

**THE MICROMORPHOLOGICAL AND ANATOMICAL PROPERTIES OF
ONOSMA ANGUSTISSIMUM HAUSSKN. & BORNM. AND
O. CASSIUM BOISS. (BORAGINACEAE)**

ÖZNUR ERGEN AKÇIN¹ AND RIZA BINZET²

*Department of Biology, Faculty of Sciences and Arts,
Ordu University, Ordu 52750, Turkey*

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Abstract

The micromorphological and anatomical properties of *Onosma angustissimum* Hausskn. & Bornm. and *O. cassium* Boiss. were investigated. *O. angustissimum* is an endemic species. All examined taxa had secondary root structure. Crystals were seen in the stem and leaves of two species. Epidermal cells containing crystal needles were observed in *O. cassium*. Studied *Onosma* species had isobilateral type of leaves. Stomata were anisocytic and anomocytic. Glandular and eglandular trichomes especially porrect-stellate and simple trichomes were densely seen on the both upper and lower epidermis of leaves in two species. Stomata index was different in *O. cassium* from *O. angustissimum*.

Introduction

The family Boraginaceae comprises about 131 genera and 2500 species in five subfamilies and seven tribes. The Boraginaceae are distributed throughout the tropical, subtropical and temperate regions of the world. The centers of highest diversity in the North Temperate Zone are the Irano-Turanian and Mediterranean regions, and in the tropics are Central America and northern and Central South America. In Turkey there are about 300 species (Al-Shehbaz, 1991). The genus *Onosma* is represented by about 102 taxa (97 species) in Turkey and the proportion of endemism among native species is 50% including 50 endemic species and 1 endemic variety (Riedl, 1978; Davis *et al.*, 1988; Yıldırım, 2000; Riedl *et al.*, 2005; Binzet and Orcan, 2007). The roots of many species of Boraginaceae, particularly in the genera *Alkana* Tausch, *Anchusa* L., *Arnebia* Forssk., *Cynoglossum* L., *Echium* L., *Lithospermum* L. and *Onosma* L. yield red to purple naphthaquinone dyes commonly known as alkannins. Several members of the Boraginaceae have commonly been used as pot herbs and for their medicinal and culinary values (Al-Shehbaz, 1991).

Previous studies on the anatomy of this genus are limited. Metcalfe and Chalk (1979) and Watson and Dallwitz (1991) reported the characteristic properties of the family Boraginaceae. Anatomical data of the genus *Onosma* are scarce and scattered in literature

¹Corresponding author. E-mail: <oysakcin@yahoo.co.uk><Oakcin@omu.edu.tr>

²Department of Biology, Faculty of Art and Science, Adiyaman University, Adiyaman, Turkey.

dealing more generally with the Boraginaceae family (Metcalfé and Chalk, 1979). It has been noted that various types of hairs (setae) occur in Boraginaceae and it shows great variation within *Onosma* species. The anatomical and ecological properties of some *Onosma* species were studied by Akçin and Engin (2001, 2005) and Akçin (2004). Binzet and Orcan (2003) investigated the anatomical structure and palynological characteristics of *O. roussaei* DC. and *O. giganteum* Lam. The chromosome numbers of different species of *Onosma* were reported by Teppner (1981, 1988). Recently nutlet micromorphologies of some *Onosma* species were studied (Akcin, 2007a).

We began this research after perceiving the need for biological characteristics of plants to be supported by anatomical and morphological research in order to shed more light on evolutionary and systematic relationships. Therefore, the aim of this study was to investigate the micromorphological and anatomical characteristics of *Onosma angustissimum* Hausskn. & Bornm. and *O. cassium* Boiss and its application to systematics.

Materials and Methods

Plant materials were collected from different localities of southern Turkey (Table 1). Voucher specimens were 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 fixed 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). The photographs were taken with Nikon FDX-35 microscope. All measurements and observations were made using imaging software (Table 2). Stomata index was calculated according to the method described by Meidner and Mansfield (1968) (Table 3). For scanning electron microscopy, dried leaves were mounted on stubs using double-sided adhesive tape. Samples were coated with 12.5-15.0 nm of gold. Coated leaves were examined and photographed with JMS-6400 Scanning Electron Microscope.

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

| Taxa | Locality |
|-------------------------|--|
| <i>O. angustissimum</i> | C3 Antalya: Gazipaşa, Sugözü plateau, 1230 m, 36° 26' N 032° 28' E, 19.05.2005, Binzet 59; C4 Mersin: Anamur, Abanoz plateau-Bardat plateau, Fersakan around, 1300 m, 36° 21' N 032° 49' E, 20.05.2005, Binzet 60. |
| <i>O. cassium</i> | C5 Hatay: İskenderun, Arsuz, Işıklı village-Kale village, Rocky slopes and open forest, 36° 19' N 035° 47' E, 29.06.2004, 150 m, Binzet 11; 24.04.2005, Binzet 12. |

Results and Discussion

O. cassium Boiss.

Transverse sections taken from the root were observed as follows. Fellogen is uniseriate and distinguishable. Felloderm is 1-2 layered. Cortex is multilayered and

composed of parenchymatous cells and phloem. Cambium cells are 1-3 layered and distinguishable. Xylem is composed of sclerenchymatous cells and trachea and covers a large area in root. Pith rays are multi-layered. In the pith, primary xylem tissue is present (Table 2, Fig. 1).

Table 2. Anatomical properties of various tissues of *O. cassium* and *O. angustissimum*.

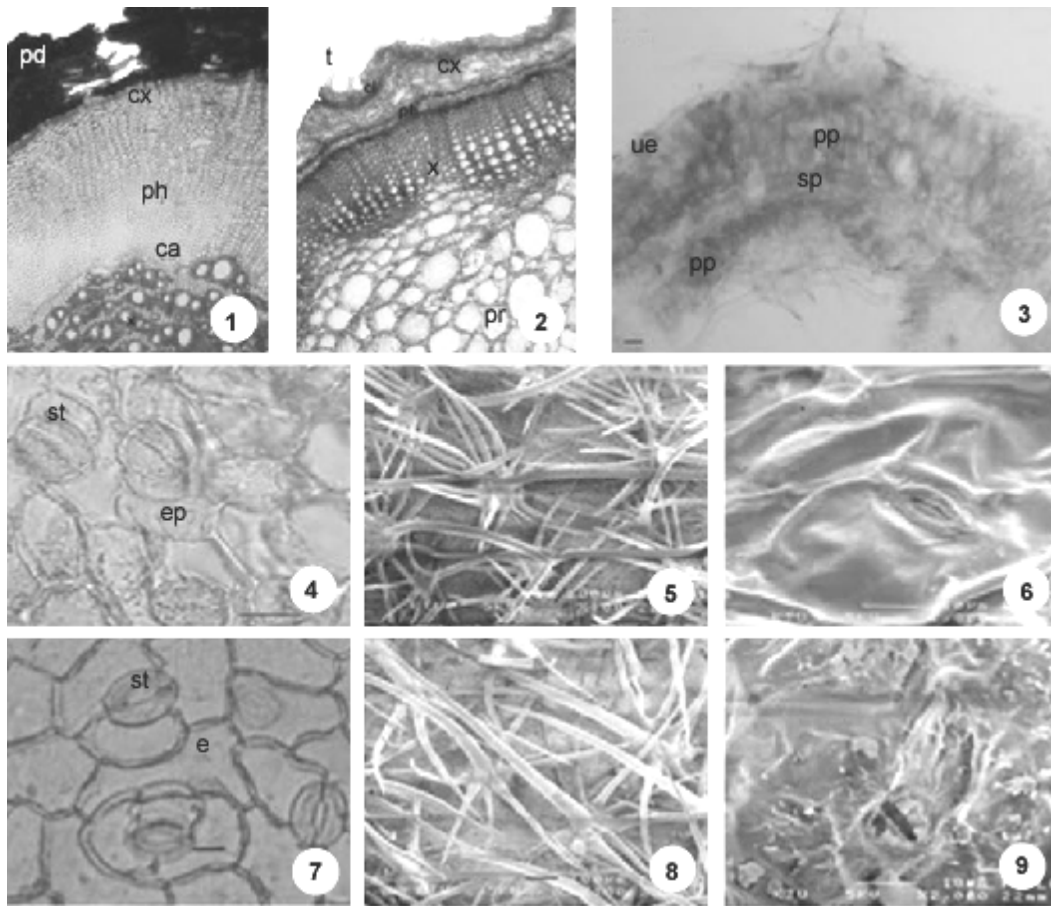
| Parameter | <i>O. cassium</i> | | <i>O. angustissimum</i> | |
|---------------------------|--|---|--|---|
| | Breadth (μm) Mean \pm SE | Length (μm) Mean \pm SE | Breadth (μm) Mean \pm SE | Length (μm) Mean \pm SE |
| Root | | | | |
| Periderm cells | 38.90 \pm 14.58 | 23.90 \pm 5.73 | 30.55 \pm 7.75 | 16.84 \pm 2.76 |
| Parenchyma cells | 25.82 \pm 5.20 | 16.27 \pm 1.66 | 25.12 \pm 3.72 | 14.67 \pm 2.39 |
| Diameter of trachea | 37.38 \pm 7.01 | | 65.65 \pm 15.21 | |
| Stem | | | | |
| Epidermis cells | 25.57 \pm 5.31 | 20.78 \pm 2.61 | 26.21 \pm 4.32 | 28.99 \pm 5.92 |
| Collenchyma cells | 44.50 \pm 7.36 | 28.10 \pm 4.45 | 34.03 \pm 6.49 | 31.44 \pm 4.99 |
| Parenchyma cells | 66.88 \pm 15.18 | 46.06 \pm 8.64 | 61.23 \pm 12.23 | 52.43 \pm 6.30 |
| Diameter of trachea | 28.47 \pm 6.16 | | 26.39 \pm 5.89 | |
| Diameter of pith cells | 77.74 \pm 20.51 | | 53.35 \pm 9.87 | |
| Leaf | | | | |
| Upper epidermis cells | 17.68 \pm 2.68 | 13.29 \pm 2.46 | 29.96 \pm 9.04 | 19.04 \pm 5.99 |
| Lower epidermis cells | 18.99 \pm 3.65 | 13.30 \pm 2.53 | 19.38 \pm 5.55 | 23.69 \pm 6.38 |
| Palisade parenchyma cells | 27.30 \pm 2.93 | 11.76 \pm 2.63 | 18.82 \pm 5.93 | 63.80 \pm 12.31 |
| Spongy parenchyma cells | 9.2 \pm 0.69 | 11.93 \pm 1.49 | 20.68 \pm 4.99 | 12.56 \pm 3.89 |

Table 3. Stoma features on the upper and lower epidermis of *O. cassium* and *O. angustissimum*.

| Parameter | <i>O. cassium</i> (Mean \pm SE) | | <i>O. angustissimum</i> (Mean \pm SE) | |
|--|--------------------------------------|------------------|--|------------------|
| | Upper surface | Lower surface | Upper surface | Lower surface |
| Number of stomata (1 mm ²) | 52 \pm 5.20 | 46 \pm 4.60 | 34 \pm 3.40 | 66 \pm 6.60 |
| Number of epidermis cells (1 mm ²) | 248 \pm 4.8 | 271 \pm 7.1 | 247 \pm 4.7 | 346 \pm 4.6 |
| Stoma index | 17.33 | 14.51 | 12.09 | 16.01 |
| Stoma length (μm) | 24.71 \pm 2.9 | 13.92 \pm 3.18 | 15.11 \pm 4.17 | 17.24 \pm 1.93 |
| Stoma width (μm) | 22.66 \pm 2.49 | 16.29 \pm 3.32 | 13.74 \pm 2.71 | 13.57 \pm 1.91 |

Epidermis is covered by cuticle with glandular and eglandular trichomes on the stem. Eglandular trichomes are simple and stellate. The epidermis is composed of uniseriate rectangular or oval cells. Collenchyma is 2-3 layered. There are 3-5 layered compressed parenchyma cells and 2-4 layered parenchyma cells in the cortex. Endodermis is distinguishable. Sclerenchymatous cells are present between phloem and parenchyma. Cambium is distinguishable. Pith rays are 1-6 layered. The pith consists of large and cylindrical parenchymatous cells (Table 2, Fig. 2).

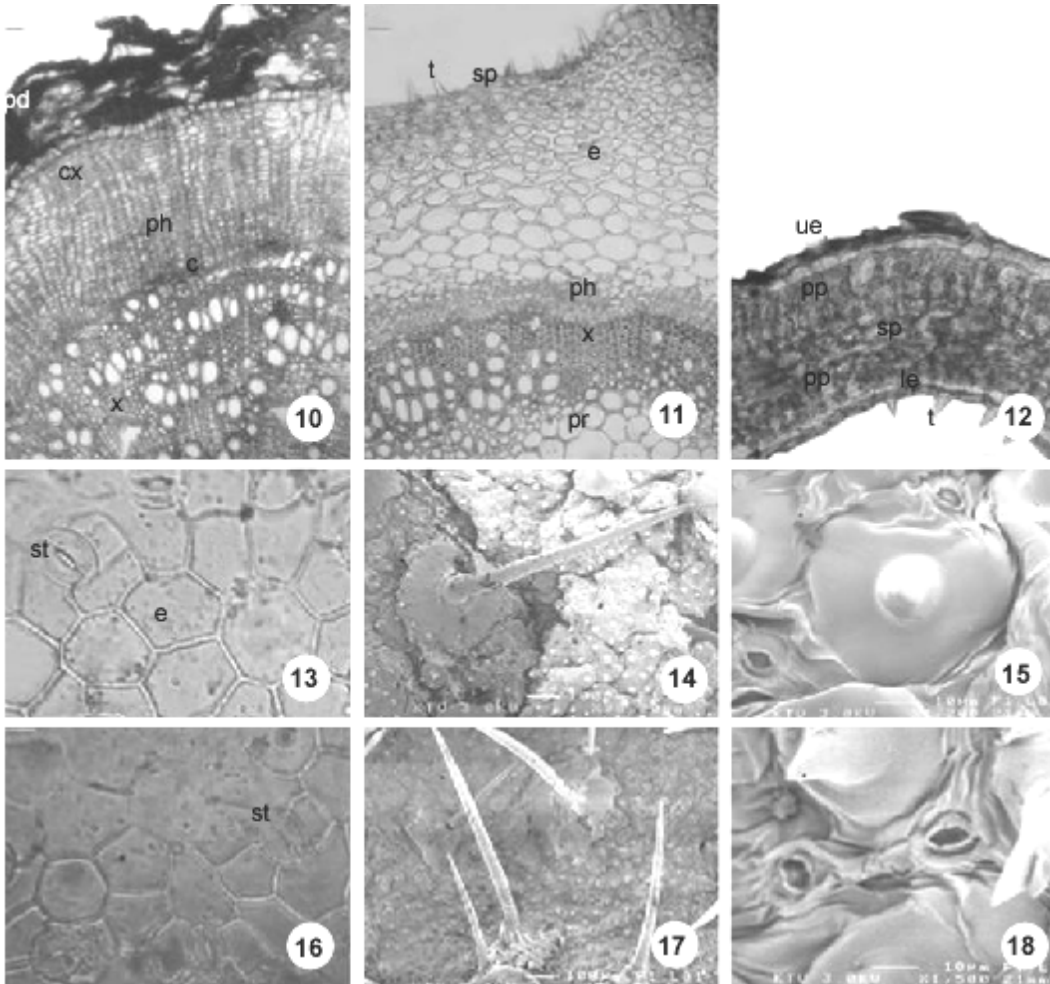
The transverse and surface sections taken from the leaves were observed (Figs. 3-9). There is a thick cuticle. Epidermal cells are isodiametric and oval. Some epidermal cells are larger and located higher than the others on the upper surface. Epidermal cells contain crystal needles. Glandular and eglandular trichomes especially porrect-stellate trichomes are seen on the both upper and lower epidermis. The stellate trichome base elevate above the epidermis. These trichomes have cystoliths in the bases. Palisade parenchyma cells are 2-(3) layered on the upper surface and single layered on the lower surface. Spongy parenchyma cells are 2-(3) layered. Stomata types are of anisocytic and anomocytic. There are more anisocytic stomata on the upper epidermis. Vascular bundles are collateral and surrounded by a parenchymatic bundle sheath (Tables 2-3).



Figs. 1- 9: *O. cassium*. 1. Transverse section of root. 2. Transverse section of stem. 3-9. Leaf (LM, SEM). pd = peridermis; cx = cortex; c = cambium; x = xylem; e = epidermis; cl = collenchyma; sc = sclerenchyma; en = endodermis; ph = phloem; ue = upper epidermis; pp = palisade parenchyma; sp = spongy parenchyma; le = lower epidermis; st = stomata. Bars: 1,2 = 50 μ m; 3,4,7 = 20 μ m; 5,8 = 100 μ m; 6,9 = 10 μ m.

***O. angustissimum* Hausskn. & Bornm.**

Cortex is multi-layered and parenchymatous under the periderm of root. Clear and large phloem is seen in the upper portion of the cambium. Xylem is composed of untidy concentric rings. The pith consists of tracheary elements (Table 2, Fig. 10).



Figs. 10-18. *O. angustissimum*. 10. Transverse section of root. 11. Transverse section of stem. 12-18. Leaf (LM, SEM). pd = peridermis; cx = cortex; c = cambium; x = xylem; e = epidermis; cl = collenchyma; sc = sclerenchyma; en = endodermis; ph = phloem; ue = upper epidermis; pp = palisade parenchyma; sp = spongy parenchyma; le = lower epidermis; st = stomata. Bars: 10 = 60 μ m; 11 = 65 μ m; 12 = 30 μ m; 13, 16 = 15 μ m; 14, 17 = 100 μ m; 15, 18 = 10 μ m.

Cuticle layer is thick on the stem. The epidermis consists of uniseriate, flattened, rectangular or orbicular cells. Glandular and eglandular trichomes are present on the epidermis. The eglandular trichomes include simple trichomes and porrect-stellate

trichomes with ornamental cuticle. The glandular trichomes are capitate types with unicellular or bicellular stalk and a head. Capitate trichomes are dense on the stem. Collenchyma is generally 2-3 layered (sometimes 4-5 layered). Endodermis is distinguishable and 1-2 layered. Xylem and phloem elements are clear. Cambium is distinguishable. Tracheids with thick walls are dense in xylem tissue. Pith cells are large and cylindrical (Table 2, Fig. 11).

The adaxial and abaxial epidermis of the leaf consist of uniseriate, oval or rectangular cells in transverse section. Both epidermis are covered with short simple trichomes, short and long porrect-stellate trichomes and glandular trichomes. Eglandular trichomes have ornamental cuticle and contain crystals in the bases. Stomata are anisocytic and anomocytic on both epidermis. Anisocytic stomata are more dense than ones on the upper epidermis. Pallisade parenchyma cells are 3-layered on the upper surface and 1-2 layered on the lower surface. Spongy parenchyma cells are 2-3 layered. Vascular bundles are collateral and surrounded by a bundle sheath (Tables 2-3, Figs. 12-18).

Several authors paid attention on anatomical features of *Onosma*. The pith region of root consists of generally primary xylem elements in *Onosma* species (Akçin and Engin, 2005; Binzet and Akçin, 2009). In the present study we found that *O. cassium* and *O. angustissimum* had primary xylem elements in pith region. Some *Onosma* species have parenchymatic pith region (Binzet and Orcan, 2003; Akçin, 2007b; Binzet and Akçin, 2009).

The presence of crystals in Boraginaceae is an important phenomenon (Metcalf and Chalk 1979). Azizian *et al.* (2000) reported that crystal (calcium carbonate) present in two forms in *Onosma* species; a. deposited in cell wall of hairs, or b. located in the base of large hairs. Crystals were clearly seen in the bases of stellate trichomes in *O. cassium* and *O. angustissimum*. *O. sieheanum* Hayek has crystals in the pith region and trichomes (Binzet and Akçin, 2009). In *O. intertextum* Hub.-Mor. crystal needles is present in epidermal cells (Binzet and Akçin, 2009). This characteristic was found in *O. cassium*. Glandular and eglandular trichomes especially porrect-stellate and simple trichomes are densely seen on both the upper and lower epidermis of leaf in two species. According to Öztürk and Seçmen (1996), plants from dry habitats possess more pubescent leaves or densely covered with trichome. *O. cassium* and *O. angustissimum* grow in dry habitats and they show the same features. Metcalfe and Chalk (1979) pointed out that the Boraginaceae have both bifacial and isobilateral leaves. According to Azizian *et al.* (2000) two distinct leaf anatomical structures are present within the genus *Onosma*: in the sections *Protonosma* and *Podonosma*, leaf is dorsiventral, and in the section *Onosma*, leaf type is isobilateral. In our study (section *Onosma*), leaves are isobilateral. *Onosma* species generally have isobilateral leaf (Akçin and Engin, 2001, 2005; Binzet and Orcan, 2003; Akçin, 2004). Metcalfe and Chalk (1979) reported that there is both anomocytic and anisocytic stomata in Boraginaceae. The leaf anatomy and trichome features of

fourteen *Onosma* species were investigated by Azizian *et al.* (2000), who observed that the stomata are mainly anomocytic. Akçin (2007b) reported that stomata are anisocytic and anomocytic in *O. armenum*. According to Zarinkamar (2007) dominant stomatal type is anomocytic, with anisocytic cells present as a subordinate type in some species such as *O. microcarpum* DC. and *O. dichroanthum* Boiss. Our findings are similar with Metcalfe and Chalk (1979) and Akçin (2007b). Stomata type was anisocytic and anomocytic in two species. There are more anisocytic stomata on the upper epidermis of *O. cassium*. Stomata in *O. cassium* are larger than *O. angustissimum*. Stomata index in upper surface of *O. cassium* is bigger than *O. angustissimum*. However, *O. angustissimum* has bigger stomata index in lower surface. Stomata index is important as the number of stomata may be changed by the age of the leaf, but the stomata index remains constant for a species (Trease and Evans, 1982).

We believe that important discoveries unearthed during the study of anatomy and morphology will lead to a better understanding of the species, and provide a contribution to any further study.

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