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Original Article

An anatomical study of *Lepidium graminifolium* L. (Brassicaceae)

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ABSTRACT

Background and Aims: Many taxonomic studies on *Lepidium* species grown in Türkiye have been conducted to illuminate its morphological characteristics. Although many morphological studies occur, only a few anatomical studies are found on *Lepidium* species worldwide. The aim of this study is to reveal the anatomical features of *Lepidium graminifolium.*

Methods: In order to determine the anatomical features of *L. graminifolium*, plant samples were collected and preserved in 70% ethanol, and then all sections were cut separated by hand with a razor blade and stained with Sartur reagent. Microscopic slides were prepared and examined using the light microscope, Olympus BH-2. Photographs were taken with the light microscope and measurements were made using the ImageJ© program. Stomatal indices were calculated using the formula SI (%) = (S/S+E) × 100, where S is the number of stomata per unit area, and E is the number of epidermal cells per unit area.

Results: The results elucidate on the anatomical features of the root, stem, leaf and petiole. A table was prepared by taking measurements of the anatomical structures to present the anatomical characteristics of *L. graminifolium*. This study is the first to elucidate upon the anatomical features of *L. graminifolium*.

Conclusion: Two important structures are mentioned among *Lepidium* species that can be used as distinguishing characteristics: the morphology of the trichomes and the type of stomata. Compared with other studies, the stoma type of *L. graminifolium* was found to differ from other *Lepidium* species. The anatomical features that may differ among other *Lepidium* species are the sclerenchymatous fibers observed in the root, the bifacial type of the leaf mesophyll, and the sparse distribution of unicellular glandular hairs on both the adaxial and abaxial surfaces of the leaves.

Keywords: Anatomy, Brassicaceae, *Lepidium*, Türkiye

INTRODUCTION

Brassicaceae (or Cruciferae), commonly known as the mustard family, is a large plant family comprised of 372 plant genera and 4,060 accepted plant species (International Plant Names Index [IPNI], 2023). Cruciferae are annual, biennial, or perennial grasses and shrubs containing glucosinolates of great scientific and economic importance (Koch & Mummenhoff, 2006). Most of these are distinctly woody (Simpson, 2019). The flowers are actinomorphic and usually consist of four free sepals, four free petals, and six free tetradynamous stamens (Appel & Al-Shehbaz, 2003; Hedge, 1976). The major distribution centers of the family occur in the Irano-Turanian, Mediterranean, and Saharo-Sindian regions (Bona, 2014). Among the distribution regions of the family, the largest number of endemic taxa is found in the Irano-Turanian phytogeographical region and represented by 150 genera and 530 endemic species, followed by the Mediterranean phytogeographical region with 110 genera and 290 endemic species (Al-Shehbaz, Mutlu, & Dönmez, 2007).

The genus *Lepidium* L. is one of the largest genera of the Brassicaceae, consisting of 262 species (IPNI, 2023). It is distributed worldwide, primarily in temperate and subtropical regions. The genus is poorly represented in Arctic climates and grows in mountains in tropical areas. In Türkiye, the genus *Lepidium* is represented by 15 taxa, one of which is considered a naturalized invasive alien plant (Bona, 2013).

Anatomical data have been applied to better understand the interrelationships of plants, and combined analyses have provided confirming evidence in the molecular age of the natural relationships plant families have. Anatomical characteristics are most useful when determining the relationship between different genera, families, orders, and/or other taxonomic categories. Anatomical data have also solved several phylogenetic

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problems. Metcalfe identified several herbarium specimens using vegetative anatomy (Chaffey, 2008). Many studies are also found to have addressed the importance of leaf anatomy and trichome morphology as a distinctive feature for identifying *Lepidium* species (Abdel, 2005; Al-Shehbaz, Beilstein, & Kellogg, 2006).; Beilstein, Al-Shehbaz, & Kellogg, 2006).

Lepidium species have medicinal importance for their sulfur heteroside content. *Lepidium meyenii*, known as Peruvian maca, is used to regulate sexual dysfunction and for a memory enhancement and antidepressant; it also has neuroprotective, antioxidant, anti-cancer, and anti-inflammatory effects (Peres et al., 2020). According to another study, maca is used to manage anemia, infertility, and female hormone balance (Lee, Shin, Yang, Lim, & Ernst, 2011). Due to the *Lepidium* species potential for medical use, determining its morphological and anatomical characteristics and elucidating upon its distinguishing characteristics are important.

L. graminifolium is a perennial species with a wide distribution from Europe to Northwest Africa and Southwest Asia. It grows on dry slopes up to 2000 m above mean sea level. The species is multi-stemmed and highly branched above. The stem is glabrous, and rarely has sparse hairs (Figure 1A-1B). Flowering occurs from April to September (Bona, 2014).

A number of taxonomic studies on *Lepidium* species have been carried out in Eastern Europe (Smirnov, 1948; Dorofeev; 2012; De Carvalho & Vasconcellos, 1964). Hewson (1981) also studied the Australian *Lepidium* species. German (2014) mentioned unresolved taxonomic complexity regarding *Lepidium* species in Central and Southwest Asia, and Bona (2014) made a taxonomic revision of the genus in Türkiye.

Although many morphological studies have occurred, only a few anatomical studies are found on *Lepidium* species worldwide (Grigore & Toma, 2008; Sangekar, Devarkar, Shaikh, Shahane, & Kshirsagar, 2018) The anatomical study of *Aethionema lepidioides* conducted by Tekin (2022) shows the importance of the anatomical characteristics in plant taxonomy for Brassicaceae family members. This is the first detailed study to elucidate on the root, stem, and leaf anatomical features of *Lepidium graminifolium.*

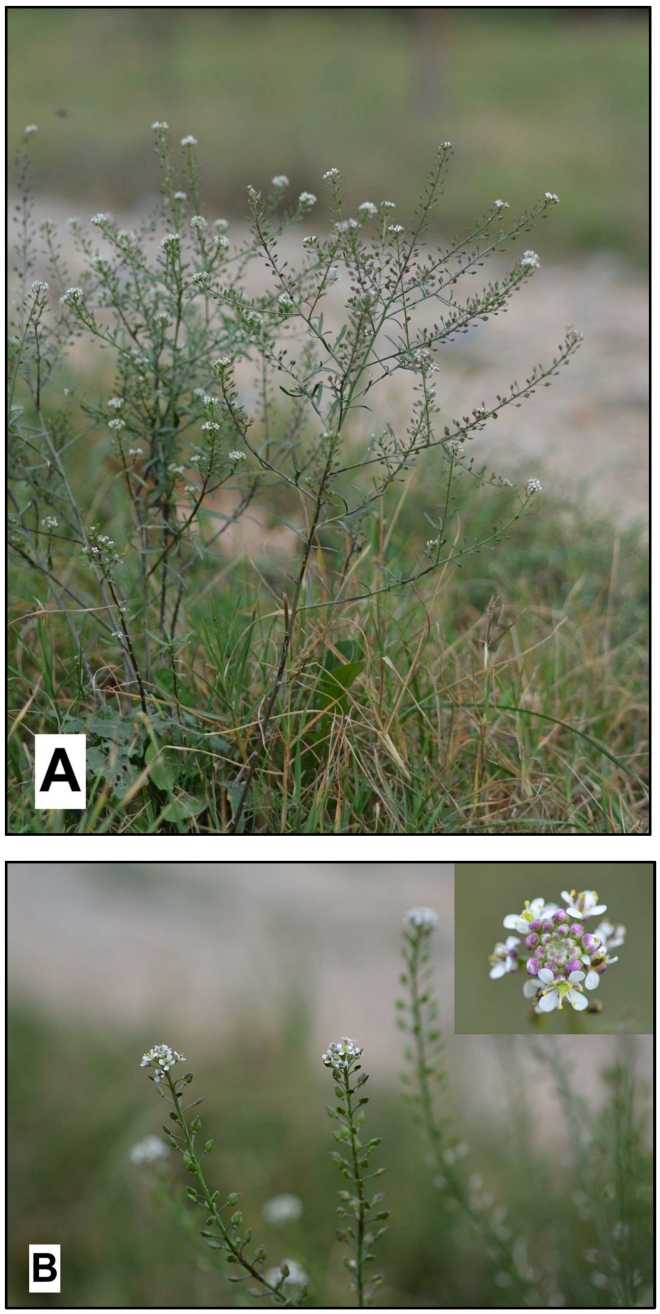

Figure 1. General view of *Lepidium graminifolium.* (A) General view of *L. graminifolium* in its own habitat; (B) Stem and flowers of *L. graminifolium.*

MATERIALS AND METHODS

Plant samples were collected by the first author on November 19, 2022 from Validebağ Grove in Istanbul's Üsküdar municipality. Photos of the plant were taken during its flowering period with a Nikon D7100 camera and 60 mm Nikkor macro lens. The collected plant samples were identified using *Flora of Turkey and the East Aegean Islands* (Vol. I; Hedge, 1965). The prepared herbarium specimens were deposited in the Herbarium of the Faculty of Pharmacy, Istanbul University (ISTE) and recorded with number ISTE: 118370. For anatomical investigations, plant materials were preserved in 70% ethanol, and then all sections were cut separated by hand with a razor blade. All sections were stained using Sartur reagent (Çelebioğlu & Baytop, 1949; Özkan, 2017) and examined with the Olympus BH-2 light microscope. Photographs were taken with the light microscope and measurements were made using the ImageJ© program. Stomatal indices were calculated using the formula: SI (%) = (S/S+E) × 100, where S is the number of stomata per unit area, and E is the number of epidermal cells per unit area. For anatomical descriptions, the study follows the terminology proposed by Metcalfe & Chalk (1957).

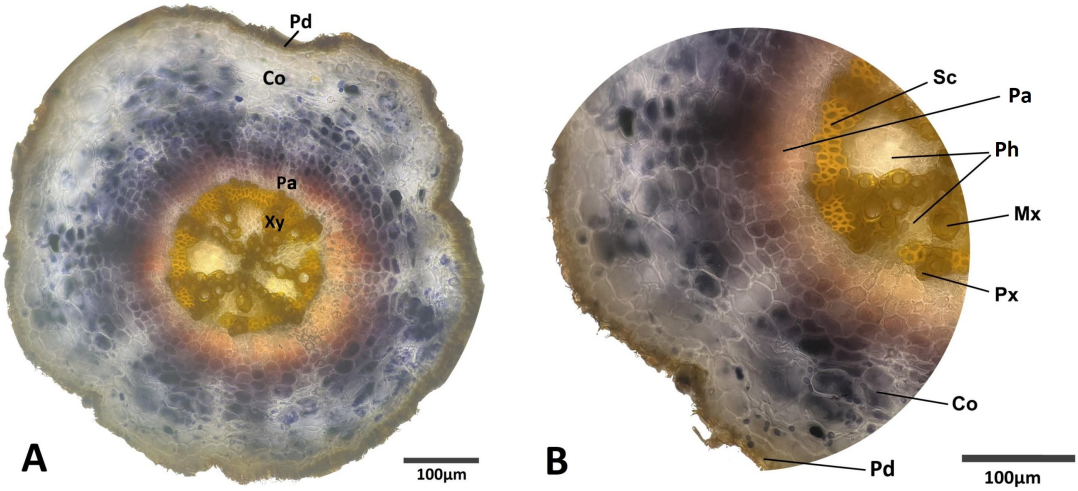

Figure 2. Root cross section of *Lepidium graminifolium*: (A) General view (B) Cortex and vascular bundles (Co = cortex, Mx = metaxylem, Pa = parenchyma Pd = periderm, Ph = phloem; Sc = sclerenchymatous fibers, Px = protoxylem, Xy = xylem).

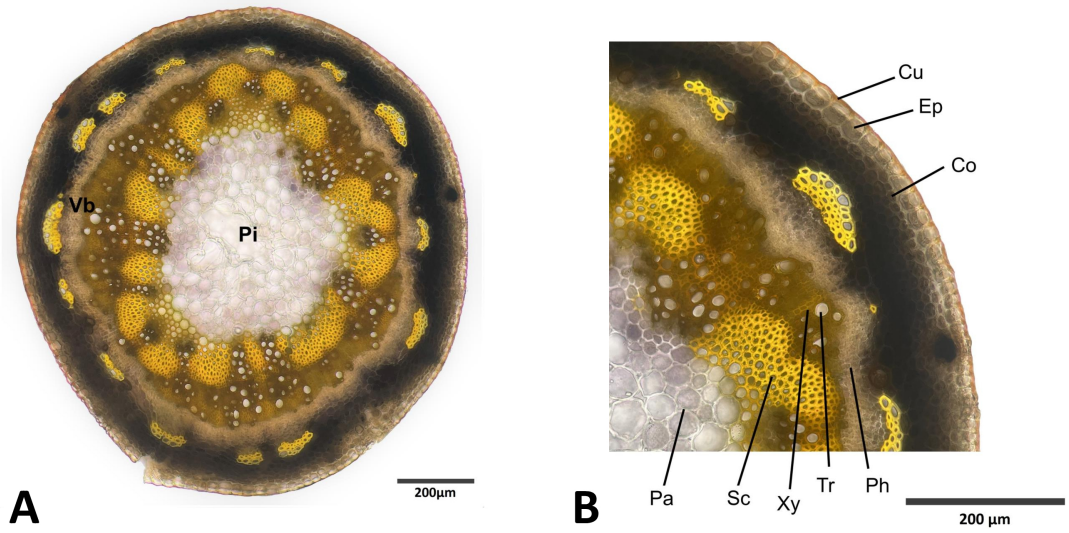

Figure 3. Stem cross section of *Lepidium graminifolium*: (A) General view; (B) Pith and vascular bundles (Cu = cuticle, Ep = epidermis, Co = cortex, Xy = xylem, Ph = phloem, Sc = sclerenchyma, Tr = trachea, Pa = parenchyma, Pi = pith, Vb = vascular bundle).

RESULTS AND DISCUSSION

Anatomical properties of the root

The periderm, which replaces the epidermis as the outer protective tissue, consists of 2 to 3 flattened, unformed cell layers with an average thickness of 17.31 µm (± 7.78 µm SD). The inner layer after the periderm is a cortex consisting of 10-15 layers of isodiametric or irregularly shaped cells with intercellular spaces. The cortex includes sclerenchymatous fiber cells. The inside of the cortex has a well-developed central cylinder, with phloem elements arranged radially between the polyarch xylem arms. The central cylinder is surrounded by 4-6 rows of sclerenchymatous fiber cells. The tracheary elements have an average diameter of 11.09 µm (± 5.36 µm SD; Figure 2). The measurements are listed in Table 1.

Anatomical properties of the stem

The outer protective tissue consists of a single layer of epidermis covered with a thin cuticle (2.15 ± 1.02 µm SD). The average width of the epidermis cells is 19.70 µm (± 6.46 µm SD), and the average length is 20.88 (± 3.44 µm SD). Four to seven layers of collenchyma cells are observed below the epidermis. The cortex, which is an outer layer of the stem, lies below the epidermis and is composed of 5-7 rows of large thin-walled parenchyma cells, followed by sclerenchymatous fibers. These sclerenchymatous fiber groups are located opposite the vascular bundles and above the phloem, which consists of 4-6 rows of isodiametric cells. The xylem and phloem are arranged side by side on the same radius. The xylem is located under the phloem, and no cambium was observed between the xylem and phloem. The xylem has tracheary elements with an average diameter of 11.79 µm (± 3.17 µm SD). The vascular bundles are collateral in type. Between these vascular bundles, sclerenchymatous fibers are seen to spread throughout the stem in the form of a ring. The pith consists of isodiametric parenchyma cells (Figure 3). The measurements are listed in Table 1.

Anatomical properties of leaf

The leaf is bifacial. The mesophyll has two distinct parts. Below the adaxial surface of the leaf is a single line of isodiametric epidermis cells under the outermost thin cuticle layer. The epidermis is followed by palisade parenchyma cells consisting of 3-4 layers of elongated cells. Beneath the palisade parenchyma cells is a sponge parenchyma cell consisting of 3-4 layers of oval or oval-rectangular cells, followed by isodiametric epidermis cells arranged in a single row on the abaxial surface. Stomata are present on the both surfaces of the leaf, so the leaf is amphistomatic. The stoma is anisocytic in type. The stomatal index was calculated as 32.2 for the adaxial surface and as 28.1 for the abaxial surface. In the mesophyll, elongated palisade parenchyma cells are arranged in 2 rows. This is followed by oval-shaped sponge parenchyma cells arranged in 4-5 rows. The mean mesophyll thickness was calculated as 185.70 µm (± 9.72 µm SD). Collateral-type vascular bundles are present within the mesophyll tissue. These represent the midrib and veins of the leaf. Unicellular eglandular trichomes are sparsely distributed on both the adaxial and abaxial surfaces of the leaves. The petiole has a single line of epidermis cells under the outermost thin cuticle layer. From the epidermis towards the center parenchyma, cells are observed to be lined up in 5-6 rows. The center and edges of the petiole have 3-5 vascular bundles, one big central vascular bundle followed by 2-4 little lateral vascular bundles (Figure 4). The measurements are listed in Table 1.

German's (2014) study mentions two important characters that can be used as distinguishing characteristics: the morphology of the trichomes and the type of stomata. When comparing the anatomical data obtained in this study with the anatomical data on different *Lepidium* species found in other studies, anatomical differences are observed to be present. For example, Sangekar's (2018) study on *L. sativum* stated the stoma to be of the tetracytic type, while the current study observed anisocytic-type stoma in *L. graminifolium*. Although most of the anatomical structures of *L. sativum* species are similar to *L. graminifolium*, the difference between stoma types for these two species shows that more anatomical studies can play a role in resolving the taxonomic issues surrounding *Lepidium* species around the world.

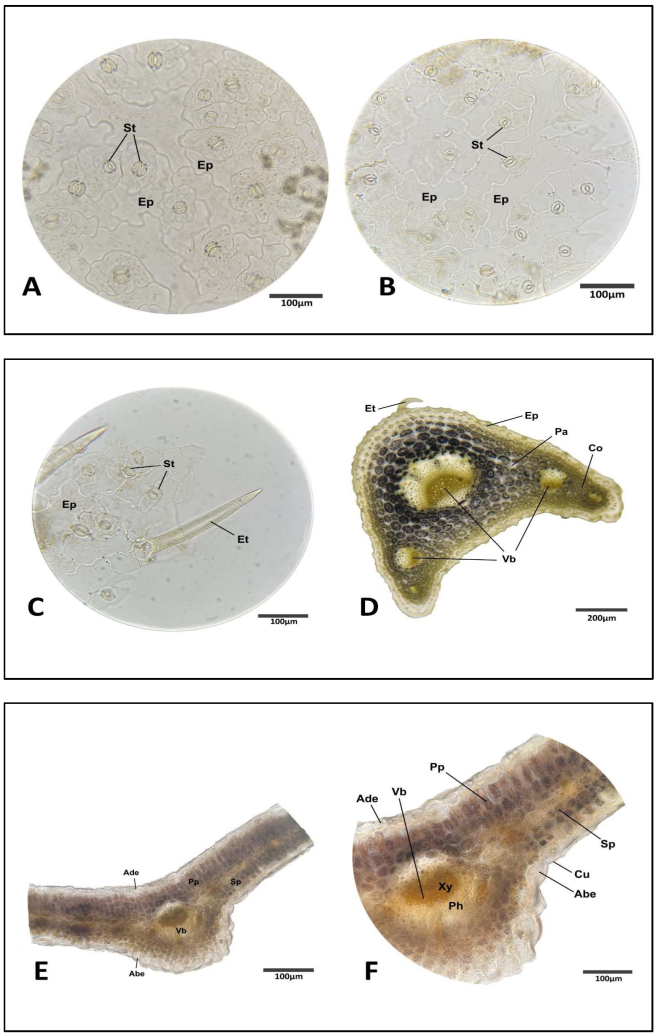

Figure 4. Leaf superficial and cross sections of *Lepidium graminifolium*: (A) Superficial section of abaxial epidermis, (B) Superficial section of adaxial epidermis, (C) eglandular trichome on the adaxial surface, (D) Cross section of the petiole, (E) and (F) Cross sections of the leaf lamina (Ade = adaxial epidermis, Abe = abaxial epidermis, Pp = palisade parenchyma, Sp = sponge parenchyma, Cu = cuticle, Xy = xylem, Ph = phloem, St = stoma, Ep = epidermis, Co = collenchyma, Pa = parenchyma, Vb = vascular bundle, Et = eglandular trichome, St = stoma, Ep = epidermis).

Table 1. Anatomical measurements of *Lepidium graminifolium*

		Width (µm)		Length (µm)	
		Min-Max	**Mean ± *SD***	**Min-Max**	**Mean ± *SD***
Root	Periderm thickness	10.24-28.46	17.31 ± 7.78	–	–
	Cortex cells	6.59-26.32	14.61 ± 7.58	12.98-47.94	31.07 ± 11.28
	Sclerenchymatous fiber cells	2.93-7.79	4.90 ± 1.76	4.73-8.64	7.07 ± 1.67
	Trachea (diameter)	3.44-20.18	11.09 ± 5.36	–	–
Stem	Cuticle thickness	2.66-5.33	3.77 ± 1.09	–	–
	Epidermis cells	9.43-27.55	19.70 ± 6.46	15.61-24.34	20.88 ± 3.44
	Endodermis cells	11.40-13.20	12.36 ± 0.82	14.02-24.05	18.15 ± 3.86
	Trachea (diameter)	6.86-16.23	11.79 ± 3.17	–	–
	Parenchyma cells(diameter)	13.73-49.71	29.54 ± 11.75	–	–
Leaf	Adaxial epidermis cells	20.87-37.67	27.16 ± 6.26	18.42-27.42	22.45 ± 3.90
	Abaxial epidermis cells	24.53-53.47	36.51 ± 12.12	18.91-46.15	32.41 ± 10.87
	Palisade parenchyma cells	11.04-18.43	15.15 ± 3.01	22.11-40.53	34.26 ± 6.88
	Spongy parenchyma cells	12.71-24.53	19.44 ± 3.98	11.71-20.16	15.93 ± 3.26
	Mesophyll thickness	175.47-200.34	185.70 ± 9.72	–	–
	Eglandular trichome	24.40-35.63	30.74 ± 4.38	244.67-297.56	267.01 ± 21.27
	* *SD* = Standard Deviation				

CONCLUSION

This is the first study to elucidate upon the anatomical features of *L. graminifolium.* The sclerenchymatous fibers observed in the root, the bifacial type of leaf mesophyll, and the sparse distribution of unicellular glandular hairs on both the adaxial and abaxial surfaces of the leaves are the anatomical features that differ from other *Lepidium* species. This study believes that elucidating upon the anatomical features of other *Lepidium* species may reveal results that are able to support morphological data regarding taxonomy.

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