The Anatomical Characteristics of Endemic Aethionema dumanii Vural & Adıgüzel (Brassicaceae)

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ABSTRACT
The present study deals with the anatomical properties of Aethionema dumanii, which is an endemic species growing in Salt Lake (Konya). The stem cross-sections have a single-layered epidermis, parenchymatous cortex, vascular bundles, and pith cells. An equifacial mesophyll type was observed in the leaves with multiple-layered palisade tissue, but the spongy parenchyma was reduced. Vascular bundles were smaller. The cross-sections of fruit had single exocarp, 3- or 4-layered mesocarp, and single-layered endocarp. Transverse sections of the seed showed an epidermis, palisade tissue, endosperm, and embryo. The anatomical characteristics of A. dumanii has been found to have valuable taxonomic importance.

INTRODUCTION
The family Brassicaceae is represented by 321 genera and about 3660 species worldwide, and at such high numbers, it is known as a significant dicot family (Al-Shehbaz, 2012). In Turkey, the list of this family is composed of 91 genera and 686 taxa (Günner et al. 2012). The major distribution centres of the family are the Mediterranean, Irano-Turanian, and Saharo-Sindian regions (Hedge, 1976). Turkey is one of the richest countries in this respect, with 653 native species belonging to 61 genera in the family Brassicaceae (Al-Shehbaz et al. 2007).

The genus Aethionema W.T. Aiton is represented by about 43 taxa in Turkey, including 20 endemic species (Ertuğrul, 2012). Hence, the endemism rate for this genus is approximately 47%.

Turkish Aethionema species have been the subject of different research areas such as palynology, seed micromorphology, and molecular studies (Pınar et al., 2007; Sunar et al., 2016; Karaismailoğlu, 2017; Çeter et al., 2018). Little known about the vegetative organs and anatomical traits of the genus Aethionema (Atçeken et al., 2016; Karaismailoğlu, 2019). Aethionema dumanii is a perennial, dwarf, and endemic plant that only grows in salty areas. There have been no anatomical studies about this halophytic species.

In this study, A. dumanii endemic plant, which is only known from Salt Lake, were evaluated using their anatomical characteristics, both vegetative organs, fruit, and seeds.

MATERIAL and METHODS
The plant materials were collected from Salt Lake in the province of Konya. Some were prepared according to standard herbarium techniques, while others were placed into 70% ethyl alcohol. The paraffin method was
used for the vegetative organs, fruit and seed of the studied taxa for cross-sections (Johansen, 1940). 5 plant samples were used and the experiments were done at least 3 times. The vegetative and generative organs of the species were cut into small pieces. Next, they were treated through an increasing alcohol series to remove the water from the tissues. As the next step, a portion of paraffin was added to small glass flasks. The paraffin blocks were made and 12–16-µm-thick transverse sections were cut using a Leica microtome with disposable blades. The safranin-fast green were used for dyeing anatomical sections. Under a light microscope (Leica DM 1000), the best sections were chosen and photographed at magnifications of 10x, 20x, and 40x. The measurements, which were made with the Kameram 21 software programme, were based on at least 30 or more cells per specimen. The mean values of the measurements of all of the investigated taxa were given (Table 1). If the paraffin method was unsuccessful, cross-sections were taken by hand using a razor blade. Permanent slides were observed under a Leica DM 1000 light microscope (LM). Measurements were made using the Kameram 21 programme and photos were taken with a Canon EOS 450D camera that was attached to the LM. The stomata were classified based on at least 30 or more cells per specimen.

RESULTS

Stem anatomy

Four main anatomical regions were determined in the cross-sections of the stems. These layers could be counted from the peripheral to the centre, as the epidermis, cortex, vascular bundles, and pith regions (Figure 1-A). The outermost surface of the stems was limited to rectangular-shaped epidermis cells with

|     | Width | Length |
|-----|-------|--------|
|     | Min   | Max    | Mean±SD | Min   | Max    | Mean±SD |
| Stem |       |        |         |       |        |         |
| Cuticle | 8.16 | 9.79 | 8.92±0.82 |       |        |         |
| Epidermis | 12.03 | 25.05 | 18.02±4.8 | 8.47 | 12.08 | 9.83±1.36 |
| Collenchyma | 4.45 | 17.11 | 10.67±3.04 | - | - | - |
| Cortex | 32.41 | 44.99 | 37.16±3.3 |       |        |         |
| Trachea | 16.29 | 31.87 | 24.79±5.6 | - | - | - |
| Pith cells | 35.7 | 70.8 | 59.02±10.12 | - | - | - |
| Leaf |       |        |         |       |        |         |
| Upper cuticle | 3.63 | 5.52 | 4.3±0.62 | - | - | - |
| Lower cuticle | 2.3 | 6.3 | 3.87±1.32 | - | - | - |
| Palisade cells | 18.36 | 41.4 | 25.7±5.7 | 40.82 | 112.7 | 76.6±19.5 |
| Spongy cells | 41.22 | 84.94 | 57.0±14.4 | - | - | - |
| Thickness of mesophyll | 451.7 | 901.8 | 675.7±155.1 | - | - | - |
| Upper epidermis | 22.3 | 62.8 | 44.3±14.4 | 18.9 | 27.1 | 21.9±2.87 |
| Lower epidermis | 21.8 | 63.2 | 37.82±10.4 | 14.78 | 27.76 | 22.5±4.1 |
| Fruit | Pericarp thickness | 132.1 | 188.5 | 157.8±24.1 | - | - | - |
| Seed | Testa thickness | 86.34 | 130.4 | 103.2±16.4 | - | - | - |

Leaf anatomy

The cross-sections of the leaves of the studied species had 2 epidermis with mesophyll tissue and their shapes were determined as rectangular-oval (Figure 2). The mesophyll was equifacial and composed of 4 or 5 rows of palisade and reduced spongy parenchyma cells. The vascular bundles of the leaf were arranged in a single line and the median one is the biggest. The other vascular bundles were smaller than the median one.

The leaves of A. dumanii are amphistomatic and the stomata was observed as anizocytic type according to with the level of epidermis (Figure 3). The shapes of epidermis cells are irregular or polygonal. The cell walls of epidermis cells are smooth (Figure 3-A,B). The stomatal index in upper surface was calculated as 33.3 and, 29.3 in lower surface and the ratio of stomata index 1.13.

Fruit anatomy

The transverse sections of the fruit were triangular-shaped and the pericarp was composed of a lined exocarp, 3 or 4 rows of mesocarp, and a single-layered endocarp (Figure 4-A). The vascular bundle was located in the endocarp layer (Figure 4-B).
Figure 1. The cross sections of stem of *Aethionema dumanii*. A. c: cuticle, ep: epidermis, co: cortex parenchyma, sc: sclerenchyma, ph: phloem, x: xylem B. p: pith cell, pi: pith region

Şekil 1. *Aethionema dumanii* gövde enine kesitleri. A. c: kutikula, ep: epidermis, co: korteks parenkiması, sc: sklerenkima, ph: floem, x: ksilem B. p: öz hücresi, pi: öz bölgesi

Figure 2. The cross section of leaf of *Aethionema dumanii*. c: cuticle, ue: upper epidermis, le: lower epidermis, pp: palisade parenchyma, sp: spongy parenchyma, x: xylem, ph: phloem, st: stomata.

Şekil 2. *Aethionema dumanii* yaprak enine kesitleri c: kutikula, ue: üst epidermis, le: alt epidermis, pp: palizat parenkiması, sp: sünger parenkiması, x: ksilem, ph: floem, st: stoma

Figure 3. The surface sections of leaf of *Aethionema dumanii*. a. Upper surface, b. Lower surface. ep:epidermis, st:stomata

Şekil 3. *Aethionema dumanii* yaprak yüzeysel kesitleri. a. Üst yüzey, b. Alt yüzey. ep:epidermis, st:stoma
Figure 4. The cross sections of fruit of *Aethionema dumanii*. A. v: fruit valve, s: seed B. en: endocarp layer, me: mesocarp layer, ex: exocarp layer, r: replum.

Şekil 4. *Aethionema dumanii* meyve enine kesitleri. A. v: meyve valfi, s:tohum B. en:endokarp tabakası, me:mezokarp tabakası, ex:ekzokarp tabakası, r:replum

Figure 5. A. The general view of seed transverse sections ep: epidermis, cp: crushed palisade cells, p: single layered palisade tissue, en: endosperm layer, em: embryo. B. close view of seed layers.

Şekil 5. A. Tohum enine kesitlerinin genel görünümü ep:epidermis, cp:parçalanmış palizat hücreleri, p:tek tabakalı palizat dokusu, en:endosperm tabakası, em: embriyo. B. Tohum katmanlarının yakın görünümü
Seed anatomy

The cross-sections of the seed determined the integuments, endosperm, and embryo (Figure 5-A). The seed cross-sections showed that there was a single-layered epidermis. Single-layered palisade tissue was under the epidermis, as well as crushed palisade tissue. The endosperm layer was composed of rectangular–oval-shaped cells (Figure 5-B). The embryo was present in the seeds.

DISCUSSION and CONCLUSION

The present work is the first report about the anatomical traits of the vegetative organs, fruit, and seeds of A. dumanii in the family Brassicaceae. Investigations conducted regarding the anatomical traits of the genus Aethionema are little-known (Atçeken et al., 2016, Karaismailoğlu, 2019). The epidermis in the stem was similarly shaped, but they were different with regards to the number of layers. A. dumanii had a single-lined epidermis, similar to A. arabicum and A. karamanicum (Atçeken et al., 2016). However, some Aethionema taxa (A. cordatum and A. armenum) have a 2- or 3-rowed epidermis in their stem anatomy (Atçeken et al., 2016). The cortex was parenchymatous, and scleranchymatic groups were placed above the vascular bundles in A. dumanii. Atçeken et al. (2016) declared that A. karamanicum and A. armenum had scleranchyma above the vascular bundles, but that was not present in A. arabicum and A. cordatum. Unifacial and equifacial mesophyll in the genus Aethionema were reported by Atçeken et al. (2016). According, in our study, A. dumanii had equifacial mesophyll in its leaf anatomy. Moreover, the palisade parenchyma was well-developed in the leaves of A. dumanii and increasing palisade tissue occurred in the habitat (salty soils) of A. dumanii.

The fruit and seed anatomical properties contained essential information about the taxonomy of the family Brassicaceae (Mummenhoff et al., 2008; Mühlfansen et al., 2010; Lenser et al., 2016). In many Brassicaceae species, a living endosperm layer around the embryo plays an essential role in the regulation of the germination and dormancy of the seeds (Müller et al. 2006; Graeber et al. 2012). A. dumanii had a single-lined endosperm layer around the embryo, as Lenser et al. (2016) reported with A. arabicum.

Although the epidermis of testa in A. dumanii is single layered Karaismailoğlu (2019) had declared that testal epidermis in the examined Aethionema taxa is scleranchymatic and consists of two layers including outer and inner epidermis. The outer epidermis indicated markedly variations among the examined taxa. The same researcher indicated that the testa thickness could be change in great variation for Aethionema genus. A. dumanii has 103.2 μm testa thickness in seed congruent with Karaismailoğlu (2019)'s study. Anatomical characteristics might be useful in the definition of A. dumanii. Nevertheless, these characteristics will be more valuable if other species of Aethionema are also examined. In conclusion, A. dumanii was studied in the present work and the anatomical characteristics of stem, leaf, fruit and seed were determined for the first time.

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