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Comparative Root and Stem Anatomy of Four Rare *Onobrychis* Mill. (Fabaceae) Taxa Endemic in Turkey

Mehmet TEKİN[1]*, Gülden YILMAZ[2]

[1]*Cumhuriyet University, Faculty of Pharmacy, Department of Pharmaceutical Botany, 58140, Sivas, Turkey; mtekin2280@gmail.com (*corresponding author)*
[2]*Trakya University, Faculty of Science, Department of Biology, Balkan Campus, 22030, Edirne, Turkey; guldenyilmaz2009@yahoo.com*

Abstract

Four endemic taxa of *Onobrychis* Mill. genus, some of them being classified in the endangered threat category, were investigated for root and stem anatomy. *Onobrychis quadrijuga*, *O. argyrea* subsp. *argyrea*, *O. tournefortii* and *O. albiflora* were studied in regard to specific anatomy for the first time within the hereby study. Anatomical characters as the size and shape of the periderm, cortex, cambium cells in root and epidermis, collenchyma, cortex, cambium and pith cells in stem belonging to these four *Onobrychis* taxa were determined in detail. Based on the roots and stems measurements and analysis, specific anatomical differences between species were revealed.

Keywords: anatomy, endemic, *Onobrychis*, root, stem

Introduction

Fabaceae, represented by 750 genera and more than 18,000 species, is one of the largest dicot families, having species of profound economic importance (ILDIS, 2001). *Onobrychis* Mill., in the family Fabaceae, subfamily Faboideae, tribe *Hedysareae* DC. (Chase and Reveal, 2009) comprises 342 perennial and annual species. This genus is distributed throughout Northern temperate regions, Eastern Mediterranean region and Southwest Asia (Hedge, 1970; Hejazi *et al.,* 2010). The diversity and concentration of these species are high in the region around Anatolia–Iran–Caucasia. There are 23 species of *Onobrychis* listed in The Flora Europaea (Tutin *et al*., 2001) , whereas the main centre of diversity extended from Central Asia to Iran, were the number of species is increased to 56 species, 27 of which being endemic (ILDIS, 2005). In Turkey, the genera *Onobrychis* is represented by 55 species under five different sections and 28 of them are endemic (Hedge, 1970; Davis *et al.,* 1988; Duman and Vural, 1990; Aktoklu, 2001; Yıldırımlı, 2004).

The species of *Onobrychis* (sainfoin) are economically important plants that are grown in order to produce high-protein fodder for animals. As these species can fix atmospheric nitrogen they are important for farm animal nourishment and soil fertility. Also, they have a great potential for pasture and meadow improvement, land conservation and rehabilitation, besides being important genetic sources for breeding (Cavallarin *et al.,* 2005; Parlak and Parlak, 2008). *Onobrychis* play an important role in the soil environment by increasing the nutritive value of drought-resistant pasture (Abou-El-Enain, 2002).

There are several studies about palynology, taxonomy, karyology and cytotaxonomy of some *Onobrychis* taxa in the literature. The pollen morphology was used as the most important microscopic character in the taxonomy of *Onobrychoidei* (Pınar *et al.,* 2009). A cytotaxonomic study of some *Onobrychis* (Fabaceae) species and populations in Iran determined the karyology of 20 taxa (45 populations) of the genus *Onobrychis* from different geographic origins (Hejazi *et al.*, 2010). Ovule ontogenesis and megagametophyte development in *Onobrychis schahuensis* Bornm. (Fabaceae) were also studied (Chehregani and Tanaomi, 2010). Genetic relationships of the genera *Onobrychis, Hedysarum,* and *Sartoria* using seed storage proteins were determined (Arslan and Ertugrul, 2010). The pollen size and morphology of 20 *Onobrychis* taxa from Turkey were studied using both light and scanning electron microscopy (Avcı *et al*., 2013). Seed and germination characteristics of wild *Onobrychis* taxa in Turkey were determined (Avcı and Kaya, 2013). Even so, based on the authors' bibliographical observations, the anatomical characteristics of *Onobrychis* were not studied well enough. There was a study including quantitative and qualitative characters of peduncle anatomy on 20 species of *Onobrychis* Miller sect. *Heliobrychis* Bunge from Iran by light microscopy (Karamian *et al.,* 2012). Nodal anatomy of *Onobrychis grandis* (Fabaceae) and anatomy of *Onobrychis cornuta* (L.) Desv. sprout were determined (Zhogoleva, 1976, 1980).

However, there are no studies about root and stem anatomy of *Onobrychis* taxa in Turkey. Therefore, the aim of the current study was to determine the anatomy of root and stem of endemic taxa *Onobrychis quadrijuga, O. argyrea* subsp. *argyrea*, *O. tournefortii* and *O. albiflora*. Endemic species are both globally and locally important.

Conserving areas of high endemism might give a chance to further study the ecological, life history and physiological factors influencing endemics, while simultaneously protecting areas of

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Table 1. Natural habitat of investigated specimens of *Onobrychis* taxa

Taxon	Locality, Collector, Collector number, Date
O. quadrijuga	B6 Sivas: Kangal-Gürün , 8 km; 1,530 m, N 39° 07' 52,2" E 37° 14' 33,4" M. Tekin 1250, 21.06.2012; ibid. M. Tekin 1284, 30.06.2012
O. argyrea subsp. *argyrea*	B6 Sivas: Ulaş, Ziyarettepe; 1,406 m, N 39° 33' 08,9" E 37° 01' 12,1" M. Tekin 1287, 30.06.2012
O. tournefortii	B6 Sivas: Sivas to Hafik, Emre village; 1,317 m, N 39° 49' 37,5" E 37° 17' 05,1" M. Tekin 1290, 08.07.2012
O. albiflora	B6 Sivas: Sincan to Kangal, 5 km; 1,220 m, N 39° 28' 01,9" E 37° 50' 34,5" M. Tekin 1291, 08.07.2012

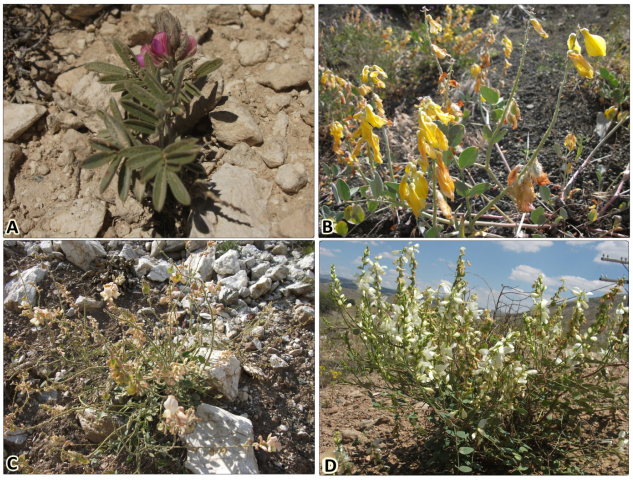

Fig. 1. General view of examined *Onobrychis* taxa in natural habitat: A) *O. quadrijuga*; B) *O. argyrea* subsp. *argyrea*; C) *O. tournefortii*; D) *O. albiflora*

variable habitat. Anatolia (Turkey) with its adjacent lowland environment can be considered in reference to the patterns and processes that characterize plant diversity, evolution and distribution (Fahn, 1990; Hassan and Heneidak, 2006; Mehrabian *et al.,* 2007). Also, the morphological and anatomical studies on taxa are very important because some of them are threatened or under extinction. According to Red Data Book of Turkish Plants, treat category of *O. albiflora* is critically endangered (CR), *O. quadrijuga* is endangered (EN), while *O. argyrea* subsp. *argyrea* and *O. tournefortii* have the same threat category which is lower risk/conservation dependent (LR/cd) (Ekim *et al.,* 2000).

Materials and Methods

The specimens used in this study were collected from natural habitats in Sivas province (Turkey). Localities and photos in natural habitat of investigated specimens of *Onobrychis* taxa were given in Table 1 and Fig. 1 respectively. Some specimens were prepared as herbarium materials and deposited in Cumhuriyet University Herbarium (CUFH). For anatomical studies, the root and stem 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, and with Safranine O (Sigma) for lignin in the ratio of 3/2 (AB/SO). They were left in dye about 5 minutes for staining. The stained sections were mounted in glycerin-gelatine to obtain permanent preparations (Jensen, 1962). Sections were examined using an Olympus light microscope BX51. Photographs were captured using a digital camera (Olympus DP70) connected with the light microscope.

Results and Discussion

In this study, four *Onobrychis* taxa, endemic in Turkey, were studied for anatomical properties. General views with habitats of all examined taxa are given in Fig. 1.

Root and stem anatomy of *Onobrychis quadrijuga*, *O. argyrea* subsp. *argyrea*, *O. tournefortii* and *O. albiflora* were determined for the first time within this study. Anatomical characters, which were selected and quantified here, included the size and shape of the periderm, cortex, cambium cells in root and shape of epidermal cells, the number of collenchyma layers, the number of parenchyma layers in cortex, the shape of parenchymatous cells in pith and the secretory material in the cavities of stem in cross sections.

The order of listing the species in text, tables and photos was determined according to their evolutionary stage of development.

Root anatomy

Periderm cells were 8-14 layered on the outer surface of the root in *O. quadrijuga*, 3-8 layered in *O. argyrea* subsp. *argyrea*, 4-7 layered in *O. tournefortii* and 7-12 layered in *O. albiflora*. There were individually or as a bundle of sclerenchymatic fibre cells in periderm and among periderm with cortex in *O. quadrijuga*, while there was not sclerenchymatous tissue in periderm in all the others species examined hereby (Fig. 2; Table 2).

Parenchyma cells were generally irregular, occasionally rectangular oval shaped in *O. quadrijuga,* generally irregular or occasionally depressed rectangular and depressed oval shaped in *O. argyrea* subsp. *argyrea* and *O. tournefortii*, irregular shaped in *O. albiflora* (Fig. 2; Table 2). There were individually or as a bundle of sclerenchymatic fibre cells in cortex, secondary phloem and secondary xylem in all examined species.

O. quadrijuga had the largest secondary phloem among the *Onobrychis* species studied in this study, while the amount of sclerenchymatic fibre cells in *O. albiflora* was more than in the rest of the examined species (Fig. 2; Table 2).

Cambium was generally distinguishable, but occasionally indistinguishable in *O. quadrijuga*, distinguishable in *O. argyrea* subsp. *argyrea*, in *O. tournefortii* and in *O. albiflora*. Secondary pith rays consisted of 2-4 layered parenchymatous cells, which were heterogenous in *O. quadrijuga.* In secondary xylem, diameter of trachea was 10-63 μm in *O. quadrijuga*, 15-100 μm in *O. argyrea* subsp. *argyrea,* 17-95 μm in *O. tournefortii*, 15-88 μm in *O. albiflora*. In the center of the root there were many sclerenchymatic fibre cells in *O. albiflora*, distinctive than in the other taxa examined (Fig. 2; Table 2).

Stem anatomy

When cross sections of stems were examined it was noted that the epidermis consisted of square, rectangular or rectangular oval cells in *O. quadrijuga*, square or rectangular, occasionally rectangular oval cells in *O. argyrea* subsp. *argyrea*, square or rectangular cells in *O. tournefortii,* generally depressed

Table 2. The length and width measurements of cells (µm) and cell layer number in root and stem of examined *Onobrychis* taxa

			O. quadrijuga		*O. argyrea* subsp. *argyrea*		*O. tournefortii*		*O. albiflora*	
			min	max	min	max	min	max	min	max
Root	Periderm cell	Length	17	43	17	45	12	93	12	28
		Width	3	10	4	13	5	18	5	15
	Periderm cell layer		8	14	3	8	4	7	7	12
	Cortex cell	Length	10	75	17	73	17	140	17	73
		Width	7	38	10	43	7	108	12	40
	Cortex cell layer		6	9	7	10	10	18	8	13
	Cambium cell	Length	10	25	10	15	10	23	12	25
		Width	2	6	3	6	2	8	2	7
	Cambium cell layer		2	3	2	6	3	7	2	6
	Seconder pith ray layer		2	4	3	7	2	8	3	6
	Diameter of trachea		10	63	15	100	17	95	15	88
Stem	Epidermis cell	Length	5	15	7	20	4	25	7	43
		Width	5	13	4	10	4	20	5	10
	Cortex cell	Length	12	50	10	40	10	50	10	53
		Width	5	25	7	30	7	43	7	28
	Cortex cell layer		6	10	8	11	8	11	8	12
	Cambium cell	Length	7	15	10	23	10	18	12	18
		Width	2	5	2	8	2	8	3	7
	Cambium cell layer		3	5	1	3	2	4	1	3
	Pith cell	Length	50	160	35	200	20	120	17	125
		Width	40	130	28	175	20	105	15	115

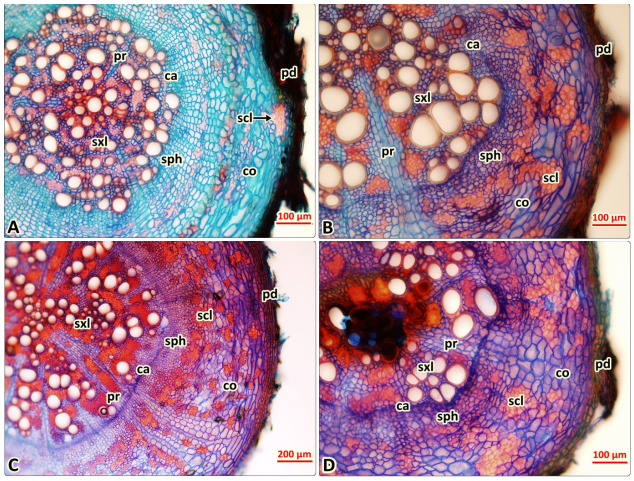

Fig. 2. Cross sections of roots of examined *Onobrychis* taxa: A) *O. quadrijuga*; B) *O. argyrea* subsp. *argyrea*; C) *O. tournefortii*; D) *O. albiflora*; ca: cambium; co: cortex, pd: periderma; pr: pith ray; scl: sclerenchyma; sph: secondary phloem; sxl: secondary xylem

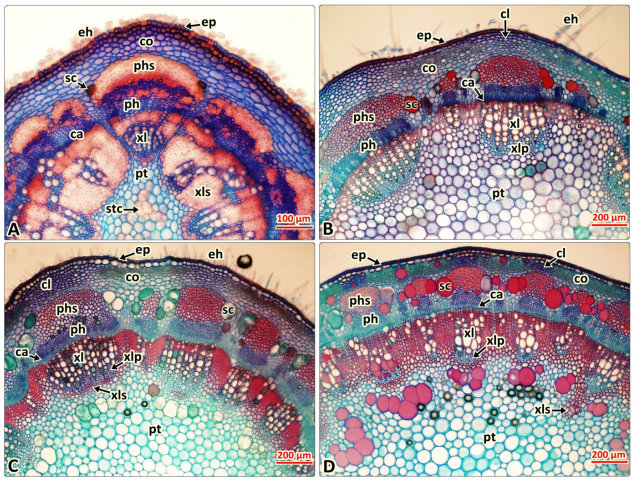

Fig. 3. Cross sections of stems of examined *Onobrychis* taxa: A) *O. quadrijuga*; B) *O. argyrea* subsp. *argyrea*; C) *O. tournefortii*; D) *O. albiflora*; ep: epiderma; eh: eglandular hair; ca: cambium; cl: collenchyma; co: cortex parenchyma; pt: pith parencyma; ph: phloem; phs: phloem sclerenchyma; sc: secretory cell; stc: stone cell; xl: xylem; xlp: xylem parenchyma; xls: xylem sclerenchyma

rectangular or occasionally square cells in *O. albiflora*. There were single celled eglandular hairs on the epidermis in all examined species except *O. albiflora*. Underneath the epidermis there was collenchyma with single layered cells, while collenchyma in cortex was multilayered, among epidermis and phloem schlerenchyma in all of the taxa. In addition to this, phloem sclerenchyma was larger in *O. tournefortii* and *O. albiflora* than in the other species (Fig. 3; Table 2).

Cortex consisted of parenchyma cells in all of the species. Cells of cortex were oval, rectangular oval and occasionally irregular shaped in *O. quadrijuga,* oval and circular shaped in all of the other species in the study. There were sclerenchymatic fibre bundle in floem and more intensely in xylem in *O. quadrijuga* (Fig. 3; Table 2).

Cambium was distinguishable in *O. quadrijuga*. There were pith rays which consisted of 2-5 layers, between pith region and cortex in *O. quadrijuga*. There were secretory storage cells, larger than the other cells in cortex, especially close to the phloem sclerenchyma and in pith region in all of the examined species except *O. quadrijuga*. Pith cells were parenchymatous, oval or circular shaped in all species. In pith region, as a distinct feature among studied taxa, some parenchymatous cells turned into stone cells in *O. quadrijuga*.

There is no anatomical study on *Onobrychis* taxa in Turkey. There was a study about morphological, anatomical and palynological characters for *O. cornuta* (L.) Desv., *O. arnacantha* Boiss., *O. verae* Sirj. var. *verae*, *O. verae* Sirj. var.

rechingeri Sirj., *O. ptycophylla* (Del.) DC.*, O. amoena* subsp. *amoena* Popov & Vved, *O. amoena* subsp. *meshhedensis* Sirj. & Rech.f.*, O. chorassanica* Bunge ex Boiss. growing in Northeast Iran (Amirabadizadeh *et al.,* 2014). Morphological study, shape, size and color of vegetative organs and legumes were assessed, while for the anatomical study, cross sections of mature fresh or dried stems, leaflets and petioles were prepared and stained (Amirabadizadeh *et al.,* 2014). Schweingruber *et al*. (2011) reported that in the *Onobrychis* stems, vessels and fibers were arranged in tangential layers and were surrounded by paratracheal and marginal parenchyma. The large rays were not lignified.

The distribution of axial parenchyma cells can be apotracheal. In te current study cortex was made of parenchyma cells in all species as 6-10 layered in *O. quadrijuga,* 8-11 layered in *O. argyrea* subsp. *argyrea* and in *O. tourneforti* and 8-12 layered in *O. albiflora.* The results are similar with the findings of the stem anatomy study of some *Onobrychis* species belonging Northeast Iran (Amirabadizadeh *et al.,* 2014): 9-12 layered in *O. cornuta*, 4-9 layered in *O. arnacantha,* 5-8 layered in *O. verae* var. *verae,* 5-7 layered in *O. verae* var. *rechingeri,* 4-9 layered in *O. ptycophylla,* 3-7 layered in *O. amoena* subsp. *amoena,* 5-9 layered in *O. amoena* subsp. *meshhedensis,* 6-10 layered in *O. chorassanica*.

Other researchers studied the peduncle anatomy of four groups of *Onobrychis* taxa in Iran (Karamian *et al.,* 2012). It was noted the existence of some cavities within cortex parenchyma and also around pericyclic fibres. In the current study, cavities were also observed in cross sections of stem, while no cavity was seen in root sections. These cavities were filled with secretory material from the secretory cells (sc), stained red with safranine dye (Fig. 3).

Conclusions

In this study, anatomical properties of roots and stems of *O. quadrijuga, O. argyrea* subsp. *argyrea*, *O. tournefortii* and *O. albiflora*, some of them being classified in the endangered threat category, were determined in detail for the first time. 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* species in the study; the amount of sclerenchymatic fibre cells 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*.

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