

Ecology and Morpho-Anatomical Investigations on *Orchis purpurea* Huds. and *Orchis mascula* (L.)L.

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ABSTRACT

In this study, in the present study reveals the morphological, anatomical and ecological characteristic of *Orchis purpurea* Huds. and *Orchis mascula* (L.)L. in Turkey. Plant materials of *Orchis* L. species were collected from one population, between 2018 in Turkey. In Turkey, for many centuries, various kinds of *Orchis* have been extracted from the tubers and consumed as hot drink with the name of “salep” and it has been exported as well. 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. In anatomical investigations, the structure of root, stem and leaf were determined. Also, stomata and epidermal cell of leaves were investigated. The anatomical results were discussed with similarities and differences with the anatomical structure of other *Orchis* L. species. According to habitat definition, *O. purpurea* and *O. mascula*. grew up to 900 m to 1000 m. Also, *Quercus* forests were most common habitat of *O. purpurea* and *O. mascula*.

Keywords: *Orchis* , Ecology, Morphology, Anatomy, Orchidaceae

INTRODUCTION

The flora of Turkey is represented 1.220 genus and 11.707 species and sub-species which belong to 154 family. Turkey is one of the country that has the richest flora in the World with 11.707 species and sub-species taxa. Floristic Statement of Steppe in Central Anatolia According to the study of Turkey’s Flora the number of species is more than 2.000. The number of endemic species is higher in Central Anatolia as well as floristic richness (Akman, 2014). In the steppes of Central Anatolia particularly species of Labiatae, Scrophulariaceae, Caryophyllaceae, Crucifera, Boraginaceae, Cistaceae and Leguminosae families are predominate (Akman, 2014).

Orchidaceae is the most famous and attractive plant family among all plant families of the world (Arditti, 1992; Prigdeon, 1992). The family Orchidaceae comprises approximately 19,500 species distributed all around the world. Turkish orchidaceae were introduced in the volume 8 and 11 of Flora of Turkey. *Comperia* is a minor genus of Orchidaceae family in Flora of Turkey, represented by one species (Renz and Taubenheim, 1984; Kreutz, 2000). 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) Most cultivars are tropical or sub-tropical. Many orchidaceae species are threatened in wild due to over collection and habitat degradation. Therefore, orchidaceae studies have got considerable importance. This species is known as salep in Turkey likes many other members of Orchidaceae (Baytop, 1997; Tuzlaci, 2006). Dried and powdered roots of this species are used to produce hot beverage “salep” and in preparing ice cream. For this reason many individuals of this species are collected and sold by local people (Toroglu *et al.*, 2010).

ORCHIS L.

Erect perennials with globose to ellipsoid undivided tubers. Leaves unspotted or spotted, ±arranged near base. Emerging spike by spathe-like leaves, many flowered, ±cylindrical. Flowers in various shades of red, purple, and yellow, rarely white. Bracts membranous. Lateral sepals spreading to reflexed or all sepals connivent with petals, forming a hood. Labellum ±directed down-wards, entire or 3-lobed, with entire or ±divided middle lobe, glabrous or ±papillose above, with saccate to filiform spur. Anther firmly attached to short, erect column, folded median part of rostellum placed between the parallel anther cells (loculi). Pollinia 2, clavate, narrowed below to caudicles, attached to separate viscidia, which are enclosed in a single pouch (bursicula). Ovary cylindrical, sessile, twisted, glabrous.

Ecology, morphology and anatomy of several Turkish Orchid species have been studied earlier (Durmuskahya, 2013; Durmuskahya et al., 2014; Aybeke et al., 2010). But information about various orchid species is merge. 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.

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 *Orchis mascula* and *Orchis purpurea*. Moreover the research contributes to the taxonomy of the genus *Orchis* and *Orchidaceae* family.

MATERIALS AND METHODS

Orchis mascula and *Orchis purpurea* was collected from Eskişehir: Sarıcakaya-Hekimdağ, below *Quercus* trees, 980 m., 19.05.2018 and stored in the Eskişehir Osmangazi University Herbarium as a herbarium specimen (OUFE 12507- OUFE 12508). Determination of the species was made according to the Davis (1978 & 1988). Morphological descriptions are based on living plants and herbarium specimens.

In anatomical studies, the collected plant were stored in 70% alcohol, and made permanent with glycerine-gelatin by taking cross-sections from their root, stem, leaf and surface sections of the leaf (Vardar 1987). A light microscope was used to examine the sections and photographs were made using a Olympus CX41, diagnostic digital camera. For each plant sample, morphological and ecological features were recorded. . In this study, total 7 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).

CONCLUSION

- *O. mascula* (L.) L., (Figure 1)

Plant 20-40 cm. Stem slender, often flexuous. Basal leaves 4-6, obovate to oblong, spreading, c. 8-12 x 1.5-3 cm, shining-green, unspotted (seldom faintly marked with dark lines near base). Spice cylindrical, lax, many-flowered. Flowers mauve to red. Sepals obruse, lateral ones spreading to reflexed.

- *Orchis purpurea* Huds., Fl. Angl. ed. 1:334 (1762). (Figure 2)

Stout plant, 40-80 cm, with 4-6 ovate-oblong leaves near base, to 16 x 6 cm. Spike ± dense and many-flowered, flowers large. Sepals forming a globose hood, uniformly coloured or with conspicuous dense dark reddish-brown spots outside.



Figure 1. *Orchis mascula* (L.)L.,
 A. Herbarium image, B. Habitat view.



Figure 2. *Orchis purpurea* Huds.,
 A. Herbarium image, B. Habitat view.

Orchis mascula (L.)L.

Labellum 3-lobed, convex, with few darker dots near base or undotted; middle lobe elongate, clearly exceeding the ovate lateral lobes, reniform to nearly 2-lobed. Spur slightly curved upwards, \pm dilated at tip, \pm equalling ovary. **Fl.** 5-6. Glades and edges of coniferous forest, *Quercus* scrub, 950-1000 m. (P.H. Davis, 1978) (Fig. 3).

Orchis purpurea Huds.

Labellum flat, to 20 mm, 3-lobed, whitish or pale rose, densely spotted with tufts of reddish-purple papillae; lateral lobes linear; middle lobe obcordate or obovate-cuneate, slightly bilobed; lobules broadly rhombic, rounded or truncate, irregularly crenulate, mostly with a tooth in between. Spur cylindrical, curved forwards, nearly half as long as ovary. **Fl** 4-5. *Glades and edges of deciduous forests, scrub, on calcareous soils, 100-1750 m.* (P.H. Davis, 1978) (Fig. 4).

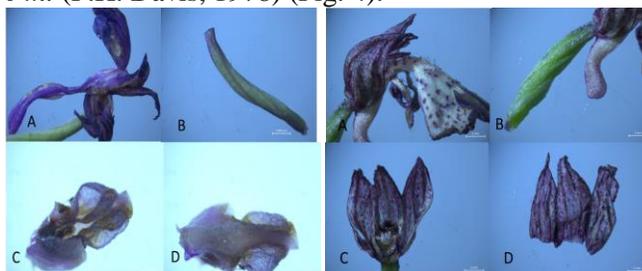


Figure 3. *O. mascula* morphology: **A** flower, **B** Spur, **C** lateral lobes, lip, anther cap and column, **D** dorsal lobes sepal, dorsal sepal,

Figure 4. *Orchis purpurea* morphology: **A** flower, lateral **B** Spur, **C-D** lip

Root transverse section

Cross section of root showed single layered epidermal cells. The cell length was longer than width (20-45 \times 35-50 μ m). Cortex consisted of 8-10 layered parenchymatous cells. The shape of cortex cells ranged from oval to globular. These cells were 55-110 μ m in diameter and their walls were thin. Parancymatic cells located far from center were smaller than the ones close to center (Fig. 1A). These cells contained starch grains and cluster raphide crystals (Fig. 1F). Fungal pelotons and hyphae were observed in big cortical cells. 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 (25.5-40 \times 20-40 μ m) with thickened wall were seen but were not visible clearly. Pericycle was single-layered and located under endodermis. Xylem consisted of radially arranged 6 to 7 vessels, while pith was made up of parenchymatous cells. (Fig. 5).

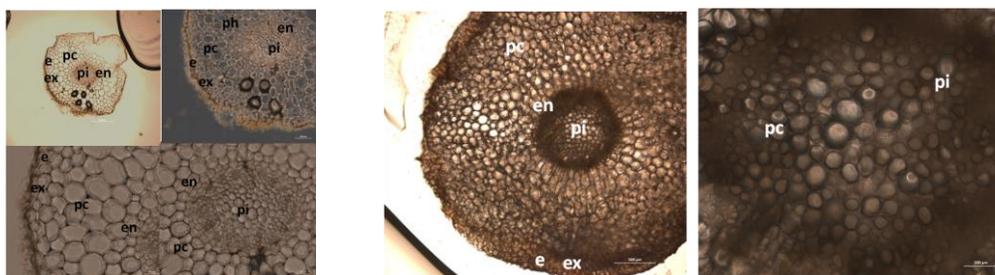


Figure 5. Root transverse section of *Orchis mascula* (L.)L. **Figure 6.** Root transverse section of *Orchis purpurea* Huds. . e epidermis, ex exodermis, pc parenchymatic cells, en endodermis, ph phloem, xy xylem, pi pith (Bar 100 μ m)

Anatomical Section (Stem)

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, 20-60 \times 10- 30 μ m. 4-7 layers of cortex cells were found under epidermis with 45-75 μ m diameter. Few collenchyma cells with thick and lignified cell walls were found in cortex layer. 5-6 layered collenchyma cells gave durability to the stem. Cortical parenchyma, a thinner layer than collenchyma was found with large amount of starch grains (Fig. 7-8). Presence of collenchyma cells in *Ophrys* L. and *Dactylorhiza* Necker ex Nevski have been reported previously in other studies (Altundag *et al.*, 2012; Aybeke *et al.*, 2010). 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. 7-8).

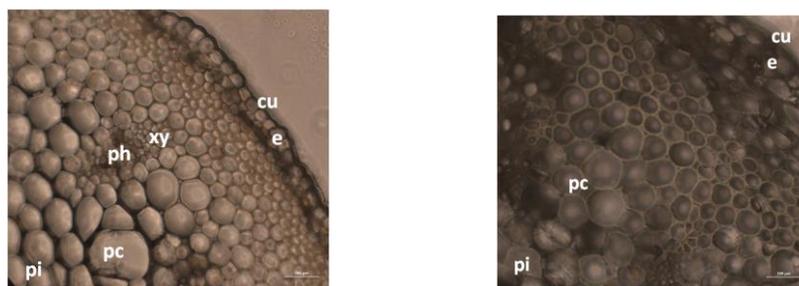


Figure 7. Stem transverse section of *Orchis mascula* **Figure 8.** Stem transverse section of *Orchis purpurea* Huds. (L.)L. cu cuticle, e epidermis, pc parenchymatic cells, ph phloem, xy xylem, pi pith (Bar 100 μ m)

Anatomical Results (Leaf)

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 \times 80-85 μ m) with smooth cuticle. Abaxial epidermis was 55-80 \times 35-50 μ m. Adaxial epidermis cells were bigger than abaxial epidermis cells and adaxial cuticle thicker than abaxial. These features were observed in *Orchis laxiflora* Lam and *O. purpurea* Hudson by Aybeke *et al.* (2010). But Sevgi *et al.* (2012a) observed that these species had thicker cuticle on abaxial side and they had similar thickness on both adaxial and abaxial surface. According to former studies, adaxial epidermal cells of *O. spitzelii* were transformed to water-storage tissues. Mesophyll layer was homogenous, without any differentiation in to palisade and sponge parenchyma tissues (Fig. 9-10).

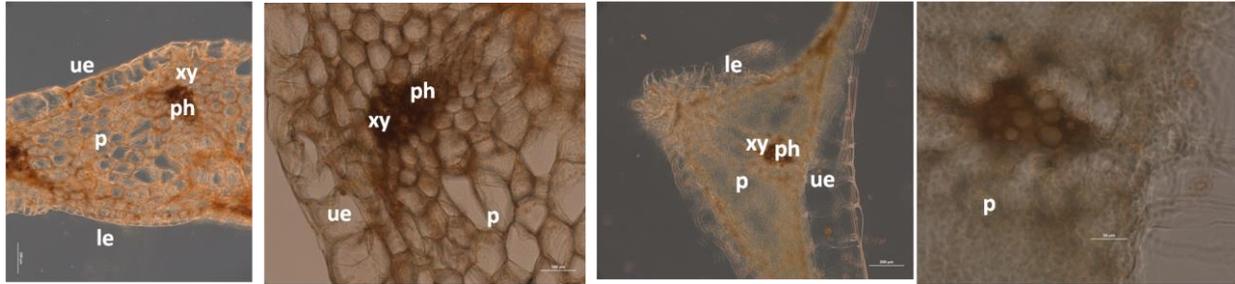


Figure 9. Leaf transverse section of *Orchis mascula* (L.)L. **Figure 10.** Leaf transverse section of *Orchis purpurea* Huds. . **ue** upper epidermis, **p** parenchyma, **ph** phloem, **xy** xylem, **le** lower epidermis (Bar 100 μ m) **xy** xylem, **le** lower epidermis (Bar 200 μ m)

In this study *Orchis mascula* and *Orchis purpurea* 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).

The leaf is unifacial and compose of parenchymatic cells with intensively chloroplast. Vascular bundle is collateral and there hasn't bundle sheath. Stoma cells are located only on the abaxial side of the epidermis, as shown in Figure 9-10; just as Sevgi et al. (2012) were reported. As a whole this study increase the knowledge of the morphology and anatomy in *Orchis* reporting data about the *Orchis mascula* and *Orchis purpurea* (Fig. 9-10).

As far as ecological status is concerned, this species is rare as compared with other *Orchis* species like, *Orchis anatolica*, *Orchis italica* Poiret and *Orchis simia* Lam. It is generally found in small groups. Due to its extreme habitat, its population are limited. Therefore, some scientist accept that it is an endangered species. Because of local use of this species like in preparing *salep* (Tecimen et al., 2010), natural population is decreasing day by day. As compared to other orchid species, which are used for salep making, such as *Serapias vomeracea*, *Orchis anatolica*, *Anacamptis pyramidalis* (L.) Rich., it was found that in different habitat it blossoms in later time and these reason makes it easy target for plant collectors.

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