



Comparative leaf and peduncle anatomy of four Turkish endemic *Onobrychis* Mill. taxa.

Mehmet TEKİN^{*1}, Gülden YILMAZ²

¹ Cumhuriyet University, Faculty of Pharmacy, Department of Pharmaceutical Botany, 58140, Sivas, Turkey

² Trakya University, Faculty of Science, Department of Biology, Balkan Campus, 22030, Edirne, Turkey

Abstract

In this study, leaf and peduncle anatomy of four Turkish endemic taxa of the genus *Onobrychis* from sect. *Onobrychis*, including *Onobrychis quadrijuga*, sect. *Heliobrychis* including *Onobrychis argyrea* subsp. *argyrea*, and sect. *Hymenobrychis* including *Onobrychis tournefortii*, and *Onobrychis albiflora* were investigated for the first time. Numerous quantitative and qualitative characters in the leaf and peduncle cross sections were determined and studied. Results showed that, for the leaves quantitative characteristics and for the peduncle qualitative characteristics are more distinctive between examined taxa. As the main, while in the leaf, mesophyll thickness, stomata and epidermis cells features, in the peduncle, especially, the contour of the peduncle and location of cavities in cross section, and density of papillae on hair surface were represented considerably variation between examined taxa.

Key words: anatomy; endemic; leaf; *Onobrychis*; peduncle; Turkey

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Türkiye endemiği olan dört *Onobrychis* Mill. taksonunun karşılaştırmalı yaprak ve pedünkül anatomisi

Özet

Bu çalışmada, Türkiye’de yayılış gösteren *Onobrychis* cinsine dâhil olan, sect. *Onobrychis*’e ait *O. quadrijuga*, sect. *Heliobrychis*’e ait *O. argyrea* subsp. *argyrea*, sect. *Hymenobrychis*’e ait *O. tournefortii* ve *O. albiflora* taksonlarının yaprak ve pedünkül anatomisi ilk defa incelenmiştir. Yaprak ve pedünkül enine kesitleri üzerinden birçok nitel (kalitatif) ve nicel (kantitatif) karakterler tespit edilerek çalışılmıştır. Sonuçlara göre yapraklar için nicel, pedünkül için ise nitel karakterler, taksonlar arasındaki ayırt edici özellikler açısından önemlidir. Temel olarak, yapraktaki mezofil tabakası kalınlığı, stoma ve epidermis hücrelerinin özellikleri, pedünkülde ise özellikle enine kesit şekli, tüy yüzeyindeki papilla yoğunluğu, boşlukların konumları gibi özellikler incelenen taksonlar arasında dikkate değer şekilde varyasyon göstermektedir.

Anahtar kelimeler: anatomi; endemik; *Onobrychis*; pedünkül; Türkiye; yaprak

1. Introduction

The Fabaceae is one of the largest dicot family, represented by 730 genera and about 19.500 species, having usually economically important plants (Simpson, 2006). *Onobrychis* is one of the huge genus of Fabaceae family, tribe *Hedysareae*, subfamily *Faboideae*. The genus *Onobrychis* has 170 species which densely distributed in the Anatolia-Iran-Caucasian triangle, South West Asia, Mediterranean region and in temperate Europe and Asia in the world (Cronquist, 1981; Zohary, 1987; Aktoklu, 2001). There are only, 23 species of *Onobrychis* are listed in The Flora Europaea (Ball, 1968), while the main diversification center ranges from Central Asia to Iran, so the number of species is increased to 56 species, 27 of which are endemic (Ildis, 2005).

In Turkey, *Onobrychis* genus is divided into five sections and is represented by 55 species, 28 of them are endemics (Hedge, 1970; Davis et al., 1988; Duman and Vural, 1990; Aktoklu, 2001; Yıldırım, 2004). The species of *Onobrychis* (sainfoin) are economically important plants that are grown to produce high-protein food for many animals.

* Corresponding author / Haberleşmeden sorumlu yazar: Tel.: +905434421321; Fax.: +902842354010; E-mail: mtekin2280@gmail.com

As these species can fix atmospheric nitrogen, they are important not only for farm animal nourishment but also for soil fertility. In addition, *Onobrychis* species have a great potential for pasture and meadow improvement, land conservation and rehabilitation and have importance as genetic sources for breeding (Cavallarin et al., 2005; Özaslan Parlak and Parlak, 2008). There were lots of studies about palynology, taxonomy, karyology and cytotaxonomy of some *Onobrychis* taxa in the literature. Pmar et al. (2009) have suggested that pollen micromorphological characteristics have a great importance in the taxonomy of sect. *Onobrychoidei*. A cytotaxonomic study of some *Onobrychis* (Fabaceae) species and populations in Iran that determined the karyology of 20 taxa (45 populations) of the genus *Onobrychis* from different geographic locations (Hezaji et al., 2010).

Based on our bibliographical observations the anatomical characteristics of *Onobrychis* were not studied well enough. Karamian et al. (2012) were investigated some quantitative and qualitative characteristics of peduncle anatomy of 20 Iranian *Onobrychis* species from sect. *Heliobrychis* Bunge. Nodal anatomy of *Onobrychis grandis* Lipsky and anatomy of *Onobrychis cornuta* (L.) Desv. were determined (Zhogoleva, 1976; 1980). Amirabadizadeh et al. (2015) were studied morphological, anatomical and palynological characteristics of 8 perennial *Onobrychis* taxa growing in Northeast Iran. However, there is no study on the leaf and peduncle anatomy of *Onobrychis* taxa in Turkey. According to Red Data Book of Turkish Plants, by considering IUCN criterions, treat category of *O. albiflora* Hub.-Mor. is critically endangered (CR); *O. quadrijuga* Hedge et Hub.-Mor. is endangered (EN); *O. argyrea* Boiss. subsp. *argyrea* and *O. tournefortii* (Willd.) Desv. have the same threat category which is lower risk/conservation dependent (LR/cd) (Ekim et al., 2000). Within these endemic taxa, *O. quadrijuga* and *O. albiflora* are also known only type locality from Sivas province whereas; *O. argyrea* subsp. *argyrea* and *O. tournefortii* are grown in other some cities especially Inner Anatolia of Turkey (Hedge, 1970; Davis et al., 1988). The root and stem anatomy of these endemic *Onobrychis* taxa were studied by Tekin and Yilmaz (2015).

The aim of this study is the determination of the leaf and peduncle anatomical characteristics of Turkish endemic taxa, *O. quadrijuga*, *O. argyrea* subsp. *argyrea*, *O. tournefortii*, *O. albiflora* for the first time and its usage of contribution to the genus taxonomy.

2. Materials and methods

The specimens of four *Onobrychis* taxa were gathered from natural habitats in Sivas province (Turkey). Localities of investigated specimens of *Onobrychis* taxa were given in Table 1. Some specimens were prepared as herbarium material and were stored in the Herbarium of Cumhuriyet University, Faculty of Science (CUFH). Some selected specimens were fixed and conserved in 70% ethyl alcohol for later anatomical studies. The hand sections were taken by razor blade, from leaves and peduncle of each taxa. For double staining, Alcian blue (Sigma) and Safranin O (Sigma) were used in ratio 3/2 as a dye (Yilmaz and Tekin, 2013). The hand sections of each taxa were waited about five minutes in dye mixture. While, Alcian blue were stained the pectic components of the sections to blue, Safranin were stained the lignin components to red of each sections and, thus good contrast was obtained. The permanent preparates were prepared from selected good stained sections by using glycerin-gelatine (Jensen, 1962). The measurements were made by using Olympus BX22 optical microscopy. For microphotographs, Olympus BX51 light microscopy and fitted Olympus DP70 camera were used. Stomatal index was calculated according to study of Meidner and Mansfield (1968).

Table 1. The collection data of the studied *Onobrychis* taxa

Taxon	Locality	Date	Collector and collector number
<i>O. quadrijuga</i>	B6 Sivas: Kangal-Gürün road 8. km, 1530 m, N 39° 07' 52.2" E 37° 14' 33.4"	21.06.2012	M. Tekin, 1250
		30.06.2012	
<i>O. argyrea</i> subsp. <i>argyrea</i>	B6 Sivas: Ulaş, Ziyarettepe, 1406 m, N 39° 33' 08.9" E 37° 01' 12.1"	30.06.2012	M. Tekin, 1287
<i>O. tournefortii</i>	B6 Sivas: Sivas to Hafik, Emre village, 1317 m, N 39° 49' 37.5" E 37° 17' 05.1"	08.07.2012	M. Tekin, 1290
<i>O. albiflora</i>	B6 Sivas: Sincan to Kangal, 5. km, 1220 m, N 39° 28' 01.9" E 37° 50' 34.5"	08.07.2012	M. Tekin, 1291

3. Results

3.1. Leaf anatomy

The leaves are surrounded by single layered epidermis on the upper and lower surfaces in all examined taxa. Upper epidermis consists of rectangular oval, square or occasionally irregular shaped cells in *O. quadrijuga*; square, rectangular, occasionally oval or irregular shaped cells in *O. argyrea* subsp. *argyrea*; oval, rectangular occasionally irregular shaped cells in *O. tournefortii*; depressed rectangular or occasionally irregular shaped cells in *O. albiflora*. Lower epidermis consists of rectangular oval, occasionally square shaped cells in *O. quadrijuga*; square, rectangular, occasionally oval shaped cells in *O. argyrea* subsp. *argyrea*; oval, rectangular oval, occasionally square shaped cells in *O. tournefortii*; depressed rectangular oval shaped cells in *O. albiflora*. In both epidermises, there are eglandular unbranched trichomes in all examined taxa except for *O. albiflora* (Figure 1). The leaves are amphistomatic, the stomata are anomocytic type and the mesophyll is isobilateral in all examined taxa (Figure 2). For all examined taxa, stomatal index was calculated and stomata dimensions are given in Table 2.

Underneath of upper epidermis, there are palisade parenchyma cells. Palisade parenchyma is 6–7 layered and cells are cylindrical or rectangular oval shaped in *O. quadrijuga*; 6–8 layered and cells are usually cylindrical, occasionally rectangular oval or irregular shaped in *O. argyrea* subsp. *argyrea*; 7–9 layered and cells are cylindrical or rectangular occasionally oval or irregular shaped in *O. tournefortii*; 5–6 layered and cells are cylindrical or irregular shaped in *O. albiflora*. Under upper epidermis, between palisade parenchyma cells there are voluminous cylindrical shaped secretory cells in all examined taxa. Spongy parenchyma cells are cylindrical, oval or circular shaped in all taxa. Spongy parenchyma consists of 2–3 cell layers in *O. quadrijuga*, 3–4 cell layer in *O. argyrea* subsp. *argyrea*, 2–3 cell layers in *O. tournefortii* and 4–5 cell layered in *O. albiflora*. Under the lower epidermis, there are small secretory cells in all examined taxa. These secretory cells are circular or oval shaped in *O. quadrijuga*; circular, oval or occasionally irregular shaped in *O. argyrea* subsp. *argyrea*; circular, oval or occasionally irregular shaped in *O. tournefortii* and circular or oval shaped in *O. albiflora* (Figure 1; Table 3).

Table 2. The stomatal index and stomata measurements of the leaf adaxial (Ad) and abaxial (Ab) epidermis of examined *Onobrychis* taxa (min: minimum; max: maximum)

		<i>O. quadrijuga</i>	<i>O. argyrea</i> subsp. <i>argyrea</i>	<i>O.</i> <i>tournefortii</i>	<i>O.</i> <i>albiflora</i>
		mean	mean	mean	mean
Number of stomata (1 mm ²)	Ad	352	238	185	348
	Ab	316	288	228	284
Number of epidermis cells (1 mm ²)	Ad	2602	1742	948	1834
	Ab	2648	1916	954	1682
Stomatal index	Ad	11.916	12.020	16.328	15.948
	Ab	10.661	13.067	19.289	14.445
		min–max	min–max	min–max	min–max
Stoma length (µm)	Ad	16 – 22	23 – 28	25 – 30	17 – 27
	Ab	19 – 22	22 – 29	26 – 31	22 – 30
Stoma width (µm)	Ad	15 – 19	19 – 23	17 – 22	17 – 20
	Ab	16 – 19	19 – 25	17 – 20	18 – 22

3.2. Peduncle anatomy

When transverse sections of peduncle were investigated, it is determined that the shape of peduncle is hexagonal in *O. quadrijuga* and in *O. argyrea* subsp. *argyrea*, ±circular in *O. tournefortii* and circular in *O. albiflora*. The epidermis consists of rectangular cells in *O. quadrijuga*, square, rectangular or rectangular oval cells in *O. argyrea* subsp. *argyrea*, *O. tournefortii* and very depressed rectangular cells in *O. albiflora*. There are unicellular unbranched eglandular hairs on epidermis in all taxa except for *O. albiflora*. Hair surface is densely papillose in *O. quadrijuga*, papillose in *O. argyrea* subsp. *argyrea* and smooth or loosely papillose in *O. tournefortii*. Beneath the epidermis, there is continuous single cell layered collenchyma. The collenchyma is discontinuous and multilayered, between epidermis and phloem sclerenchyma in cortex area of all taxa. Collenchyma is 5–7 cell layered in *O. quadrijuga*, 6–9 cell layered in *O. argyrea* subsp. *argyrea*, 6–7 cell layered in *O. tournefortii* and 6–8 cell layered in *O. albiflora*. Cortex is made of parenchyma cells in all examined taxa and is 4–5 cell layered in *O. quadrijuga*, 5–8 cell layered in *O. argyrea* subsp. *argyrea* 6–8 cell layered in *O. tournefortii* and 7–9 cell layered in *O. albiflora*. There are secretory cells in cortex in all taxa except for *O. quadrijuga*. Assimilation cells of cortex are oval or circular and 3–6 layered in *O. quadrijuga*, 4–9 layered in *O. albiflora*; generally cylindrical, occasionally oval or circular and 5–9 layered in *O. argyrea* subsp. *argyrea*; cylindrical or circular and 4–9 layered in *O. tournefortii*.

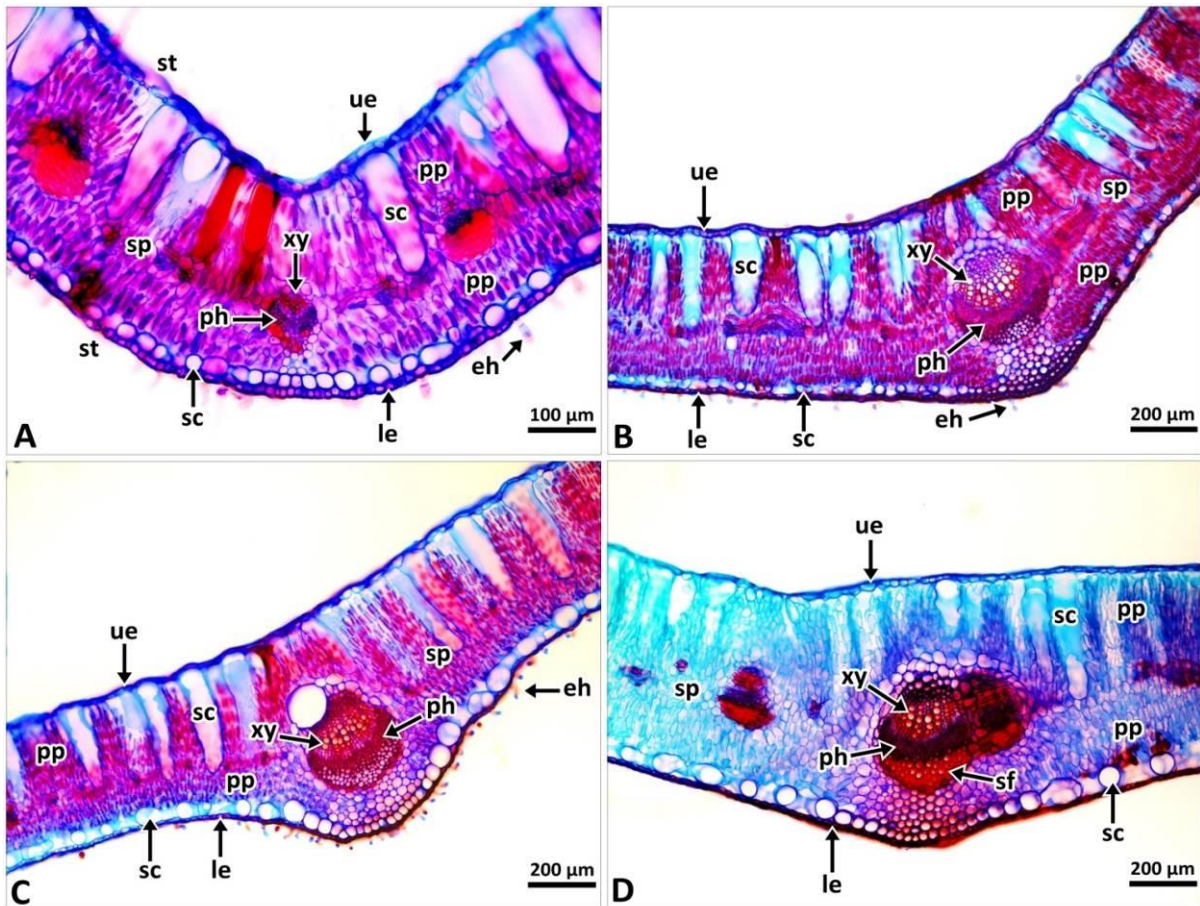


Figure 1. Microphotographs of leaf transverse sections of examined *Onobrychis* taxa: **A.** *O. quadrijuga*; **B.** *O. argyrea* subsp. *argyrea*; **C.** *O. tournefortii*; **D.** *O. albiflora* (cl: collenchyma; eh: eglandular hair; le: lower epidermis; ph: phloem; phs: phloem sclerenchyma; pp: palisade parenchyma; sc: secretory cell; sf: sclerenchymatic fibers; st: stoma; ue: upper epidermis; xy: xylem).

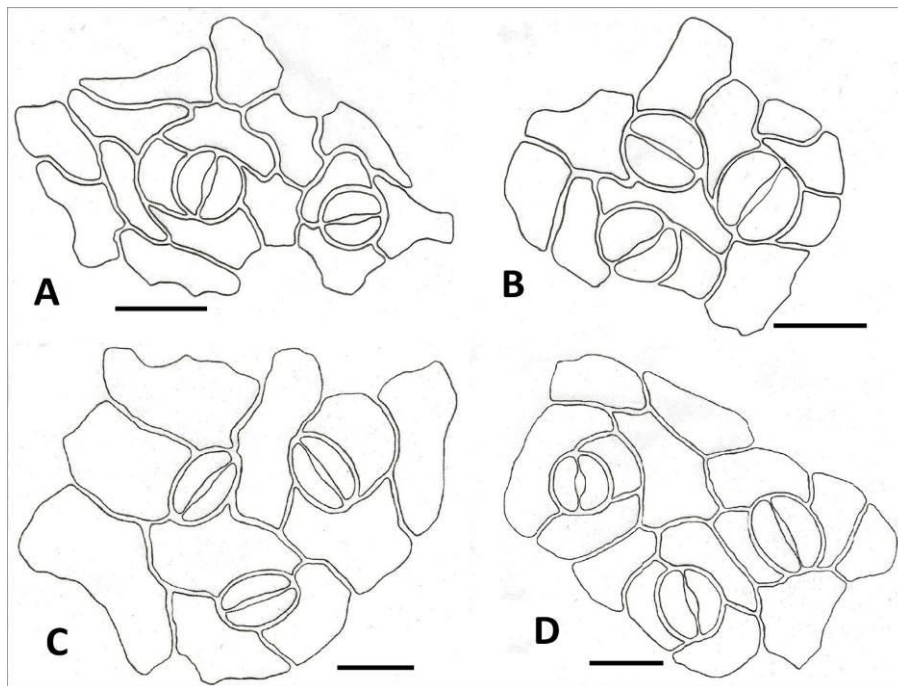


Figure 2. The illustration of epidermal cells and stomata from leaf lower surface-section of examined *Onobrychis* taxa: **A.** *O. quadrijuga*; **B.** *O. argyrea* subsp. *argyrea*; **C.** *O. tournefortii*; **D.** *O. albiflora* (Scale bars: 25 µm).

There are phloem sclerenchyma fibers which is 3–5 layered in *O. quadrijuga*, 9–12 layered in *O. argyrea* subsp. *argyrea*, 10–15 layered in *O. tournefortii* and 7–9 layered in *O. albiflora*. Cambium is distinguishable in all taxa and consists of 2–3 layered, depressed rectangular or square shaped cells in *O. quadrijuga*, 1–2 layered, very depressed rectangular shaped cells in *O. argyrea* subsp. *argyrea*, 2–4 layered, generally depressed rectangular, occasionally irregular shaped cells in *O. tournefortii*, 1–3 layered, rectangular, depressed rectangular or occasionally square shaped cells in *O. tournefortii* and 2–3 layered, occasionally depressed rectangular shaped in *O. albiflora*. Pith cells are parenchymatous and oval or circular shaped in all examined taxa (Table 3; Figure 3).

Table 3. The measurements of length (L) and width (W) of cells (μm) and cell layer number of some tissues in leaf and peduncle of examined *Onobrychis* taxa (min: minimum, max: maximum).

		<i>O. quadrijuga</i>		<i>O. argyrea</i> subsp. <i>argyrea</i>		<i>O. tournefortii</i>		<i>O. albiflora</i>	
		min	max	min	max	min	max	min	max
LEAF									
Upper epidermis cells	L	10	41	17	52	17	51	10	43
	W	10	18	7	20	6	25	7	26
Palisade parenchyma cells	L	25	58	20	61	17	54	5	63
	W	10	16	10	23	12	26	5	30
Palisade parenchyma cell layers		6	7	6	8	7	9	5	6
Spongy parenchyma cell layers		2	3	3	4	2	3	4	5
Mesophyll thickness		250	320	420	510	350	450	510	600
Lower epidermis cell	L	7	38	5	36	5	36	8	43
	W	5	13	4	13	4	18	9	16
PEDUNCLE									
Epidermis cells	L	7	36	3	20	4	28	7	36
	W	6	15	3	8	5	15	3	13
Cortex cells	L	10	30	7	30	7	33	10	30
	W	7		7	20	6	25	7	20
Cortex cell layers		3	6	5	9	4	8	4	9
Collenchyma cell layers		5	7	6	9	6	7	6	8
Phloem sclerenchyma cell layers		3	5	9	12	10	15	7	9
Cambium cells	L	5	13	5	11	5	15	4	16
	W	2	7	2	5	1	5	1	5
Cambium cell layer		2	3	1	2	2	4	2	3
Vascular bundle number		7	8	7	8	13	14	9	9
Pith cells	L	10	48	22	132	20	106	20	154
	W	8	40	22	127	20	102	18	142

4. Conclusions and discussion

In the literature, although there are lots of studies about morphology and anatomy of some endemic plants for Turkey (Yılmaz, 2014; Tekin and Meriç, 2013) there is no enough study on the leaf and peduncle anatomy of *Onobrychis* taxa.

In our previous study, anatomical properties of roots and stems of *O. quadrijuga*, *O. argyrea* subsp. *argyrea*, *O. tournefortii* and *O. albiflora* were determined (Tekin and Yılmaz, 2015). When root sections were compared within each other some differences were noted: there was not sclerenchymatous tissue in periderm in the examined species, except *O. quadrijuga*; *O. quadrijuga* had the largest secondary phloem among the *Onobrychis* taxa in the study; the amount of sclerenchymatic fibers in *O. albiflora* was more than in the other species. When stem sections were compared with each other, the differences determined were as follows: there were single-celled eglandular hairs on the epidermis in all examined species except *O. albiflora*; phloem sclerenchyma was larger in *O. tournefortii* and *O. albiflora* than in the other species; in pith region, as distinct from other three taxa, some parenchymatous cells turned into stone cells in *O. quadrijuga*.

In examined taxa, in terms of morphological characteristics, *O. quadrijuga* is a little more different than other taxa. In terms of morphology of leaf anatomy, *O. quadrijuga* has significant differences from the other examined taxa. While mesophyll thickness varies between 350–600 μm in all other taxa, *O. quadrijuga* has thinner mesophyll within the limits of 250–320 μm . Additionally, in terms of stomata features, *O. quadrijuga* is very different from the other

taxa. Stomata length and stomata width is the smallest in *O. quadrijuga* on the both surfaces of the leaf. Stomata number and epidermis cell number in a square millimeters area are significantly greater than the other species (Table 2). There is no information found that stoma index study on taxa of *Onobrychis* genus in the literature. Amirabadizadeh et. al. (2015) were studied leaf anatomy of eight taxa and grouped in two according to the existence of continuous or discontinuous vascular leaf bundle. According to their study, *O. arnacantha*, Bunge. ex Boiss. *O. amoena* Pop & Vved subsp. *amoena* and *O. chorassanica* Bunge has continuous vascular leaf bundle whereas, *O. cornuta* (L.) Desv., *O. verae* Sirj var. *varae*, *O. verae* var. *rechingeri* Sirj, *O. ptycophylla* Sirj & Rech. f. and *O. amoena* subsp. *meshhedensis* Sirj & Rech. f. has discontinuous vascular leaf bundle. In present study, we were determined that, all examined taxa have discontinuous vascular leaf bundle. Midrib is almost circular shaped in all taxa. In *O. quadrijuga* midrib is small while in other three taxa it is large.

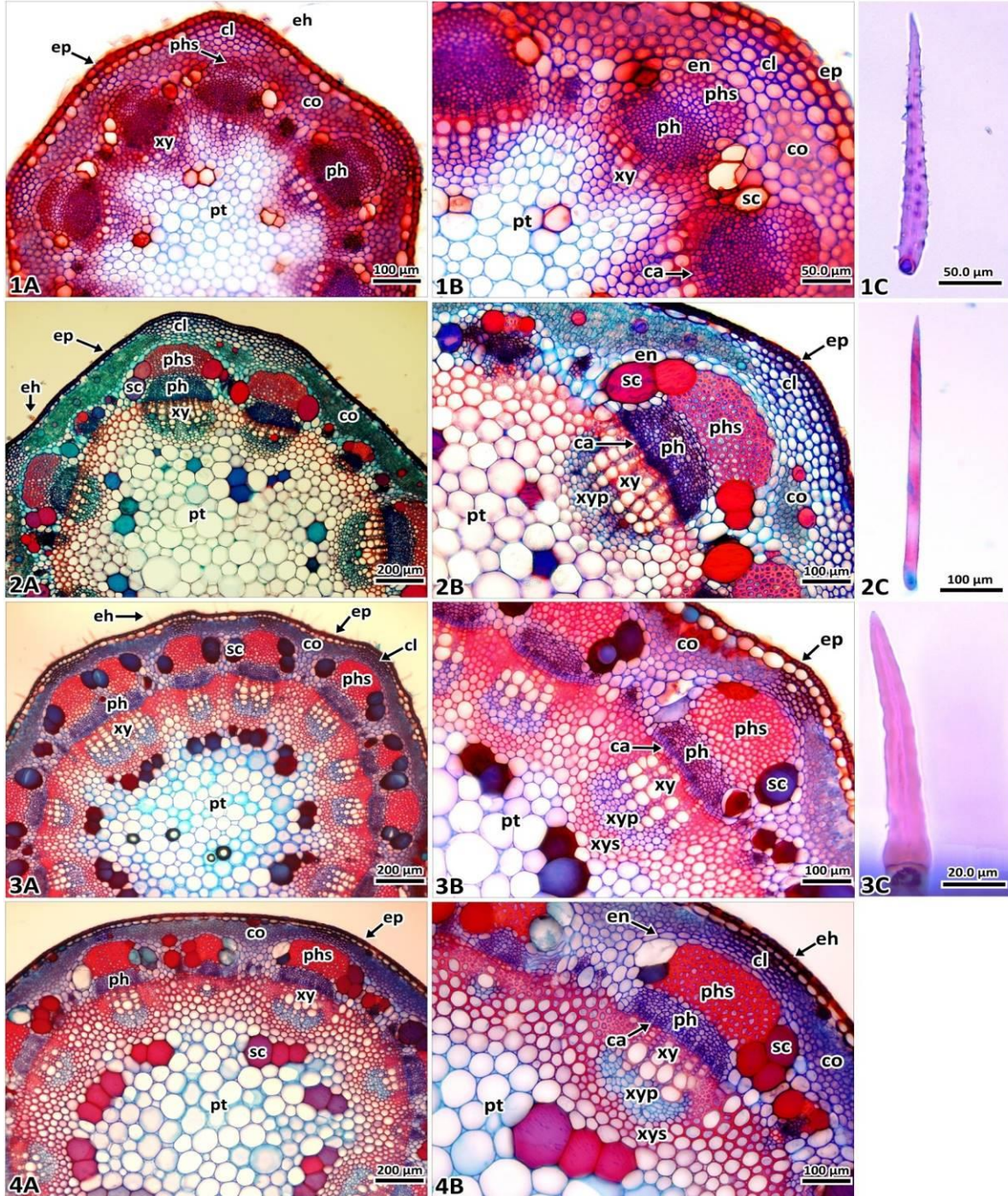


Figure 3. Microphotographs of the peduncle transverse sections of examined *Onobrychis* taxa: **1.** *O. quadrijuga*; **2.** *O. argyrea* subsp. *argyrea*; **3.** *O. tournefortii*; **4.** *O. albiflora*. **A.** general view; **B.** enlargement showing of internal structure; **C.** hair (ca: cambium; cl: collenchyma; co: cortex parenchyma; e: epidermis; eh: eglandular hair; en: endodermis; pt: pith; ph: phloem; phs: phloem sclerenchyma; sc: secretory cell; xy: xylem; xyp: xylem parenchyma; xys: xylem sclerenchyma).

There was a study about peduncle anatomy of four groups of *Onobrychis* taxa in Iran. In that study, some cavities were determined in cortex parenchyma and also around pericyclic fibers (Karamian et al., 2012). In our study, cavities were observed too in transverse section of the leaf and peduncle (Figure 1, 3). In the study of Karamian et al. (2012) on peduncle anatomy of *Onobrychis* taxa in Iran; *O. szovitsii* Boiss., *O. aurea* Ranjbar, Amirabadizadeh & Ghahremani, *O. atropatana* Boiss., *O. andalunica* Bornm., *O. scrobiculata* Boiss., *O. buhseana* Bunge ex Boiss., *O. heterophylla* C.A. Mey., *O. gaubae* Bornm., and *O. psoraleifolia* Boiss. var. *psoraleifolia* were represented a circular shape in peduncle outlines and covered with hairs which were loosely papillose or rarely smooth on their surfaces; *O. lunata* Boiss., *O. melanotricha* Boiss. var. *melanotricha*, *O. kermanensis* (Širj. & Rech. f.) Rech. f., and *O. sojakii* Rech. f. showed elliptic shape in peduncle outlines and covered with hairs which were densely papillose on their surfaces; *O. oxyptera* Boiss. and *O. plantago* Bornm. showed pentagonal shape in peduncle outlines covered with hairs which were densely papillose on their surfaces; *O. haussknechtii* Boiss. and *O. gypsicola* Rech. f. showed pentagonal or hexagonal shapes in peduncle outlines and covered with hairs which were loosely papillose on their surfaces (Karamian et al., 2012).

In our study, it was found that the shape of peduncle was hexagonal in *O. quadrijuga*, ±circular in *O. tournefortii* and circular in *O. albiflora*. Hair surface was densely papillose in *O. quadrijuga* and papillose in *O. argyrea* subsp. *argyrea*, smooth or loosely papillose in *O. tournefortii*. In peduncle anatomy, there are some important differences for *O. quadrijuga* from the other taxa. The number of phloem sclerenchyma cell layer varies in range 3–5 in *O. quadrijuga*, for other taxa it changes in range 7–15. Also, in terms of peduncle anatomy *O. quadrijuga* has the smallest pith cells with dimensions varying 10–48 × 8–40 μm (Table 3; Figure 3).

As a conclusion, anatomical characteristics of leaf and peduncle of Turkish endemics, *O. quadrijuga*, *O. argyrea* subsp. *argyrea*, *O. tournefortii*, and *O. albiflora*, were determined in details for the first time. In addition, some remarkable anatomical differences such as peduncle transverse section shape, presence and density of papillae on peduncle hair surface, thickness of leaf, stomata dimensions and stomatal index were studied of examined taxa.

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(Received for publication 23 March 2016; The date of publication 15 August 2016)