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### Anatomical Structure and Ecological of *Cephalanthera damasonium* (Mill.) Druce and Its on Contribution to the Taxonomy of Orchidaceae

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**Abstract:** In this study, in the present study reveals the morphological, anatomical and ecological characteristic of *Cephalanthera damasonium* (Mill.) Druce in Turkey. Plant materials of *Cephalanthera* species were collected from one population, between 2018 in Eskişehir/Turkey. *C. damasonium* (Mill.) Druce samples were analyzed for 7 anatomical and soil characters and habitat properties. It was investigated micrometrically in the anatomy of *C. damasonium* (Mill.) Druce. In morphological investigations, the structure of flower, lateral sepal, petal, dorsal sepal, lip, anther cap and column was determined. The findings were compared with those in Flora of Turkey. to habitat definition, *C. damasonium* (Mill.) Druce grew up to 800 m to 1200 m.

**Keywords:** Anatomical, Morphological, *Cephalanthera damasonium*, Eskişehir, Turkey.

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#### 1. Introduction

Orchidaceae is the most famous and attractive plant family among all plant families of the world. Turkey is a rich country of terrestrial orchids and represented by 150 taxa (Arditti, 1992; Prigdeon, 1992; Julou et al. 2005). Turkish orchidaceae were introduced in the volume 8 and 11 of Flora of Turkey. In Turkey, Orchidaceae is represented by 26 genus and about 166 species, of which 60 are endemic (Davis et al. 1978; Güner et al. 2000; Ataşlar, 2018; Dreesler, 1983; Pecoraro et al. 2017; Şegota et al. 2012; Stöckel et al. 2011). Terrestrial orchids have creeping, much reduced, fibrous or fleshy rhizomes or tuber like roots. *Orchis* L. taxa are terrestrial orchids and diagnosed by a basal rosette and terminal, unbranched inflorescence that is composed of small to moderately large resupinate flowers. This genus belonged to the Orchidinae tribe of which 30 species demonstrate their main distribution in the Northern regions of Europe and Asia (Renz and Taubenheim, 1984; Dressler, 1993; Kreutz, 2000; Kreutz, 2009; Abadie et al. 2006; Dafni and Ivri 1981). Most cultivars are tropical or sub-tropical. This species is known as salep in Turkey likes many other members of Orchidaceae (Baytop, 1997; Akman et al. 2014).

Orchids are nearly found in all ecosystems around the world except for glaciers, true desert and open water,

although tropical areas especially in Asia, Africa and America are the hot spots of diversity. *Orchis* is a major genus of Orchidaceae family in Flora of Turkey, represented by 22 species (Renz and Taubenheim, 1984; Kreutz, 2000). In the last description study on *Orchis* (Kreutz, 2009), some species were classified under the genus *Anacamptis* and *Neotinea* (Kreutz, 2009). In this study, the nomenclature of *Orchis* is according to the Flora of Turkey (Renz and Taubenheim, 1984).

The charisma of orchids and their biology have attracted botanists and general public as many varieties and hybrids are widely cultivated; this passion has inspired intrepid collecting expeditions and spawned hundreds of orchid societies and clubs around the world, spawning a global cultivation industry worth nine-billion dollars annually (Ziegler, 2007; Çepel N. 1988). Ecology, morphology and anatomy of several Turkish *Orchid* species have been studied earlier (Durmuskahya, 2013; Durmuskahya et al., 2014; Aybeke et al., 2010). Turkey has a rich biodiversity and has got more than 170 taxa (Kreutz, 2009). In high of the above, the aim of the present study was to investigate new ecological, morphological and anatomical information about another orchis taxa and to provide base knowledge for further studies. This species is known as salep in

Turkey likes many other members of Orchidaceae (Baytop, 1997; Tuzlaci, 2006).

The aim of the present study is to describe the morphological and anatomical structure and in addition to investigate the epidermal cell and stomata of leaves of *Cephalanthera damasonium*.

## 2. Materials and Method

*C. damasonium* was collected from Eskişehir: Sarıcakaya-Hekimdağ, below Quercus trees, 1000 m., 19.05.2018 and stored in the Eskişehir Osmangazi University Herbarium as a herbarium specimen (OUFE 12509). Determination of the species was made according to the Davis (1978 & 1988). Morphological descriptions are based on living plants and herbarium specimens.

Anatomical investigations were applied on the leaves of samples fixed in 70% alcohol. The transverse sections discarded from mid-vessels of leaves were stained with safranin and upper and lower surface layers were investigated in the media with 20% gliserin (Vardar, 1987). The well-staining sections were photographed on Leica DFC 295 color camera type, Leica DM 2500 light microscope. In this study, total 5 plant samples were collected for anatomical studies.

The surface layer of soil was removed and soil samples taken from 0-5 and 5-15 cm depth were analysed. The samples were air dried, ground, passed through a 2 mm sieve and subjected to physico-chemical analysis. Total soluble salts, pH, calcium carbonate content and texture were determined by the methods outlined in detail by Ozturk et al. (1997).

## 3. Results

Plant 15-45 cm. Basal leaves usually broadly lanceolate and unspotted. Spike ovoid to conical, with flowers opening from top downwards. Flowers whitish pink, with an upswept hood; lip conspicuous and in shape of a man with body.

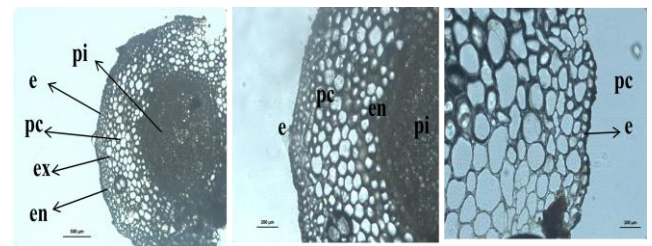


**Fig. 1** *C. damasonium* (Mill.) Druce habitat view.

*C. damasonium* (Mill.) Druce is labellum 15-20 mm, centre whitish-rose, deeply segmented near base into linear lateral lobes, middle lobe ligulate, divided into 2 linear

lobules; all lobules darker purple towards apex. Spur cylindrical. Fl. 4-5. Grassy hillsides, Scrub, on calcareous soils, Quercus scrub, 900-1000 m. (P.H. Davis, 1978) (Fig 1).

Cross section of root showed single layered epidermal cells. The cell length was longer than width ( $30-60 \times 45-100 \mu\text{m}$ ). Cortex consisted of 7-12 layered parenchymatous cells. The shape of cortex cells ranged from oval to globular. These cells were 60-100  $\mu\text{m}$  in diameter and their walls were thin. Parancymatic cells located far from center were smaller than the ones close to center (Fig. 1A). Similar results have been earlier reported by several authors in members of Orchidaceae family (Aykebe et al., 2010; Altundag et al., 2012; Durmuskahya et al., 2014). Endodermal cells ( $35-50 \times 45-100 \mu\text{m}$ ) with thickened wall were seen. Pericycle was single-layered and located under endodermis. (Fig. 2, Tab. 1).

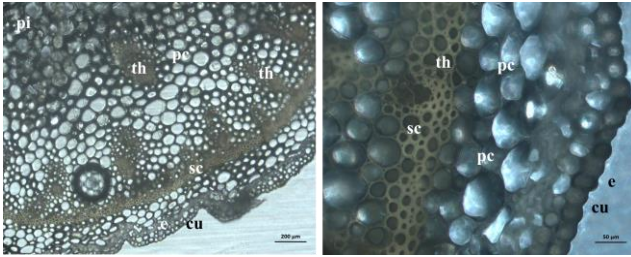


**Fig. 2** Root transverse section of *C. damasonium*. e epidermis, ex exodermis, pc parenchymatic cells, en endodermis, ph phloem, xy xylem, pi pith (Bar 100  $\mu\text{m}$ )

|             | Width ( $\mu\text{m}$ ) |      | Length ( $\mu\text{m}$ ) |      |
|-------------|-------------------------|------|--------------------------|------|
|             | Min.                    | Max. | Min.                     | Max. |
| <b>Root</b> |                         |      |                          |      |
| Epidermis   | 15                      | 25   | 25                       | 60   |
| Cortex      | 40                      | 120  | 70                       | 105  |
| parenchyma  |                         |      |                          |      |
| Endodermis  | 10                      | 35   | 20                       | 60   |

**Table 1** Root anatomy measurement of *C. damasonium* (Figure 2)

The anatomical section of stem showed thick cuticle layer. Under this layer there was a single layered epidermis. Epidermal cells were square or rectangular shaped,  $35-150 \times 30-50 \mu\text{m}$ . 8-16 layers of cortex cells were found under epidermis with 20-30  $\mu\text{m}$  diameter. Few collenchyma cells with thick and lignified cell walls were found in cortex layer. 10-24 layered collenchyma cells gave durability to the stem. (Fig. 3). Vascular bundles were collateral and located in one ring. The bundle sheath consisted of sclerenchymatic cells, at the phloem pole of vascular bundles. Pith had many lacunas in the centre of stem due to the breakup of pith into pieces (Fig. 3, Tab. 2).

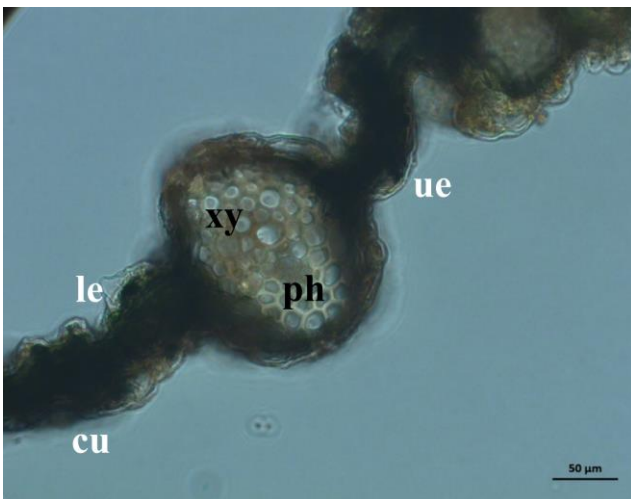


**Fig. 3** Stem transverse section of *C. damasonium* (Mill.) Druce, cu cuticle, e epidermis, pc parenchymatic cells, th trachea, pi pith (Bar 100 µm)

**Table 2** Stem anatomy measurement of *C. damasonium* (Mill.) Druce . (Figure 3)

|                    | Width (µm) |      | Length (µm) |      |
|--------------------|------------|------|-------------|------|
|                    | Min.       | Max. | Min.        | Max. |
| Stem               |            |      |             |      |
| Epidermis          | 20         | 40   | 30          | 50   |
| Cortex parenchyma  | 30         | 60   | 50          | 110  |
| Trachea (diameter) | 125        | 240  | 200         | 350  |

In leaves, adaxial epidermis consisted of a single layer of rectangular cells 25-80 × 30-60 µm with smooth cuticle. Abaxial epidermis was 55-80 × 35-50 µm. Adaxial epidermis cells were bigger than abaxial epidermis cells and adaxial cuticle thicker than adaxial. These features were observed in *O. laxiflora* Lam and *O. purpurea* Hudson by Aybeke et al. (2010). But Sevgi et al. (2012) observed that these species had thicker cuticle on abaxial side and they had similar thickness on both adaxial and abaxial surface. Mesophyll layer was homogenous, without any differentiation in to palisade and sponge parenchyma tissues (Fig. 4, Tab. 3).

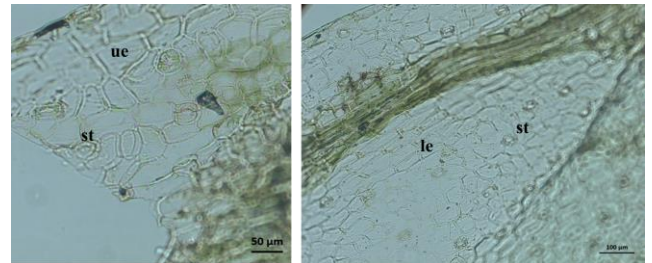


**Fig. 4** Leaf transverse section of *C. damasonium* (Mill.) Druce, c cuticle, ue upper epidermis, p parenchyma, ph phloem, xy xylem, le lower epidermis (Bar 100 µm)

**Table 3** Leaf anatomy measurement of *C. damasonium* (Figure 4)

|                    | Width(µm) |      | Length (µm) |      |
|--------------------|-----------|------|-------------|------|
|                    | Min.      | Max. | Min.        | Max. |
| Leaves             |           |      |             |      |
| Adaxial epidermis  | 5         | 10   | 10          | 15   |
| Mesophyll cells    | 15        | 20   | 30          | 50   |
| Abaxial epidermis  | 5         | 15   | 5           | 20   |
| Trachea (diameter) | 40        | 70   | 45          | 90   |

Leaves surface of investigated species was glabrous like many other orchid species. In leaves, adaxial epidermis consisted of a single layer of rectangular cells 50-110 × 80-85 µm with smooth cuticle. Abaxial epidermis was 45-100 × 55-155 µm. Adaxial epidermis cells were bigger than abaxial epidermis cells and adaxial cuticle thicker than adaxial. (Fig. 5, Tab. 4).



**Fig. 5** Leaf epidermal peeling of *C. damasonium* (Mill.) Druce, ue upper epidermis, le lower epidermis, st stomata (Bar 100 µm)

**Table 4** Leaf anatomy measurement of *C. damasonium* (Figure 5)

|                   | Width(µm) |      | Length(µm) |      |
|-------------------|-----------|------|------------|------|
|                   | Min.      | Max. | Min.       | Max. |
| Leaves            |           |      |            |      |
| Adaxial epidermis | 20        | 50   | 60         | 150  |
| Abaxial epidermis | 30        | 60   | 30         | 60   |

#### 4. Discussion

In this study *C. damasonium* (Mill.) Druce was investigated ecology, morphologically and anatomically. Morphological drawings of flower, lateral sepal, dorsal sepal, petal, lip, anther cap and column were made. The results obtained from morphological studies were

generally consistent with the description given in the Flora of Turkey (Davis 1978 & 1988). As a whole this study increase the knowledge of the morphology and anatomy in *Orchis* reporting data about the *C.damasonium* (Mill.) Druce.

## 5. Conclusion

Cross section of root showed single layered epidermal cells. The cell length was longer than width (15-25 × 25-60 µm). Cortex consisted of 10-16 layered parenchymatous cells. The shape of cortex cells ranged from oval to globular. Parancymatic cells located far from center were smaller than the ones close to center (Fig. 2). Endodermal cells (10-35 × 20-60 µm) with thickened wall were seen clearly. Pericycle was single-layered and located under endodermis. (Fig. 2). The anatomical section of stem showed thick cuticle layer. Under this layer there was a single layered epidermis. Epidermal cells were square or rectangular shaped, 20-40 × 30- 50 µm. 20-30 layers of cortex cells were found under epidermis with 40-80 µm diameter. Few collenchyma cells with thick and lignified cell walls were found in cortex layer. Vascular bundles were collateral and located in one ring. The bundle sheath consisted of sclerenchymatic cells, at the phloem pole of vascular bundles. Pith had many lacunas in the centre of stem due to the breakup of pith into pieces (Fig. 3).

Leaves surface of investigated species was glabrous like many other orchidaceae species. In leaves, adaxial epidermis consisted of a single layer of rectangular cells (5-10 × 10-15 µm) with smooth cuticle. Abaxial epidermis was 5-15 × 5-20 µm. Adaxial epidermis cells were bigger than abaxial epidermis cells and adaxial cuticle thicker than abaxial. Mesophyll layer was homogenous, without any differentiation in to palisade and sponge parenchyma tissues (Fig. 4).

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