MORPHOLOGICAL AND ANATOMICAL CHARACTERIZATION AND TRACE ELEMENTS COMPOSITION OF ONOSMA ARGENTATA HUB.-MOR. (BORAGINACEAE) ENDEMIC TO TURKEY

MUSTAFA ÖZKAN¹, ERGIN KARIPTAŞ¹, CÂNAN ÖZDEMİR², ALI KANDEMİR³ AND YURDANUR AKYOL⁴

¹Ahi Evran University, Faculty of Science and Art, Department of Biology, Kırşehir/ Turkey
²Celal Bayar University, Faculty of Science and Art, Department of Biology, Manisa/Turkey
³Erzincan University, Education Faculty, Department of Biology, Erzincan/Turkey
⁴Manisa Technical and Industrial Vocational High School, Manisa/ Turkey
*Corresponding author’s email: mustafaozkancan@gmail.com; Fax: +90 386 280 4525

Abstract

Onosma argentata Hub.-Mor. is an endemic species growing in Turkey. It is under the threat like many other endemic species in Turkey and its IUCN category is evaluated as vulnerable. According to literature, this species was known only from the two collections, in this study the plants were collected from different localities mentioned in literature. Plant is perennial. The stem is 20-35 cm, shortly hairy and rigid-setose, clothed at base with remains of leaves. The aim of this study is to provide information on the anatomical and morphological properties of O. argentata. Anatomical sections taken from the root, stem and leaves of the plants. The results have been compared with some species of Boraginaceae. In addition, the concentrations of trace elements O. argentata were determined by using atomic absorption spectrometry. Large amount of sodium (Na), aluminum (Al), potassium (K) and iron (Fe) were present in all flowers, leaf and stem of O. argentata plant.

Key words: Onosma argentata, Morphology, Anatomy, Trace elements, Vulnerable.

Introduction

In Turkey the genus Onosma L. (Boraginaceae) is represented by about 101 species of which 50 are endemic (Güner et al., 2012). Onosma argentata Hub.-Mor. is endemic to Turkey. It is under threat as many endemic species in Turkey and its IUCN category is evaluated as vulnerable (Ekim et al., 2000; Anon., 2001).

Some Onosma species are used as herbs, folk medicines and dyes. Fahad & Bano (2012) have reported, traditionally, Onosma plants are used as stimulant in rheumatism, bladder pain, kidney irritation and palpitation of heart. Dried flowers of some Onosma species are used in folk medicine to treat respiratory ailments and some of them are used in the treatment of body swellings and their flowers are eaten (Akçin & Engin, 2005). The roots of Onosma argentata are used traditionally in Turkey for wound healing and burns (Özgen et al., 2004). It is stated that O. argentata extract is effective against Staphylococcus aureus, Bacillus subtilis and Escherichia coli and the roots of O. argentata has antioxidant activity (Özgen et al., 2003).

Studies on the anatomy of this genus are limited (Metcalfe & Chalk, 1979; Binzet et al., 2012). There are several reports on the anatomy of some Onosma species (Akçin et al., 2011; Güven et al., 2013; Akçin et al., 2013; Binzet & Teke, 2014). In recent years, this species has been the subject of chemical studies (Özgen et al., 2004; Çiftçi et al., 2010). There is no detailed study on morphological and anatomical characteristics of O. argentata. Therefore the present study is undertaken to fill the gap.

Trace elements play a very important role in chemical, biological, biochemical and enzymatic reaction in the living cells of plants, animals and human bodies. The role of trace elements in the body metabolism is of prime importance (Yousufzai et al., 2001). Little is known of the trace elements of O. argentum. The study is expected to deliver preliminary data on trace element composition of O. argentum grown in Turkey and provide useful information for future studies which will be conducted on biochemistry, microbiology and physiology. Second the goal of this study was to present and investigate the composition of trace elements O. argentata by using atomic absorption spectrometry.

Material and Methods

Plant samples were collected from the following locations:

Erzincan: Sakaltutan, 2032 m, 39 52 91 N, 39 10 23 E, 17.06.2006, Serpentine, (Kandemir 7241).
Sivas: Kızılıdağ, 1700 m, 12.07.2006, Serpentine, (Kandemir 7795).

Taxonomical description of the plant specimens was prepared according to Davis (1978). Fresh samples and herbarium material were used in each case for experimental analysis and morphological measurements. Analysis of morphological and anatomical studies were made using 20 plant specimens. Each value is the average of 20 measurements. Anatomical studies were carried out on fresh samples or samples kept in alcohol. Anatomical measurements were made with the help of micrometric ocular using sections from the which of plant parts.

Results were presented by original drawings, photographs and tables. Photographs were taken with Leica DM LB microscopy.

Solutions and reagents: All solutions were prepared using ultra pure water (specific resistance 18 MΩ cm) from a Milli-Q purification system (Millipore Corporation, Massachusetts, USA). Standard solutions of analytes were prepared from their 1000 mg L⁻¹ stock solutions (Merck). Concentrated HNO₃ for digestion samples was used ultra pure grade (Merck).
**Determination of metals:** The analysis was performed by ContrAA 300 Flame Atomic Absorption Spectrometer (HR-CS AAS) (GLE, Germany) equipped with a 50 mm burner head and an injection module (SFS-6). All absorption lines of an element in the spectral range of 185-900 nm were analytically evaluated by using a Xe short-arc lamp as a continuum lamp source. Microwave-Assisted Acid Digestions have been made using a Premier microwave system.

Samples were digested using microwave system. 2.00 g portion of each sample dried at 80°C was accurately and 0.40 g directly weighted into PTFE (polytetrafluorethylene) bombs. For the samples decomposition concentrated 7 mL HNO₃ acid were added. In a tightly closed system, the following six-step microwave digestion program was applied according to Yousufzai et al. (2001). PTFE bomb was waited for an hour to cool and was carefully opened. Colorless solution was transferred into beaker and evaporated to dryness on hot plate. Afterwards final volume was diluted to 10 mL with 0.1 mol L⁻¹ HNO₃. The blank digests were carried out in the same way. Sample solutions were analyzed by HR-CS AAS.

**Results**

**Morphological properties:** Plant is perennial. Stems are numerous, up to 35 cm, shortly hairy and rigid-setose, clothed at base with remains of leaves. Basal and lower cauline leaves 70-100 x 3-7 mm, narrowly linear-lanceolate, margins revolute, subserise, densely silvery adpressed-setose on both surfaces, subadpressed to patent setose on margins, middle and upper cauline leaves similar but shorter. Inflorescence of 1-3 terminal, many flowering cymes. Bracts lanceolate to linear. Pedicels to 11 mm, long and short patent setose. Calyx 14-22 mm in flower, scarcely accrescent in fruit, densely patent-setose. Corolla whitish to cream, longer than calyx, cylindrical-inflated, sparsely hairy in upper. Anthers included. Nutlet 4 mm, ovooidal-triquetrous, apex acuminate (Fig. 1A, B).

This species can be easily recognize from other Onosma species except Onosma dissectens Hausskn. ex Bornm. with discoloured densely silvery adpressed-setose leaves. It is difficult to distinguish O. argentata from O. dissectens due to similar these floral and leaves characteristic. O. argentata is restricted with small serpentine areas around Erzincan and Sivas, located East Anatolia. It has concoloured leaves with adpressed-setose on both surfaces, patensetose on margins. But, O. dissectens have discoloured leaves that silvery beneath with almost silky dense adpressed hairs, green above hairs sparse.

**Anatomical Properties**

**Root:** Periderm is 5-16 layered on the outer surface of root. Elements of phellem are dark coloured, crushed, break up and sometimes fell out. Cortex is multilayered and parenchymatic. Cell size is larger in primary cortex than secondary cortex. Phloem elements are located in small region of root. Cambium cells are 2-4 layered, flat and distinguishable. Xylem occupies a large area. It is of sclerenchymatic cells and trachea. Trachea different in size. The center of root are occupied by primary xylem tissue. The measurements of root are given in Table 1. (Fig. 2. I-II).

**Stem:** There is a cuticular layer on epidermis. Epidermis is 1-2 layered and consist of ovoidal cell. There are glandular and aglandular hairs on epidermis. Collechyma is 1-4 layered. Cortex is 8-20 layered cells ovoidal, sometimes flatted ovoidal and different in size. Diameter of cortex cells on the endodermis are to large. Endodermis is located between cortex and vascular tissue. Cambium cells are distinguishable and 2-3 layered. Phloem covers the major part and xylem below it reaches up to the pith. There are pith rays extending up to the pith from cambium in the xylem tissue. Xylem has trachea cells different in size. The pith occupies a large space and consists of parenchymatic cells. Thicknesses of tissues in the stem were measured with the help of micrometer and results are given Table 1 (Fig. 3A, Table 1).

**Leaf:** Leaves are covered by a thick cuticular layer on both upper and lower surfaces. There is a single layered epidermis on both upper and lower surface of the leaf. Mesophyll consists of 1-2 layer of palisade parenchyma cells and 5-7 layers of spongy parenchyma cells. Palisade cells are rich in chloroplasts. Spongy parenchyma cells occupy a wide area in the mesophyll. Stornata are present on both upper and lower epidermis. Vascular bundles are surrounded by a parenchymatic bundle sheath (Fig. 3B, Table 1).

**Trace elements:** In this study, the analysis was performed by ContrAA 300 Flame Atomic Absorption Spectrometer (HR-CS AAS) (GLE, Germany). The levels of metals were calculated by using (µg·g⁻¹). Ten elements (Fe, K, Mn, Na, Cd, Cu, Pb, Cr, Al and Be) were detected in the in all flowers, leaf and stem of O. argentata. As it is known metals are unique because of their important role in metabolism (Çiftçi et al., 2009). They are essential part of many important enzymes and they also play roles as catalysts and antioxidants (Hambidge et al., 1987). The concentrations of the trace elements in the samples are presented in Table 2. According to our results, Na, Al, K and Fe contents were high in all studied plant’s sections. Mn, Cd, Cu, Pb, Cr and Be contents of same samples were low.
Fig. 2. The cross-sections of root of *Onosma argentata* (I-II)
p. periderm, c. cortex parenchyma, ca. cambium, ph. phloem, t. trachea, x. xylem

<table>
<thead>
<tr>
<th></th>
<th>Width (µm)</th>
<th>Height (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min. - Max.</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td><strong>Root</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peridermis cell</td>
<td>10.48 - 41.92</td>
<td>24.06 ± 11.25</td>
</tr>
<tr>
<td>Cortex parenchyma cell</td>
<td>15.72 - 41.92</td>
<td>29.50 ± 9.16</td>
</tr>
<tr>
<td>Pith ray diameter</td>
<td>15.72 - 104.8</td>
<td>71.90 ± 29.24</td>
</tr>
<tr>
<td>Trachea diameter</td>
<td>13.10 - 68.12</td>
<td>32.40 ± 18.76</td>
</tr>
<tr>
<td><strong>Stem</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortex parenchyma cell</td>
<td>15.72 - 94.32</td>
<td>55.30 ± 33.47</td>
</tr>
<tr>
<td>Trachea diameter</td>
<td>15.72 - 52.40</td>
<td>33.80 ± 13.89</td>
</tr>
<tr>
<td>Pith cell diameter</td>
<td>31.44 - 157.20</td>
<td>89.10 ± 51.22</td>
</tr>
<tr>
<td><strong>Leaf</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper epidermis cell</td>
<td>20.96 - 73.36</td>
<td>39.80 ± 19.82</td>
</tr>
<tr>
<td>Lower epidermis cell</td>
<td>18.34 - 52.40</td>
<td>30.00 ± 11.94</td>
</tr>
<tr>
<td>Trachea diameter</td>
<td>3.75 - 12.50</td>
<td>8.80 ± 2.65</td>
</tr>
<tr>
<td>Palisade cell</td>
<td>28.82 - 83.84</td>
<td>54.00 ± 21.19</td>
</tr>
<tr>
<td>Spongy cell diameter</td>
<td>15.72 - 36.68</td>
<td>26.20 ± 8.29</td>
</tr>
</tbody>
</table>
Fig. 3. The cross-sections of stem (A) and leaf (B) of *O. argentata*.

### Discussion

No morphological and anatomical information on *O. argentata* is available in literature except general taxonomic properties. In the results presented here, the morphological properties of *O. argentata* showed some similarities and differences compared to other findings in the Flora of Turkey. In the first description of *O. argentata*, the stem is to 35 cm, basal and lower cauline leaves 70-100 x 3-7 mm, pedicels to 11 mm and calyx 14-22 mm. The present findings showed above mentioned measured; 30 cm, 70-90 x 3-5.5 mm, 10 mm, 15-20 mm respectively.

In our anatomical studies, it was determined that the species had a typical dicotyledonous root and stem. The cambium is distinguishable and cortex is rather tight in cross-section. Metcalfe & Chalk (1979) gave information about general anatomical characteristics of Boraginaceae, pointing out that the family to which the species belongs has deeply and superficial periderm. Researchers have stated that *Onosma bracteosum* Hausskn. & Bornm. had secondary root structure, the periderm was multi-layered, the cortex was 10-15 layered and parenchymatic. The xylem was composed of scleranchymatic cells and trachea (Binzet & Akçin, 2012). In this study, it observed that investigated species had those properties. It has not been seen investigated species and *Onosma bracteosum* the stem. The same feature has not also been on the stem of *Symphytum asperum* Lepechin, *Symphytum ibericum* Steven and *Symphytum sylvaticum* Boiss. are belong to Boraginaceae family (Akçin et al., 2007). The Palisade and spongy parenchyma cells are distinguishable in a cross-section of *O. argentata*. Metcalfe pointed out that in many plants, especially among the dicots, parenchyma can be distinguished in the mesophyll palisade and spongy parenchyma.

Akçin & Engin (2005) observed that in *O. bracteosum* mesophyll had a palisade parenchyma close to the upper epidermis, four to six layers of spongy and two to three layers of palisade parenchyma. In *Symphytum asperum*, *S. ibericum* and *S. sylvaticum* the mesophyll had palisade parenchyma close to upper epidermis, single layer of palisade and three to four layers of spongy parenchyma (Akçin & Engin, 2005).

As shown in Table 2, among the metals analysed; the Na, K, Al and Fe contents of flowers, leaf and stem found to be higher than other metals. Due to the high quantity of K, Mg and Ca together with the quantity of Na plus the content of the essential elements Fe, Mn, Zn and Cu allow the seeds are considered as excellent sources of bioelements (Calixto & Canellas, 1982). The analysis of data presented in Table 2 revealed that 4 elements (Na, Al, K and Fe) exist in significant quantities. The contents of iron was found range of 34.40 and 116.58 μg.g⁻¹ in all the samples studied (Table 2). The reason of consistence of iron in leaves more than flowers and bodies is due to the fact that iron exists in chlorophyll structure of plants which has a role for photosynthesis. Existing less quantity of Beryllium in each part of the plant (flowers, leaf, body) shows that Beryllium element doesn’t play an important role in plant structure. The reason of trace element deposition can be explained by the implication of soil, water, air and various chemicals or related to the structure of genetical factors. Therefore, the studies to determine the level of trace elements in plants have contributed to basic biochemical, physiological, taxonomical, morphological and anatomical researches.

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>Metal levels in <em>Onosma argentata</em> plant (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flower</td>
<td>Fe 91.53 K 2133.06 Mn 5.04 Na 409.10 Cd 0.17 Cu 6.10 Pb 0.80 Cr 13.27 Al 62.88 Be 0.05</td>
</tr>
<tr>
<td>Leaf</td>
<td>Fe 116.58 K 1422.19 Mn 13.01 Na 279.08 Cd 0.21 Cu 3.06 Pb 0.10 Cr 25.26 Al 123.21 Be 0.05</td>
</tr>
<tr>
<td>Stem</td>
<td>Fe 34.40 K 1363.30 Mn 4.45 Na 451.47 Cd 0.25 Cu 3.98 Pb 2.57 Cr 9.60 Al 48.45 Be 0.05</td>
</tr>
</tbody>
</table>
References


(Received for publication 2 January 2015)