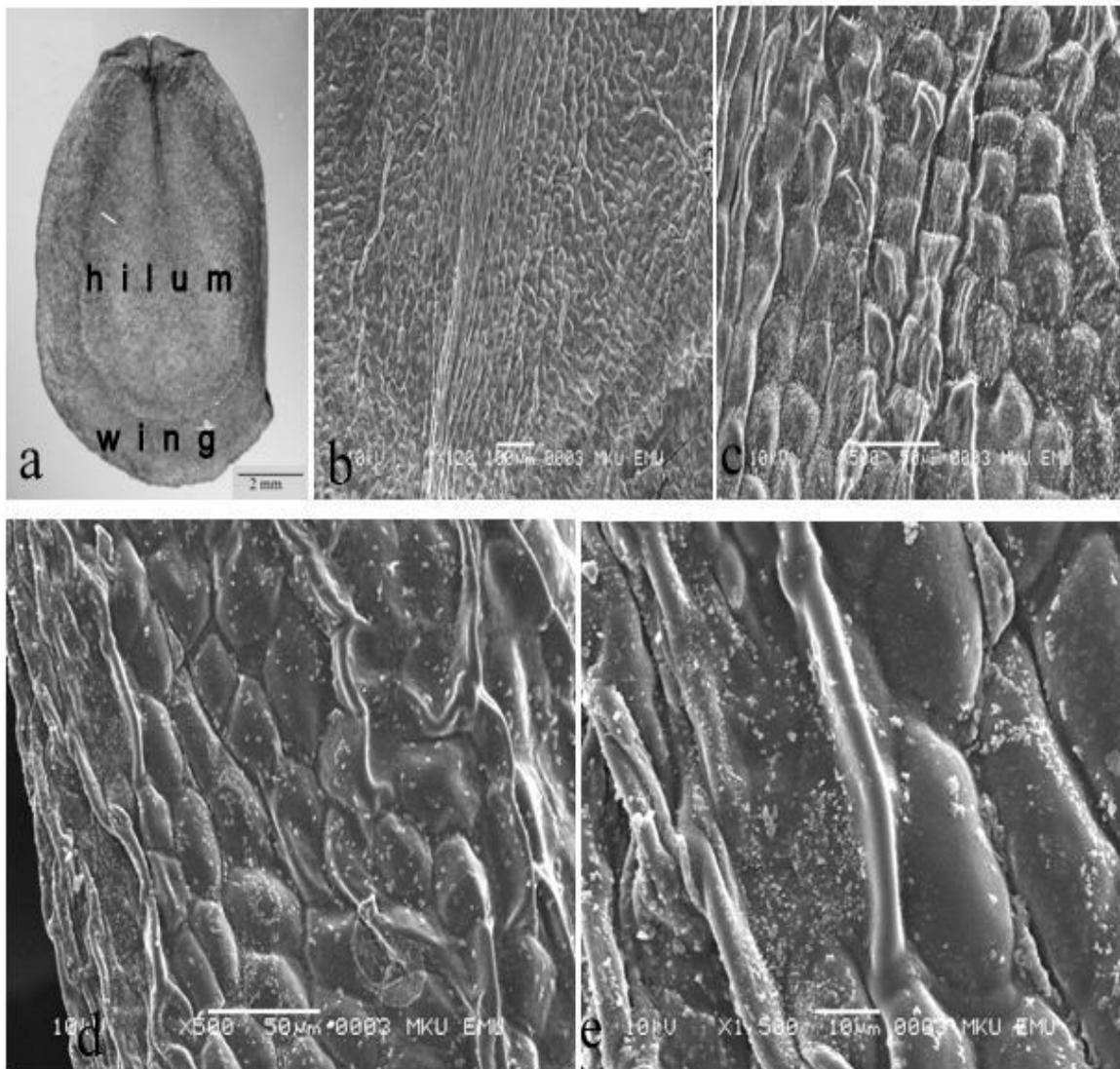


Figure 4. *V. fuscatum* subsp. *boissieri* **a.** General view of seed; **b, c.** hilum cells; **d, e.** wing cells

V. parviflorum

The seeds consists of two regions like in other species, hilum and wing region (Fig. 5. a.). Cells of this two regions differ from each other in respect to shape and ornamentation. The hilum consists of irregular, circular or quadrature cells with thickened periclinal walls. These cells have numerous grains on upper cell walls (Figs. 5. b., c.) At the middle of the hilum, lengthwise extended cells have thickened walls. Wing cells have circular or rectangular shapes. These cells have granules and flat appendages on the walls (Figs. 5. d., e.).

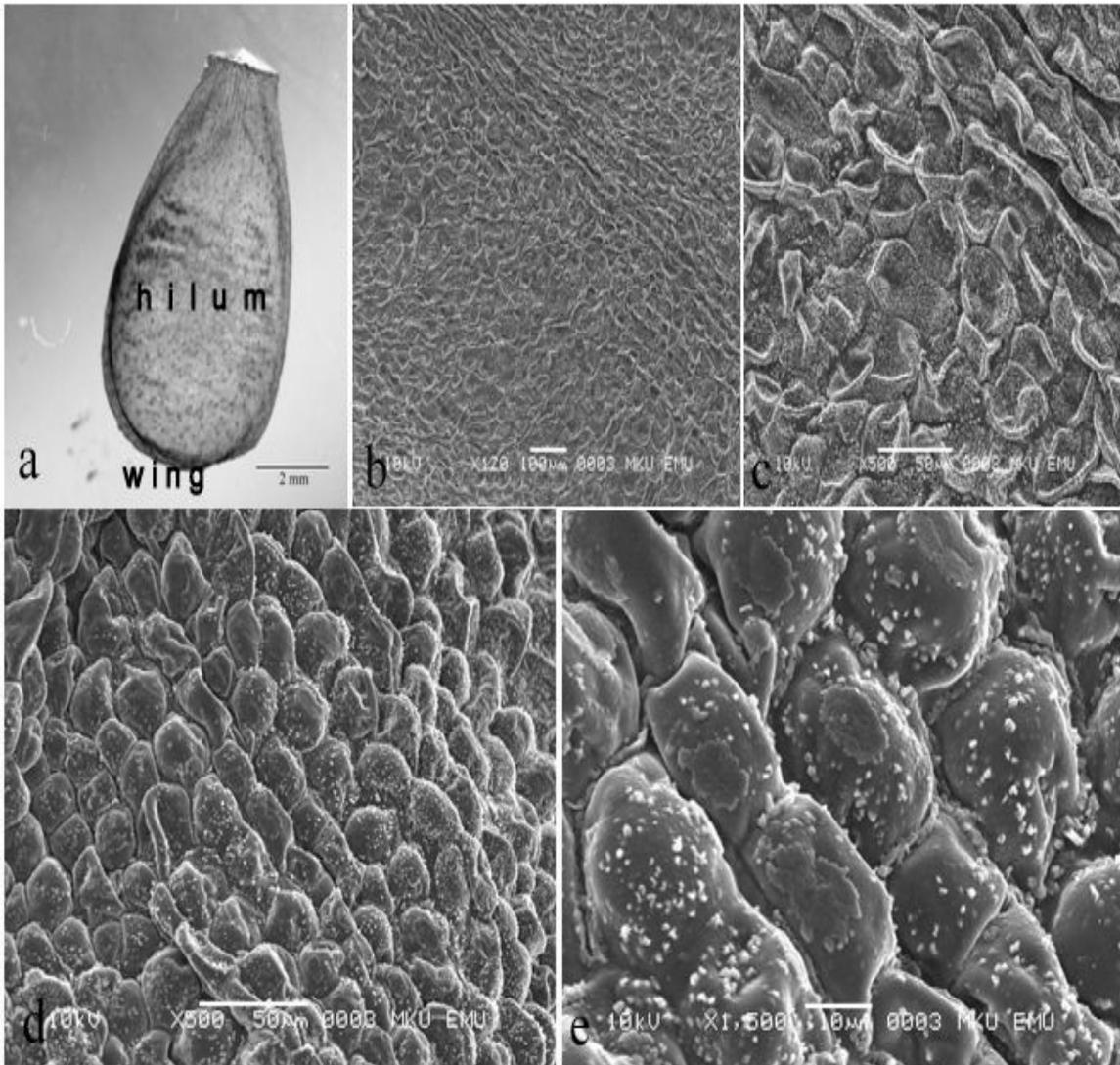


Figure 5. *V. parviflorum* **a.** General view of seed; **b, c.** hilum cells; **d, e.** wing cells

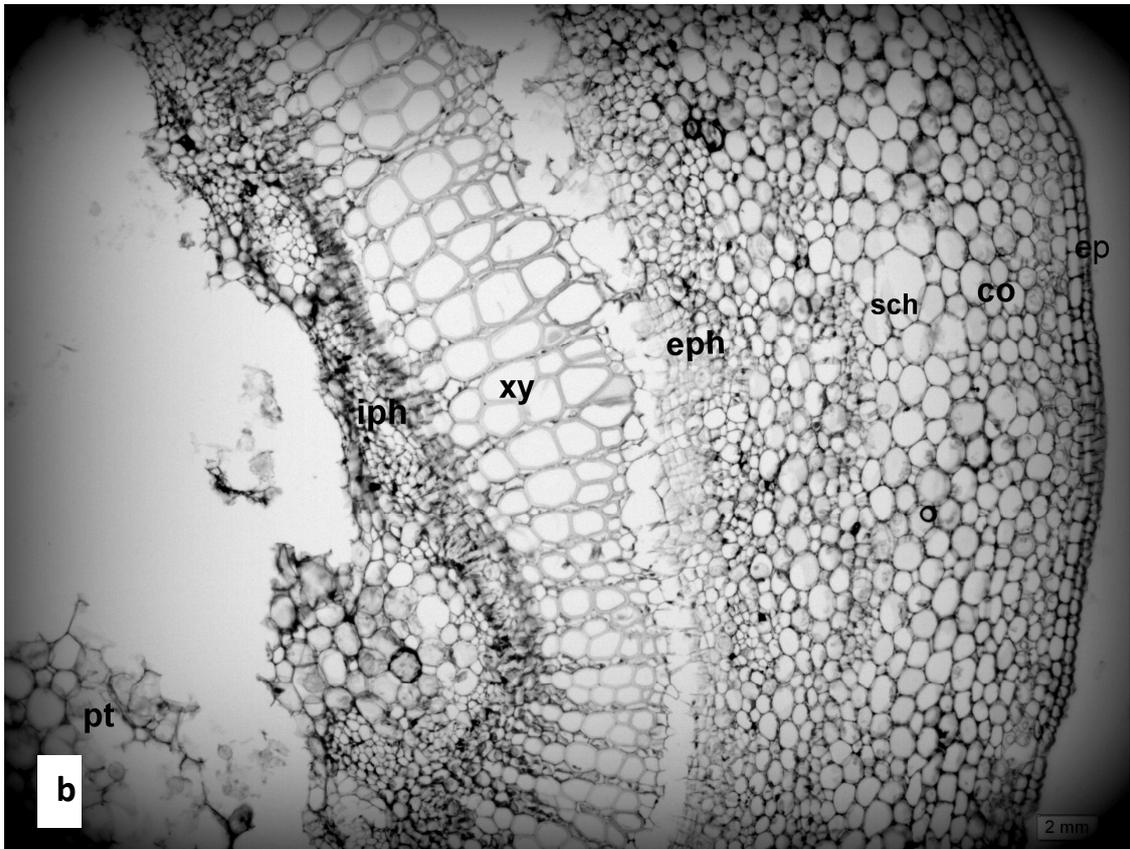
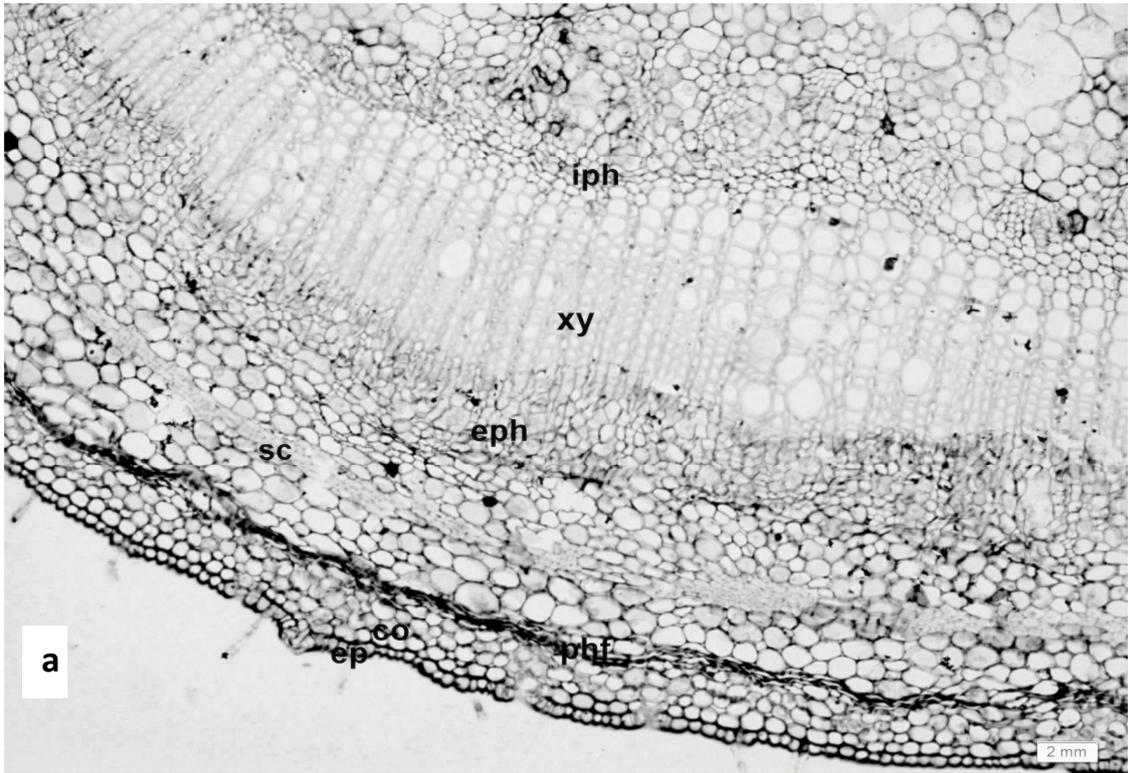
Stem Anatomy

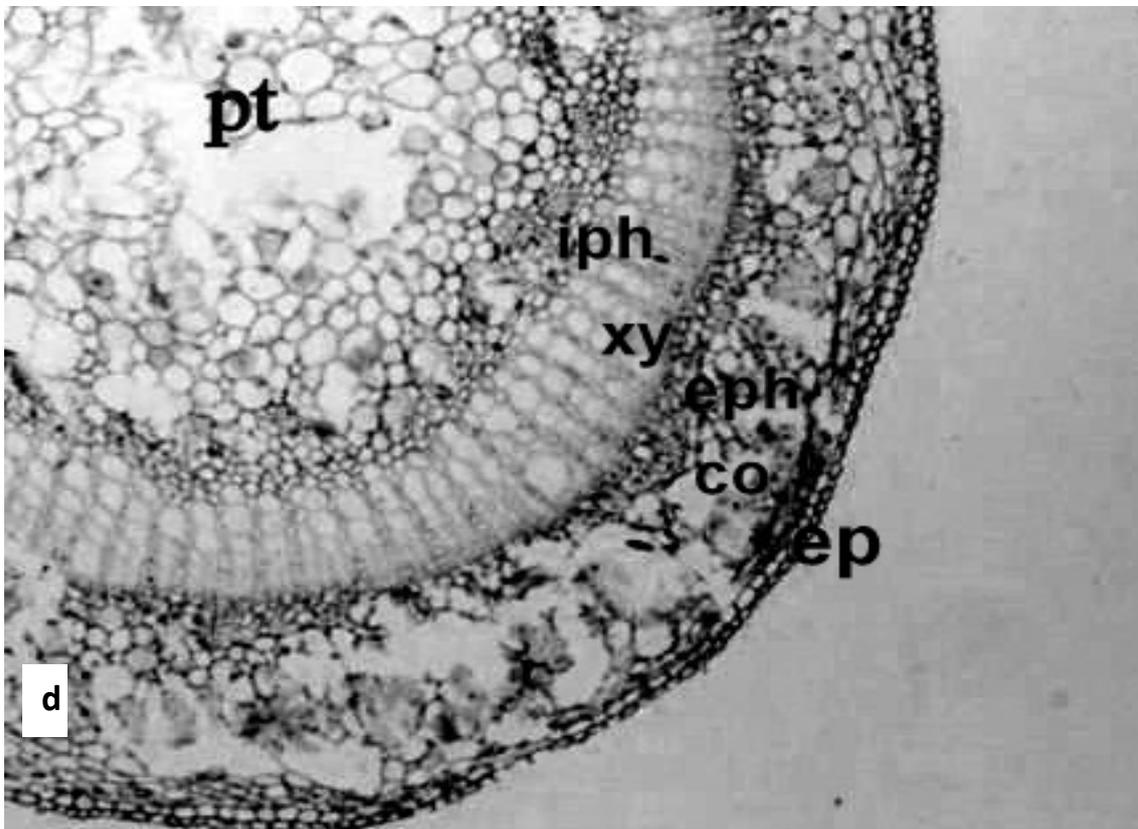
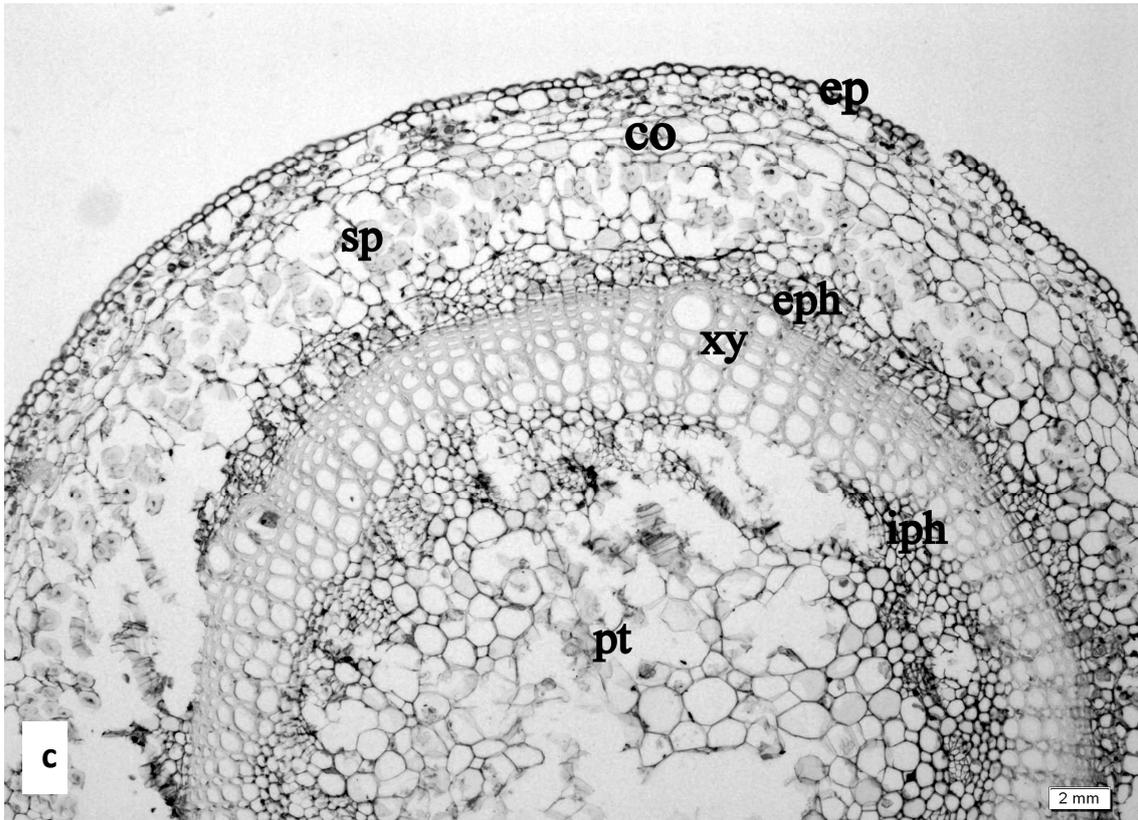
The anatomy of the herbaceous stems of five taxa are similar in general shape and tissue organisation. A thick mature stem cross section shows semi-circular outline with one or two layers of epidermis, when consisting of two layers, upper epidermal cells are smaller than, the lower ones. The stem cortex is a very large region between the epidermis and the vascular cylinder. The cortex of *V. canescens* subsp. *canescens* and *V. parviflorum* contain two layers of chlorenchyma cells under epidermal cells (Fig. 6. a.), the cortex contain secretion channels as a difference from other species in *V. canescens* subsp. *pedunculata* and *V. canescens* subsp. *canescens* stems (Figs. 6. a., b.). The patches of phloem fibres occur in the cortex of *V. canescens* subsp. *canescens*, *V. fuscatum*

subsp. *fuscatum* and *V. parviflorum* (Figs. 6. a., c., e.). Lignified cells, crystals, phloem fibres and starch grains are observed in all of the stems. In cross section, stems vascular systems of stems consist of bi- collateral bundles. In all five taxa (Figs. 6. a., b., c., d., e.) the external proto-phloem is followed by the concurrent development of protoxylem and internal phloem. The protoxylem consists of radial rows of narrow, thick-walled vessels. Tracheids of early wood are bigger than tracheids of late wood in the stem of *V. canescens* subsp. *canescens*, *V. fuscatum* subsp. *fuscatum*, *V. parviflorum* and *V. canescens* subsp. *pedunculata* (Figs. 6. a., c., e.). The vessel members consist of 7-12 row of cells. The cells usually have pentagonal shapes. Less external and internal phloem clusters occur in the stems of all taxa.

Leaf Anatomy

In all species the epidermis consists of cubical or somewhat conical shaped cells, covered by thick cuticle and sparse simple trichomes or glabrous. Stomata are of hypostomatic and anomocytic type. Mesophyll cells are differentiated into palisade and spongy parenchyma, or consist of uniform cells. Mesophyll is bifacial or monofacial. If mesophyll is bifacial, palisade parenchyma is present below the upper epidermis, consists of vertically elongated continuous single layered cells. Spongy parenchyma lies below the palisade parenchyma and is loosely arranged (Fig. 7.). While the leaf of *V. canescens* subsp. *canescens* (Fig. 7. a.) has monofacial mesophyll, the leaf of *V. fuscatum* subsp. *fuscatum*, *V. parviflorum* and *V. fuscatum* subsp. *boissieri* have bifacial mesophyll. The leaf mesophyll of *V. canescens* subsp. *pedunculata* differs from other species by isobilateral mesophyll (Fig.7. b.).





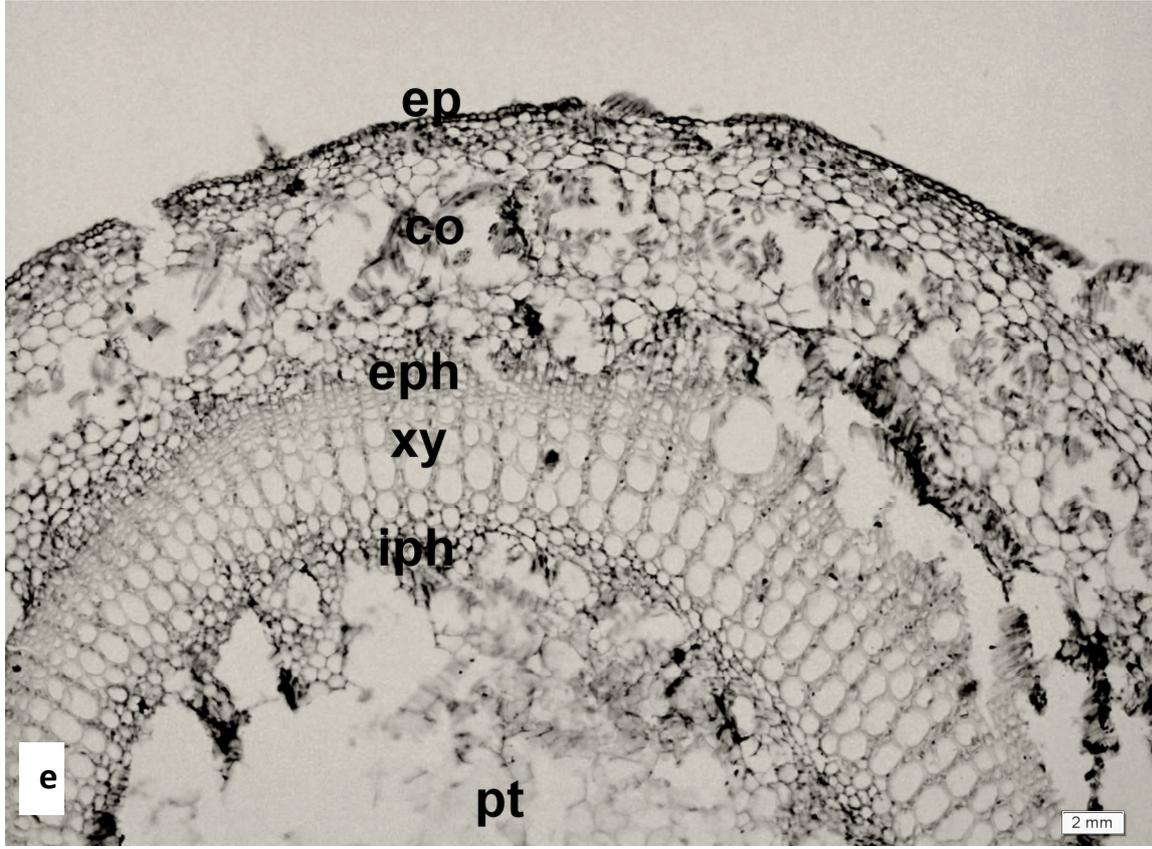


Figure 6. Stem anatomy of **a.** *V. canescens* subsp. *canescens*, **b.** *V. canescens* subsp. *pedunculata*, **c.** *V. fuscatum* subsp. *fuscatum*, **d.** *V. fuscatum* subsp. *boissieri*, **e.** *V. parviflorum*. (ep: epidermis, co: cortex, sc: secretion channel, sp: sclerenchyma patch, sc: sclerenchyma, eph: external phloem, xy: xylem, iph: internal phloem, pt: pith)

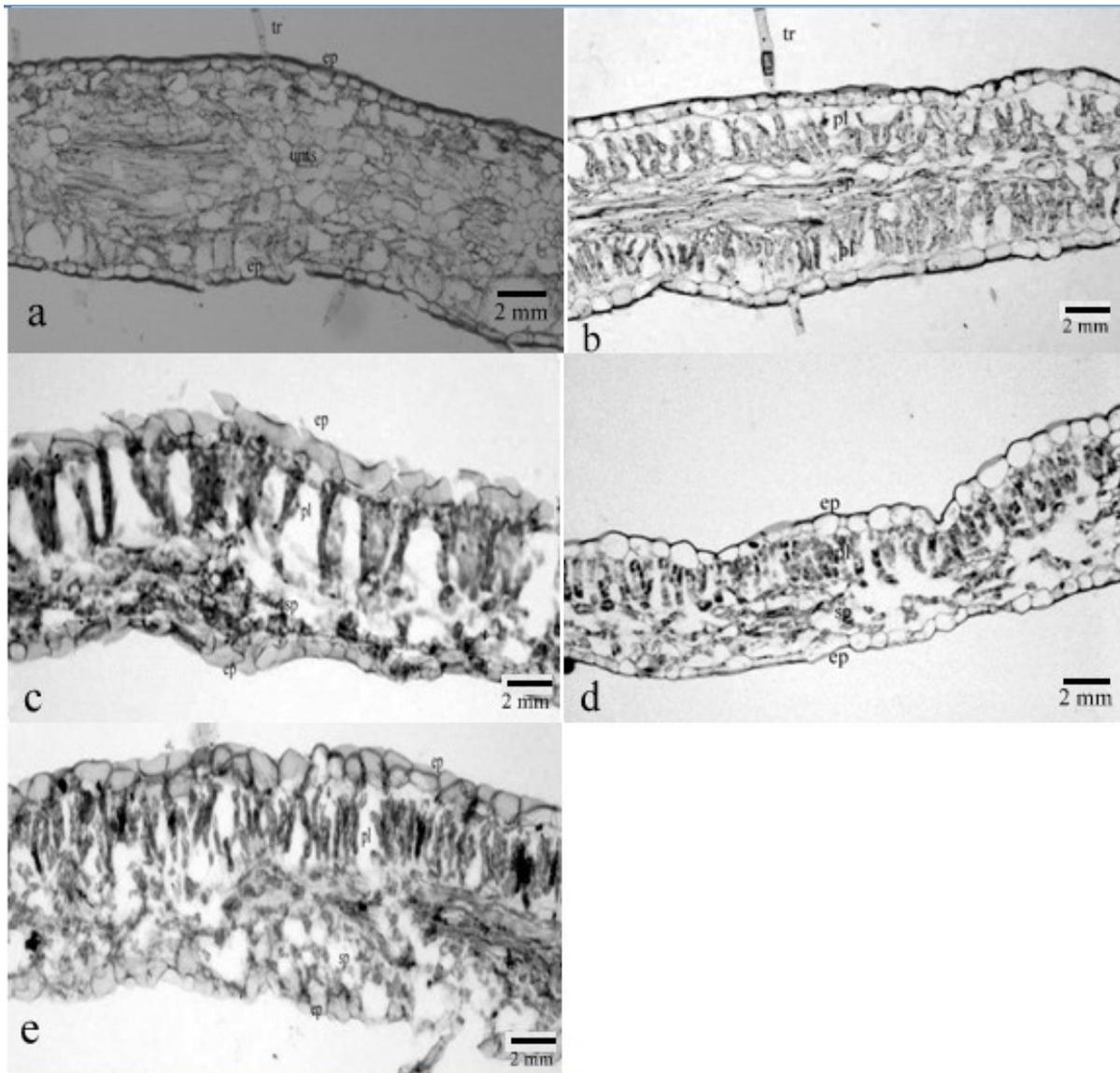


Figure 7. Leaf anatomy of **a.** *V. canescens* subsp. *canescens*, **b.** *V. canescens* subsp. *pedunculata*, **c.** *V. fuscatum* subsp. *fuscatum*, **d.** *V. fuscatum* subsp. *boissieri*, **e.** *V. parviflorum*. (ep: epidermis, tr: trichome, pl: palisade parenchyma, sp: sponge parenchyma)

DISCUSSION

The light microscopy shows that the seeds have ovate shapes. Seed dimensions vary as well; the largest dimensions (8.3-8.7 mm L. x 4.3-5.3 mm W.) are seen in *V. canescens* subsp. *pedunculata*, and the smallest dimensions (6.0-7.7 mm L. x 4.0-4.5 mm W.) in *V. fuscatum* subsp. *boissieri*. All seeds of five taxa have narrow wings and pappus. The present investigation showed

that all seeds have similar properties at outline. Seed shapes and dimensions are not usable systematically (Table 2).

Table 2. Comparison of the seed characteristics of five *Vincetoxicum* taxa.

Taxa	Seed length (mm)	Seed width (mm)	Wing width at base (mm)	Wing width at apex (mm)	Wing width at edge (mm)
<i>V. canescens</i> subsp. <i>canescens</i>	7.1-8.6	4.8-5.3	0.0-0.1	0.0-0.1	0.0-0.1
<i>V. canescens</i> subsp. <i>pedunculata</i>	8.3-8.7	4.3-5.3	0.0- 0.1	0.1-0.2	0.0-0.1
<i>V. fuscatum</i> subsp. <i>fuscatum</i>	6.7-8.5	4.0-4.8	1.0-1.9	0.5-1.1	0.5-1.0
<i>V. fuscatum</i> subsp. <i>boissieri</i>	6.0-7.7	4.0-4.5	1.0-1.4	0	0.7-1.0
<i>V. parviflorum</i>	6.7-8.4	3.6-3.8	0.2-0.5	0.3-1.5	0.2-0.3

However, micromorphological properties of studied *Vincetoxicum* taxa seeds exhibited some similarities and differences compared to each other. The seed of *V. canescens* subsp. *canescens* have two different cells on hilum. Outwards of hilum have irregular, pentagonal or hexagonal shape cells with straight and rugulate periclinal walls with irregular projections (Fig. 1. b.). The centre of the hilum consists of deep concave cells (Fig. 1. c.). However *V. canescens* subsp. *pedunculata* has homogeneous hilum cells (Figs. 2. c., d.). The seed hilum and wing consist of deep concave cells (Figs. 2. d., e.).

Hilum of *V. fuscatum* subsp. *fuscatum* is characterized by tetragonal, almost inconspicuously thickened cells with numerous small granular secondary structures (Figs. 3. b., c.) The hilum of the *V. fuscatum* subsp. *boissieri* has tetragonal and irregular cells and small granules (Figs. 4. b., c.). Wing cells of *V. fuscatum* subsp. *fuscatum* are sometimes narrowed at the middle and have grape like secondary structures on outer walls (Figs. 3. d., e.), but the wing cells of *V. fuscatum* subsp. *boissieri* only consist of polygonal cells (Figs. 4. d., e.).

Micromorphological properties of *V. parviflorum* differ from other taxa. Hilum cells of this taxa are tetragonal and concave irregular, and have numerous grains on walls (Figs. 5. b., c.). Some of the these cell walls have longitudinal lines and irregularly raised appendages. Wing cells have circular or rectangular shapes. The cells have granules and flat appendages on walls (Figs. 5. d., e.).

To the best of our knowledge there is no information on the anatomy of *Vincetoxicum* genus in the literature. But several studies can be found on *Araujia*, *Cynanchum*, *Asclepias* and

Oxystelma taxa of the same family (8, 14-17). The present study showed that *Vincetoxium* species exhibit morphological and anatomical features similar to other Asclepiadaceae genera. Secretion channels, crystals and phloem fibers observed in the studied *Vincetoxicum* taxa were found to have similar properties with *Araujia* (15), *Cynanchum* (8) *Asclepias* (16) and *Oxystelma* genera (17). Mesophyll cells are differentiated into palisade and spongy parenchyma (bifacial) like in *Oxystelma esculentum* R. Br. (17) and *Asclepias perennis* Walter (16). *Cynanchum acutum* L. and *Oxystelma esculentum* (Asclepiadaceae) seeds have narrow wings (14) like in our samples.

The results indicate that the morphological and anatomical characteristics would be helpful for the identification of *Vincetoxicum* taxa.

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