

THE ANATOMICAL PROPERTIES OF *ONOSMA MOLLIS* DC. AND *ONOSMA HALOPHILA* BOISS. & HELDR. (BORAGINACEAE) FROM TURKEY

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Abstract

The anatomical characteristics of two endemic species viz., *Onosma halophila* Boiss. & Heldr. and *O. mollis* DC. were investigated. Both of examined species are Iran-Turan Element. The root of examined species observed as secondary structure and cortex is multilayered. Raphide crystals were observed in epidermis and 2-3 layered of cortex cells bellow epidermis in *O. mollis*. leaves are isobilateral (=equifacial) in both species. Cystoliths were seen basal portions of hairs on both side epidermis of leaves in two species. Stomata types were anisocytic and anomocytic in both species. The stomata index is 11.18 of upper epidermis and 10.71 of lower epidermis in *O. mollis*. The stomata index is 10.95 of upper epidermis and 12.67 of lower epidermis in *O. halophila*. Vascular bundles were surrounded by a parenchymatic sheat in both species.

Keywords: Boraginaceae, *Onosma*, Anatomy, Turkey

Introduction

The family Boraginaceae is very large group comprising about 100 genera and 2000 species in the tropical and temperate regions of the world. Members of the family are annual, biennial or perennial herbs, rarely shrublets or trees, and are typically covered with coarse trichomes. In Turkey there is rich diversity of this family distributed in different habitats with its large economical tolerance (Riedl, 1978).

Onosma L. genus belongs to the family Boraginaceae, order Lamiales and subclass Dicotyledoneae. *Onosma* genus consisting of over 150 species is typical of xeric habitats as perennial, usually suffruticose or biennial herbs that has its greatest species diversity in southeast Europe and Asia Minor and is known to include several serpentine endemics, especially in the southern Balkans (Stevanovic *et al.*, 2003). Anatolia is an important centre of origin for *Onosma* comprising about 101 species (107 taxa), 50 of which are endemic including 1 variety to Turkey (Riedl, 1978; Davis *et al.*, 1988; Yıldırım, 2000; Riedl *et al.*, 2005 and Binzet & Orcan, 2007; Kandemir & Türkmen 2010; Aytaç & Türkmen 2011, Güner 2012). The genus *Onosma* presents considerable taxonomic problems (Tutin *et al.*, 1972). Indumentum of leaves and stem in the identification, sectional and subsectional delimitations are of great importance. Size of calyx, colours and shape of corolla and number of flowers in cymes and nutlets morphology are some other key features.

The genus has been divided into three sections: *Protonosma*, *Podonosma* and *Onosma*. *Protonosma* and *Podonosma* sections are represented by one species, most of the species are belong to *Onosma* section. This section is separated into two subsections according to solely indumentum type: *Asterotricha* (Boiss.) Gürke. and *Haplotricha* (Boiss.) Gürke. Riedl (1978) pointed out that the classification appears to be partly artificial and in need of re-investigation and new data (karyological and

palynological) may provide useful reference points in the future classification of the *Onosma* genus.

The member of this genus is used as folk medicine, herbs and dyes. *O. sericeum* Willd., *O. microcarpum* Steven ex DC. and *O. argentatum* Hub.-Mor. are used for the treatment of wounds in rural areas in Turkey (Ozgen *et al.*, 2003 and Özgen *et al.*, 2004). The flowers of some species are consumed as vegetables (Öztürk & Özçelik, 1991). Several local names are given to *Onosma* species such as “Emzik otu-Eskişehir”, “Emcek-Kemaliye-Erzincan”, Yalancı havaciva and emzik-Kemah-Erzincan” (Baytop, 1994) and mjmjmjok (Binzet, 2010).

Studies on the anatomy of *Onosma* genus are limited. Metcalfe & Chalk (1979) and Watson & Dallwitz (1991) explained the characteristics properties of family Boraginaceae. Akçin & Engin (2001; 2005) and Akçin (2004; 2007) Binzet & Akçin (2009; 2012) were studied the anatomical and ecological properties of some *Onosma* species. Binzet & Orcan (2003a; 2003b; 2009), Binzet & Akçin (2009), Binzet (2011) and Binzet & Akçin (2012) are investigated the anatomical structure and palynological characteristics of 23 *Onosma* species.

Material and Methods

In this study, the anatomical characteristics of endemic *Onosma halophila* belongs to *Haplotrichous* Subsect. and *O. mollis* belongs to *Asterotrichous*. Subsect. were investigated. Both of examined species are Iran-Turan Element. Plant samples used in this study were collected from Aksaray and Malatya respectively in 2006-2011 by the first author (Table 1.). Vouchers specimens are kept at the Herbarium of the Faculty of Art and Science of Mersin University. Taxonomical descriptions of the specimens were made according to Riedl (1978). Samples for anatomical studies were deposited in 70 % alcohol. Cross and surface sections of root, stem and leaves were excised by hand and they were covered with glycerin-gelatin (Vardar, 1987). Microphotographs were taken with Olympus BX51 microscopes.

Table 1. Locality information of the examined *Onosma* taxa.

Taxa	Locality
<i>O. halophila</i>	B4 Aksaray, Eski-Cihanbeyli 2-4 km, halophytic areas, 38° 23'N 033° 22' E, 14.06.2011, 950 m, Binzet 201129; 07.07.2011, Binzet 201146
<i>O. mollis</i>	B7 Malatya, Malatya-Yazihan 10 km, roadside, 780 m, 38° 25' N 038° 13' E, 31.05.2011, Binzet 201107; B7 Malatya, Yazihan-Hekimhan 15 km, slopes, 1075 m, 38° 42' N 038° 07' E, 26.05.2006, Binzet 92

Results

Anatomical results

***O. halophyllum*:** In transverse sections of the root, it was seen a secondary structure. Periderm consist of flattened cells with 3-7 layers. Multi-layered and fragmented cells in certain regions of the cortex tissue. Cambium with 2-3 layers squashed and flattened cell is located between phloem and xylem. Xylem is composed of tracheal elements, parenchymatous cell and sclerenchymatous cell and cover narrow area. The pith consist of tracheal cells.

The most outside; the epidermis of stem consists of uniseriate, square, oval and rectangular cells. Cuticle layer is 5 µm on the epidermis. Caudate setose trichomes are present on the epidermis. Stomata is located at the level of the epidermis. 2-4 layered squashed cells are take place the central part of the cortex tissue. Endodermis is 2-3 layered. Cambium is distinguishable and 3-4 layered. Secondary xylem covers a large area than primary xylem. Xylem tissue is composed of many obscure vascular bundle towards the pith. The pith consist of parenchymatous cells and crystals were seen in some pith cell (Figs. 1-2).

The transverse and surface sections taken from the leaf were observed as follows (Figs 3-5). The adaxial and abaxial epidermises of the leaf consist of uniseriate, rectangular, pentagonal, hexagonal and heptagonal cells in transverse section. Upper epidermis cells 27-81 µm x 20-27 µm, lower epidermis cells 126-75 µm x 11-29 µm. Cuticle layer is 5-12 µm on the abaxial and adaxial epidermis. Cystoliths were seen basal portions of hairs on both side of epidermis. Setose and glandular trichomes present on the epidermis. Stomatas are observed on both surfaces (=amfistomatic). Stomatas occur on the same level with the adaxial and abaxial epidermis. Stomata on the dorsal side outnumber those on the ventral side. Stomata are surrounded by 4-5 cells on ventral side and surrounded by 3-5 cells on dorsal side and measured 10x33 µm on the dorsal and ventral sides. Leaves are isolaral (=equifacial) in type. Palisade parenchyma has two layered cells on both sides and they are rectangular, cylindrical shape and measured as 48-126 x 16-27 µm. Spongy parenchyma with one layered cells lies between the upper and lower palisade. Vascular tissue is surrounded by a parenchymal bundle sheath. Bundle sheath extensions are seen upper and below of vascular bundles. Stomata index is 10.95 for the upper epidermis and 12.67 for the lower epidermis.

***O. mollis*:** A transverse section taken from the root was observed as followed (Fig. 6). A secondary structure is observed. Outermost, periderm tissue is composed of 10-14 layers. Periderm cells are degraded in some areas. Endodermis is distinguishable and one layered. Vascular cylinder is covering wide area. Cambium of 3-7 layers lies between phloem and xylem. Xylem tissue is composed of sclerenchymatous cells and tracheary elements and cover a wide area. The pith consist of traceal cells.

A transverse section taken from the middle part of the stem was observed as follow (Fig. 7). Outermost, cuticle layer is 5 µm. Epidermis is found with one layered. Setose trichomes and stomatas that raised above epidermis level. Raphide crystals are observed in epidermis and 2-3 layered of cortex which is under the epidermis. Endodermis is 1-2 layered. Secretory cells are rare in the phloem tissue. Cambium is distinguishable and 3-4 layered. Xylem tissue is composed of many obscure vascular bundle. The pith consist of cylindrical parenchymatous cells and cover wide area.

The adaxial and abaxial epidermises of the leaf consist of uniseriate, rectangular, pentagonal and hexagonal cells in transverse section. Cuticle layer is 10-13 µm on the abaxial epidermis and 7-10 µm on the adaxial epidermis. Upper epidermis cells 23-48 µm x 11-27 µm, lower epidermis cells 35-50 µm x 15-20 µm. Both epidermises are covered with dense adpressed hairs (reduced setae) arising from stellate-hairy tubercles. Cystoliths were seen basal portions of hairs on both side of epidermis. Stomata occur on the both surfaces and upper level with epidermis cells or same level rarely (Fig. 8). Stomata on the dorsal side outnumber those on the ventral side. Stomata are anamocytic and anisocytic. Stomata are surrounded by 3-5 cells on both side leaves and measured 10x32 µm on the dorsal side and 10x25 µm on the ventral side. Leaves are isolaral (=equifacial) in type. Palisade parenchyma has two layered cells on both sides and they are rectangular, cylindrical shape and measured as 12-15 x 38-48 µm. Spongy parenchyma with one layered cells lies between the upper and lower palisade. Vascular tissue is surrounded by a parenchymal bundle sheath. Stomata index is 11.18 for the upper epidermis and 10.71 for the lower epidermis (Figs. 9-10).

Nutlet size of *O. mollis* is 5x3.5 mm, bipyramidal, with sharp ventral keel and acute, straw coloured. Nutlet size of *O. halophila* is 3x2 mm, ovoid, subacute and lacking dorsal keel, greyish. Nutlet ornamentation is rugose type in both of species. This type is characterised by the epidermal cells of the nutlet surfaces having small or fine wrinkles (Fig. 11).

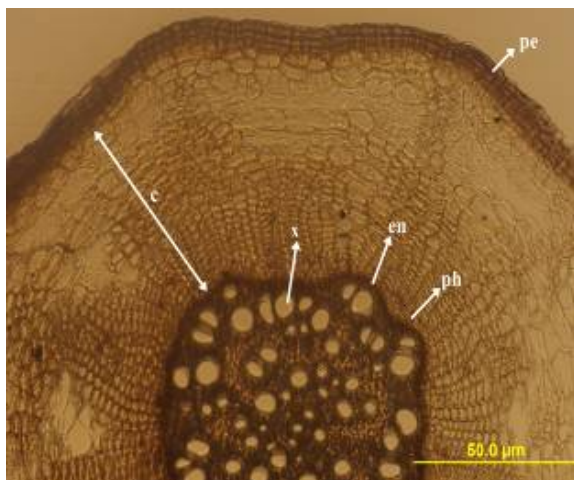


Fig. 1. *O. halophila* Cross section of root. pe: periderm, c: cortex, en: endodermis, ph: phloem, tr: trach, x: xylem.

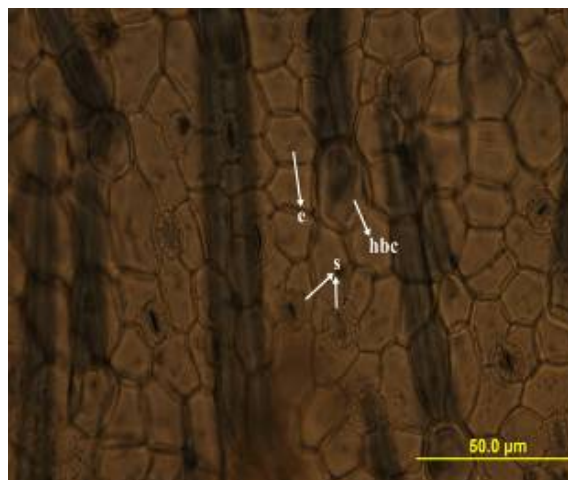


Fig. 4. *O. halophila* The upper surface of the leaves. e: epidermis, s: stomata, hbc: hair base cell.

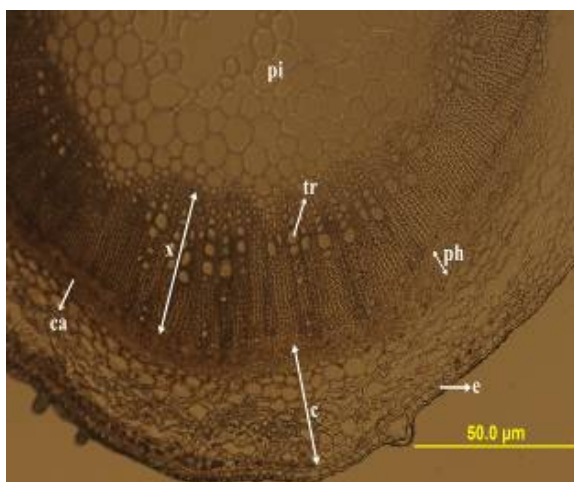


Fig. 2. *O. halophila* Cross section of stem. e: epidermis, c: cortex, ca: cambium, ph: phloem, x: xylem, tr: trache, pi: pith.

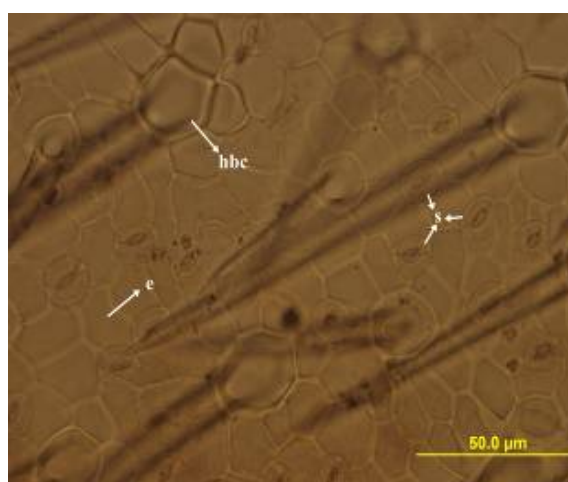


Fig. 5. *O. halophila* The lower surface of the leaves. e: epidermis, s: stomata, hbc: hair base cell.

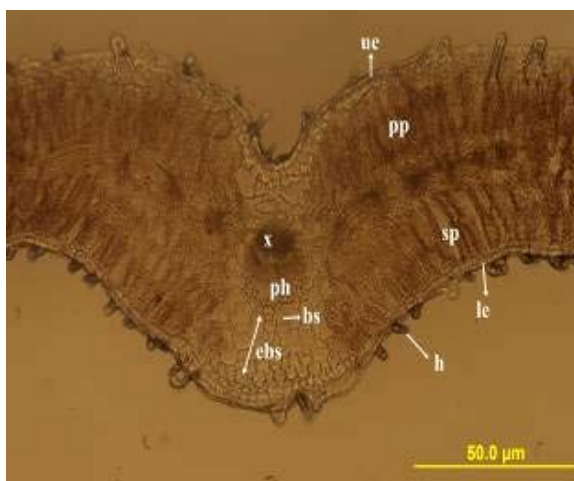


Fig. 3. *O. halophila* Transversal section of leaves. ue: upper epidermis, le: lower epidermis, h: hair, ebs: extension bundle sheath, bs: bundle sheath, ph: phloem, x: xylem.

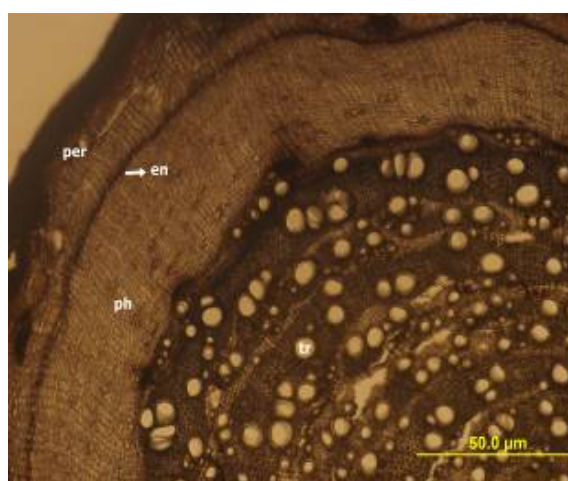


Fig. 6. *O. mollis* Cross section of root. Per: periderm, en: endodermis, ph: phloem, tr: trache.

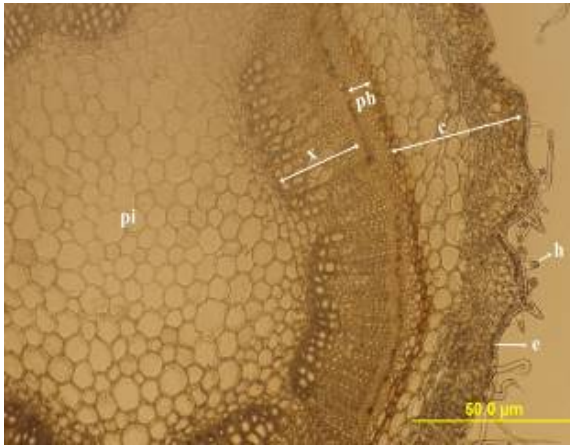


Fig. 7. *O. mollis* Cross section of stem. h:hair, e: epidermis, c: cortex, ph: phloem, x: xylem, pi: pith.

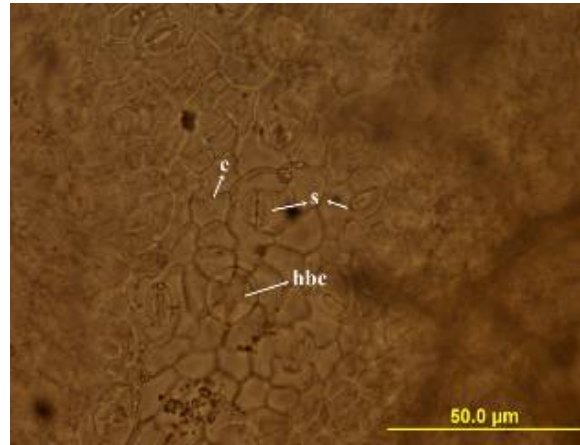


Fig. 9. *O. mollis* The upper surface of the leaves. e: epidermis, s: stomata, hbc: hair base cell.

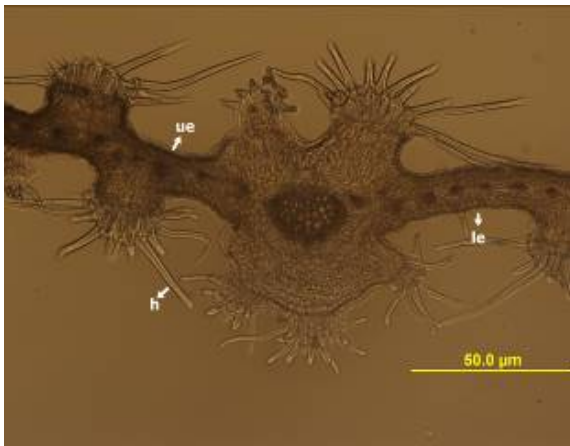


Fig. 8. *O. mollis* Transversal section of leaves. ue: upper epidermis, le: lower epidermis, h: hair.

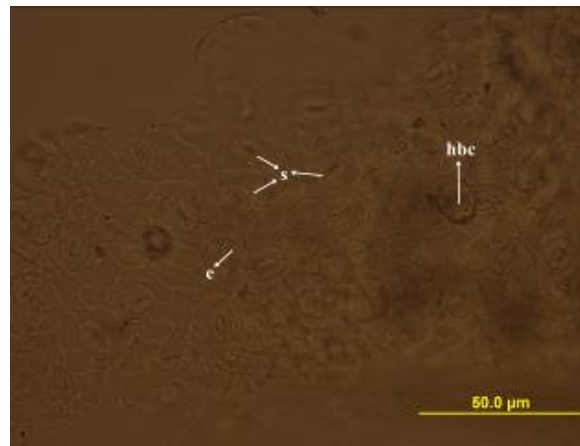


Fig. 10. *O. mollis* The lower surface of the leaves. e: epidermis, s: stomata, hbc: hair base cell.

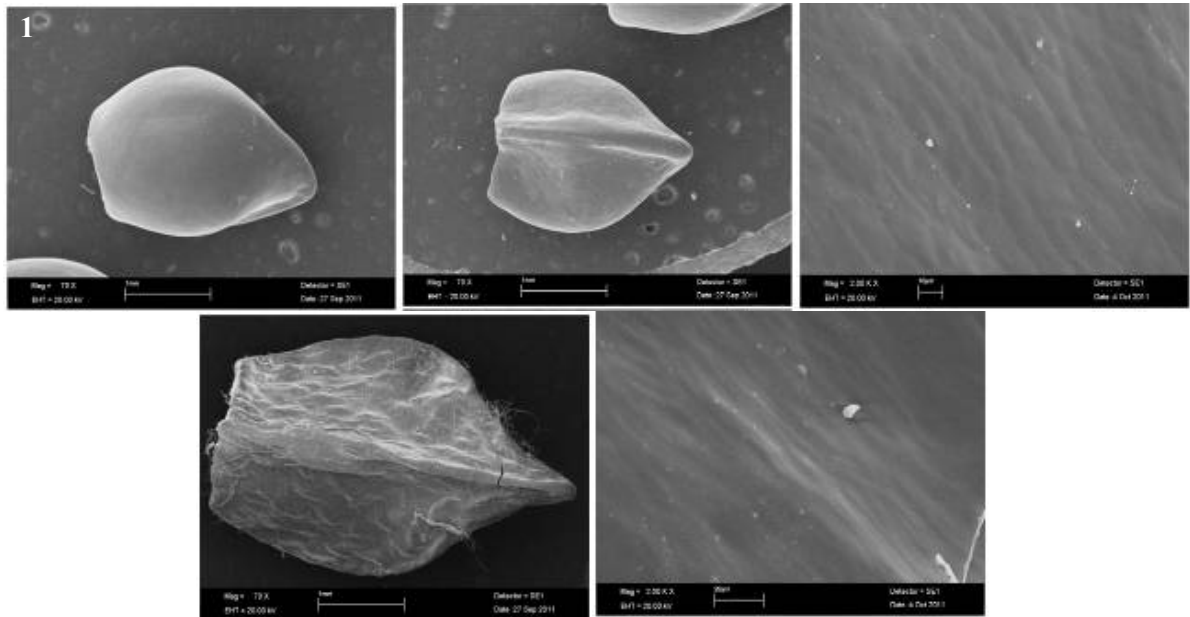


Fig. 11. SEM microphotographs of nutlet surfaces. 1-3 *O. halophila*; 4-5 *O. mollis*.

Discussion

In this study, anatomical properties of *O. mollis* belong to the subsection *Asterotricha* and *O. halophila* is endemic plant and belong to the subsection *Haplotricha* were examined. Metcalfe & Chalk (1979), Watson & Dallwitz (1991) were studied the anatomical properties of the family Boraginaceae. The anatomical properties of the both of species are suitable with the family Boraginaceae (Metcalfe & Chalk, 1979).

Analysis of the cross section of root showed that the root had a secondary structure. Xylem is composed of sclerenchymatic cells and tracheary elements in roots of *O. mollis*, and xylem is composed of tracheal elements, parenchymatic cell and sclerenchymatic cell in roots of *O. halophila*. While, the xylem tissue cover narrow area in *O. halophila*, xylem tissue cover wide area in *O. mollis*. While, the pith region root, generally consist of primary xylem elements in some *Onosma* species such as *O. giganteum* Lam. (Binzet & Orcan 2003), *O. bracteosa* Hausskn. & Bornm. (Akçin & Engin 2005), *O. sieheana* Hayek (Binzet & Akçin 2009), *O. mersinana* Riedl, Binzet & Orcan (Binzet & Orcan 2009). The pith region of root consist of parenchymatic cells in the other *Onosma* species such as *O. intertexta* Hub.-Mor. (Binzet & Akçin 2009), *O. frutescens* Lam. and *O. inexpectata* Teppner (Binzet & Akçin 2012). The pith region of *O. mollis* and *O. halophila* is composed of xylem elements, sclerenchymatic cells and parenchymatic cells. While, Upper epidermis cells are 23-48 x 11-27 µm and lower epidermis cells are 35-50 x 15-20 µm in *O. mollis*. Upper epidermis cells are 27 - 81 x 20 - 27 µm and lower epidermis cells are 75-126 x 11-29 µm in *O. halophila*. As setose trichomes and stomatas that raised above epidermis level in *O. mollis*, caudate setose trichomes and stomatas were seen the same level of epidermis in *O. halophila*. Endodermis is distinguishable and with 1-2 layered in *O. mollis* and with 2-3 layered in *O. halophila*. In all two species, cambium were seen clearly as 3-4 layered. The presence of crystals in Boraginaceae family has important characters (Metcalfe & Chalk 1979). Raphide crystals were observed in epidermis and 2-3 layered of cortex bellow epidermis in *O. mollis* only. Crystals were seen in pith cells in some *onosma* species such as *O. mutabile* (Binzet & Orcan 2003), *O. intertexta* and *O. sieheana* Hayek (Binzet & Akçin 2009). The pith is composed of parenchymatic cells and crystals were seen in some pith cell in both species. Metcalfe & Chalk (1979) pointed out that Boraginaceae family has both bifacial and isobilateral leaves. According to Azizian *et al.*, (2000), two distinct leaf anatomical structures are present within the *Onosma* genus: in sections *Protonosma* and *Podonosma*, leaf is dorsi-ventral and in section *Onosma* leaf is isobilateral. In this study (in section *Onosma*), leaves are isobilateral (=equifacial). Species of *Onosma* genus generally have isobilateral leaf (Akçin & Engin 2001, 2005; Binzet & Orcan 2003a,b; Akçin 2004, Binzet & Orcan 2009; Binzet & Akçin 2009; Binzet & Akçin 2012). Metcalfe & Chalk (1979) reported that there are both anomocytic and anisocytic stomata in Boraginaceae family. The leaf anatomies and trichome features of fourteen *Onosma* species were investigated by Azizian *et al.*, (2000), who observed that the stomata are mainly

anomocytic type. Akçin (2007b) and Binzet & Akçin (2009; 2012) reported that stomata are anisocytic and anomocytic type respectively, in *O. armena* DC., *O. intertexta*, *O. sieheana*, *O. frutescens* and *O. inexpectata*. According to Zarinkamar (2007), dominant stomatal type is anomocytic, with anisocytic cells present as a subordinate type in some species such as *O. microcarpa* DC. and *O. dichroantha* Boiss. Dasti *et al.*, (2003) explained that although anomocytic type was dominant type helicocytic, hemiparacytic, staurocytic and brachyparacytic stomata were seen in *O. stephonia*. Our findings are similar with Metcalfe & Chalk (1979), Akçin (2007), Binzet & Akçin (2009; 2012). In our study stomata type was anisocytic and anomocytic in both species. While, the stomata index is 11.18 of upper epidermis and 10.71 of lower epidermis in *O. mollis*. While, the stomata index is 10.95 of upper epidermis and 12.67 of lower epidermis in *O. halophila*. Vascular bundles are surrounded by a parenchymatic sheath in both species.

Nutlet size of *O. mollis* is 5x3.5 mm, bipyramidal, with sharp ventral keel and acute, straw coloured. Nutlet size of *O. halophila* is 3x2 mm, ovoid, subacute and lacking dorsal keel, greyish. Nutlet size, shape and surface ornamentation of 14 *Onosma* species were studied by Binzet & Akçin (2009) and they reported that rugose ornamentation seen in *O. angustissima* and *O. gigantea* nutlet surfaces. Nutlet ornamentation is rugose type in both of species.

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