Morphological and anatomical studies of *Tulipa orphanidea* (*Liliaceae*)

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Abstract. In this study, the morphological and anatomical features of *Tulipa orphanidea* (*Liliaceae*) were investigated. The specimens were collected from Edirne-Üyüklütatar village in April-May of 2009. A detailed description of the plant and of the features of its vegetative organs is provided, and the morphological features of the species are also compared with those reported in the *Flora of Turkey*. For anatomical investigation, sections of the root, stem, and leaf were cut manually with a razor blade. The adventive root, stem, and leaf anatomy of the species display the common properties of monocotyledons. The stem contains monolayer collenchyma close to the epidermis. The leaves are amphistomatic, and the mesophyll is unifacial.

Key words: Anatomy, Liliaceae, morphology, Tulipa orphanidae, Turkey

Introduction

Liliaceae includes mostly perennial herbs with starchy rhizomes, corms, or bulbs comprising about 250 genera and 3500 species (Satil & Akan 2006). They are naturally distributed in the tropical and temperate regions. The family is represented by 36 genera and 461 species in the Flora of Turkey (Selvi & al. 2008). Tulipa L. is a large genus with 113 species (Shuka & al. 2010), including some which are perennials bulbs. They grow naturally in South Europe, North Africa, the Middle East, and Central Asia and have colorful and attractive cup-shaped flowers. The genus is represented by 18 taxa in the Flora of Turkey, and two of these taxa are endemic (Marais 1984; Özhatay 2000; Terzioğlu & Coşkunçelebi 2002; Shuka & al. 2010). T. orphanidea Boiss. ex Heldr. grows in European Turkey and in other western regions of Turkey. The species is

generally distributed in the Southeastern Balkans and Crete (Marais 1984).

There are few morphological and anatomical studies on the different species of the genus Tulipa (Ocak, & al. 2004; Satıl & Akan 2006; Coşkunçelebi & al. 2008). Tulipa armena Boiss. var. lycica (Baker) Marais, an endemic species, was examined morphologically and anatomically by Ocak & al. (2004). T. aleppensis Boiss. ex Regel, which is rare, was anatomically investigated by Satıl & Akan (2006). T. gumusanica Terzioğlu and T. armena Boiss. var. armena were anatomically compared by Coşkunçelebi & al. (2008); it was found that these taxa have some important differences with regard to anatomical features. T. orphanidea has been investigated karyologically by Başak & Özhatay (1997), but there are no morphological and anatomical studies of the species. This paper aims to investigate the morphological and anatomical properties of T. orphanidea.

Material and methods

Plant samples were collected from Edirne-Üyüklütatar pasture in April 2009. The taxonomic description of the species followed Marais (1984). Some plants were prepared as herbarium materials, and voucher specimens were deposited in the Herbarium of Trakya University, Edirne (EDTU). Others were fixed in 70% ethyl alcohol for anatomical studies. Fresh and herbarium specimens were used to determine the morphological characteristics of the species and the biometric measures of bulbs, stems, leaves, and floral organs. For anatomical studies, the material was stored in 70% alcohol. The transverse sections of root, stem, and leaf, and the surface sections of leaves were investigated anatomically. Cell wall lignification tests were performed by phloroglucinol-HCl reaction. Sections were placed on slide in a drop of 0.1 g phloroglucinol in 10 ml of 95% alcohol and covered with a coverslip. Some of the solution was evaporated and then 25% HCl was diffused at the edge of the coverslip (Johansen 1940). The stained and unstained sections were mounted in glycerin-gelatin to make permanent preparations (Jensen 1962). The slides were investigated with an Olympus BH2 microscope (Tokyo, Japan), and the selected images were photographed with a Progress C12 (Jenoptik) digital camera.

Results

Morphological features

T. orphanidea Boiss. ex Heldr. in Gartenfioia i 1:309, t. 373 (1862).

Syn: *T. bithynica* Griseb. ex Baker, *T. hageri* Heldr., *T. thracica* Davidov, *T. hellespontica* Degenin, *T. hayatii* O. Schwarz, *T. whittallii* A.D. Hall.

Table 1.	Morphological	characters of	f Tulipa	orphanidea.
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	Soykan & Meric		Marais 1984	
	Width	Length	Width	Length
Plant	-	15-32 cm	-	-
Stem	4-5 mm	9–12 cm	-	-
Leaf	0.6–2.7 cm	7.5–18.6 cm	2 cm	20 cm
Bulb	14–16 mm	20–25 mm	-	-
Outer tepal	1.5–2.2 cm	4.5-6 cm	10-18 mm	30-60 mm
Inner tepal	1.2–2 cm	4.2–5.7 cm	12–21 mm	30-60 mm
Filament	-	8–10 mm	-	10–13 mm
Anther	-	24–28 mm	-	7–12 mm
Ovary	-	13–15 mm	-	-
Fruit	10–15 mm	28-31 mm	-	-

The plant is 15–32 cm high (Fig. 1). Bulb ovoid, 14–16×20–25 mm; tunicabrown (Fig. 2). Stem 9–12 cm tall and erect. Leaves 3–4, linear $0.6-2.7 \times 7.5-18.6$ cm, parallel-sided, ranging from almost as long to longer than stem. Outer tepal $1.5-2.2 \times 4.5-6$ cm; inner tepal $1.2-2 \times 4.2-5.7$ cm in length. Filament length 8–10 mm, swollen and hairy at base. Anther 24–28 mm, purple (Fig. 3); pollens dark. Ovary 13–15 mm, yellow-brown; style 1–2 mm. Fruit $10-15 \times 28-31$ mm in size (Fig. 4).



Fig. 1. General view of *Tulipa orphanidea* (bar = 50 mm).



Fig. 2. Bulb of *T. orphanidea* (bar = 10 mm).



Fig. 3. Anther of *T. orphanidea* (bar = 10 mm).



Fig. 4. Capsule of *T. orphanidea* (bar = 10 mm).

Anatomical features

Root

A cross sections of the root showed a monolayer epidermis consisting of ovoid cells at the outer side. There was a single layer of suberized exodermis without intercellular spaces under the epidermis. The 4-5 layered cortex was composed of thin-walled spherical parenchymatous cells (Fig. 5a). Endodermis was located under the cortex. The outer tangential walls were thickened in endodermal cells. This thickening was histochemically stained with Sudan III and reacted positively to phloroglucinol-HCl. Therefore, it has been assumed that it contained suberin and lignin. Casparian bands were distinct in the endodermis. Single-row pericycle was present under the endodermis. The three primary xylem ridges lay opposite to the phloem. The centre of the vascular cylinder was occupied by metaxylem elements (Fig. 5b).

Stem

The transverse sections of the stem of *T. orphanidea* showed (Fig. 6a): epidermis with a thick cuticle (9.5 μ m) composed of single-layered ovoid, or spheroid cells. Cortex was multilayered. It comprised a monolayer collenchyma close to the epidermis and spherical parenchymatous cells with intercellular spaces (Fig. 6b). The stem contained 44–49 vascular bundles of various size in the vascular cylinder. They were embedded in a disorderly manner in the parenchyma of the stem. Vascular bundles comprising the xylem and phloem were collateral in type (Fig. 6c). The pith consisted of parenchymatous cells. The stem had sparse single-celled, nonglandular hairs.

Leaf

There was single-layered epidermis on both sides of the leaf. The upper epidermis had a thicker cuticle than the lower one. Epidermal cells were orbicular in cross section. The mesophyll was unifacial, about 1024 μ m thick. It comprised 10–12 layers of round-shaped parenchyma cells. Vascular bundles had different size and were arranged in one row. The xylem faced the upper surface, while the phloem faced the lower epidermis (Fig. 7). Sclerenchyma fibers were absent in the vascular tissue. The leaf was amphistomatic. The stoma type was anomocytic, and the stoma cells were located on the same level as the other epidermis (Fig. 8a) and 35 for the lower epidermis (Fig. 8b).



Fig. 5. Root anatomy of *Tulipa orphanidea*: **a**, section of root (bar = 100 μ m); **b**, vascular cylinder (reacted to phloroglucinol-HCl; bar = 40 μ m). Legend: **co** – cortex; **en** – endodermis; **ep** – epidermis; **ex** – exodermis; **mx** – metaxylem; **pe** – pericycle; **ph** – phloem; **xy** – protoxylem; **vc** – vascular cylinder.





Fig.7. Transverse section of the leaf of *Tulipa orphanidea* (bar = 100 µm). Legend: **le** – lower epidermis; **ms** – meso-phyll; **ph** – phloem; **sc** – stoma cell; **ue** – upper epidermis; **xy** – xylem.



Fig. 8. Leaf surface sections of *T. orphanidea* (bar = 50 μ m): a, upper epidermis; b, lower epidermis. Legend: sc – stoma cell.

Discussion

Morphology and anatomy of *Tulipa orphanidea* were examined in this study. There are few morphological and anatomical studies related to the *Tulipa* species (Ocak & al. 2004; Satıl & Akan 2006; Coşkunçelebi & al. 2008). *T. armena* Boiss. var. *lycica* was morphologically and anatomically investigated by Ocak & al (2004); *T. aleppensis* was studied only anatomically by Satıl & Akan (2006). Coşkunçelebi & al. (2008) have compared the anatomical, palynological and ecological features of *T. gumusanica* and *T. armena* Boiss. var. *armena*.

The morphological features of T. orphanidea in this study are similar to those given by Marais (1984), except for the anther length. Our results have shown that the unopened anthers were 24-28 mm, while Marais (1984) had reported that the anthers were 7-12 mm long. The difference may be caused by shrinkage after anther dehiscence. The anatomical features of the root of T. orphanidea are similar to the studied Tulipa species (Ocak & al. 2004; Satil & Akan 2006). The common anatomical traits of the stem of T. orphanidea are in accordance with the anatomical features of the studied Tulipa species (T. gumusanica, T. armena var. armena, T. armena var. lycica and T. aleppensis) (Coşkunçelebi & al. 2008; Ocak & al. 2004; Satıl & Akan 2006). However, some anatomical characteristics of the stem were different, such as the supporting tissues. T. gumusanica and T. armena var. armena have a distinct monolayer collenchyma under the epidermis (Coşkunçelebi & al. 2008). While T. armena var. lycica does not contain collenchyma layers in

the stem; it has 4–5-layered sclerenchyma on the inner side of the cortex (Ocak & al. 2004). *T. aleppensis* contains neither a collenchyma, nor a sclerenchyma in the stem (Satil & Akan 2006). *T. orphanidea* is similar anatomically to *T. gumusanica* and *T. armena* var. *armena* in the stem. The stem cortex of *T. orphanidea* has a monolayer collenchyma under the epidermis.

Anatomical features of the leaf of *T. orphanidea* were similar to the examined *Tulipa* species (Ocak & al. 2004; Satıl & Akan 2006; Coşkunçelebi & al. 2008). The mesophyll is not differentiated into a palisade and a spongy parenchyma. It is composed of isodiametric parenchymatic cells. Both *T. gumusanica* and *T. armena* var. *armena* have a monolayer hypodermis beneath the upper epidermis (Coşkunçelebi & al. 2008). It has not been reported in *T. armena* var. *lycica* by Ocak & al. (2004) and in *T. aleppensis* by Satıl & Akan (2006).

For morphologically similar and closely related taxa, anatomical traits are very important diagnostic characteristics that can be used to distinguish the species from each other. The anatomical features of *T. orphanidea* are reported for the first time in this study and are compared to other studied *Tulipa* species. Some differences related to anatomical properties have been found.

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