

Morpho-anatomical studies on *Bellevalia paradoxa* Boiss. belonging to Liliaceae

¹*Ahmet Kahraman,¹Ferhat Celep,¹Musa Dogan,²Mehmet Koyuncu

¹Middle East Technical University, Department of Biological Sciences, 06531, Ankara-Turkey

²Ankara University, Pharmaceutical Botany, Faculty of Pharmacy, 06100, Ankara-Turkey

*Corresponding author: ahmetk@metu.edu.tr

Abstract

This current study presents investigations on the morphological and anatomical characteristics of *Bellevalia paradoxa* Boiss., a very attractive geophyte growing in the eastern and northeastern Turkey. For morphological studies, a detailed description as well as a distribution map of *B. paradoxa* are given. The morphological features of the species are also compared to those reported in *Flora of Turkey*. For anatomical studies, tranverse sections of the roots, scapes and leaves, and surface sections of the leaves are studied for the first time. The results show that the plant has roots composed of 7 protoxylem arms and 3-4 large metaxylem, amphistomatic and equifacial leaves, 2-3-layered palisade parenchyma, three-layered leaf margins and anomocytic stomata. Raphide crystals in the leaves of *B. paradoxa* and their distribution were determined using a light microscope.

Key words: Liliaceae; *Bellevalia paradoxa*; Morphology; Anatomy; Turkey

Introduction

Bellevalia Lapeyr., a genus of spring-flowering bulbous plants in the family Liliaceae, mostly occurs in the Mediterranean and the central-west Asiatic region. 74 species of *Bellevalia* are presented in *The World Checklist of Seed Plants* (Govaerts, 1996). The largest number of species in the genus is found in the Irano-Turanian phytogeographical region (Feinbrun, 1938-1940). In *the Flora of Turkey and the East Aegean Islands* (Volume 8), Wendelbo (1984) recognized 18 species of *Bellevalia*. Later, he (1985) reduced *B. latifolia* Feinbrun to a synonym of *B. olivieri* (Baker) Wendelbo. With the second supplement to the *Flora* (Volume 11), Ozhatay (2000) represented three more species. Subsequently, Johnson (2003) reduced *B. pycnantha* (K.Koch) Losinsk. to a synonym of *B. paradoxa* Boiss. With a new Turkish endemic species *B. leucantha* K.Perss. described by Persson (2006), the total number of species has now reached 21, eleven of which are endemic. The main characters such as leaf width and pubescence, raceme shape and density, pedicel/perianth length ratio, perianth tube/lobe ratio and colour, anther and bud features can be used to distinguish the species of the genus *Bellevalia* (Cowley et al., 1994). In flowering material, the shape of the filaments is the most significant morphological character for separating *Bellevalia* from its taxonomically closely related genera *Hyacinthella* Schur, *Hyacinthus* L. and *Muscari* Mill. (Persson and Wendelbo, 1979). *Bellevalia paradoxa* Boiss., a perennial geophyte, is geographically distributed in East and Northeast Anatolia between altitudes of 500 m and 3000 m. *B. paradoxa* may be used as an ornamental plant in gardens on account of its attractive dark, dull violet brown or blue violet flowers. It is known as 'çayır sümbülü' in Turkey. *B. paradoxa* seems to be closely related to endemic *B. rixii* Wendelbo. It differs from *B. rixii* by its straight leaves, nodding perianth, yellow anthers, longer fruiting pedicels (up to 14 mm) and narrower valves of capsule (up to 10 mm). Some studies have been carried

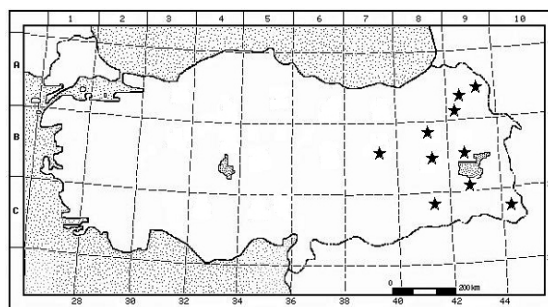


Fig 1. Distribution map of *Bellevalia paradoxa* in Turkey

out by Feinbrun (1938-1940), Wendelbo (1980, 1984), Bothmer and Wendelbo (1981), Vaikos et al. (1989), Johnson (2003), Kaviani (2008) and Rohman et al. (2009) on *Bellevalia* and other genera in the Liliaceae. However, studies on anatomy of *Bellevalia* are rather limited (Kandemir et al., 2000; Lynch et al., 2006). Some significant morphological peculiarities of *B. paradoxa* and its anatomical ones have not been provided before. Therefore, this present study aims to provide a comprehensive description of the morphological and anatomical properties of *B. paradoxa* for the first time.

Material and methods

The plant specimens were collected from Tunceli (B7 Tunceli: Ovacık to Tunceli, Ovacık valley, 1050 m, MKoyuncu 15030) and cultivated in Nezahat Gökyiğit Botanic Garden (NGBB) in İstanbul. A distribution map of *B. paradoxa* is given in Fig. 1. Morphological studies were carried out on fresh material and dried samples stored in AEF, ANK and GAZI herbaria in Ankara and the results were compared to *Flora of Turkey* (Wendelbo, 1984). For anatomical studies, the samples were kept in 70% alcohol until the sections were prepared. The paraf-

Table 1. Comparison of morphological characters of *Bellevalia paradoxa* (with its synonym *B. pycnantha*) based on our study and the Flora of Turkey

| Morphological characters | Our findings | Flora of Turkey |
|--------------------------------------|------------------------------|-----------------|
| Bulb shape | Globose-ovoid | Not recorded |
| Bulb diameter (cm) | (1-) 1.5-3 | Not recorded |
| Tunic texture | Membranous | Not recorded |
| Tunic colour | Pale to dark brown | Not recorded |
| Leaf length (cm) | (6-) 10-30 (-45) | Not recorded |
| Leaf width (mm) | (2-) 3-20 | 4-18 wide |
| Leaf shape | ± linear-lanceolate | Not recorded |
| Scape number | 1 (-3) | Not recorded |
| Scape length (cm) | 8-45 | 10-40 |
| Flower number on raceme | 10-25 (-45) | 10-18 |
| Raceme length x width in flower (cm) | 1.5-8 x 1-3 | Not recorded |
| Raceme length in fruit (cm) | Up to 12 | Not recorded |
| Rachis colour | Green to purplish | Not recorded |
| Bract/pediceal ratio | Bracts shorter than pedicels | Not recorded |
| Pediceal length in flower (mm) | 3-8 | 2-6 |
| Pediceal length in fruit (mm) | Up to 14 | Up to 12 |
| Perianth length (mm) | (3-) 5-6.5 (-7) | 6-6.5 |
| Tube length (mm) | 2.5-6 | Not recorded |
| Lobe length (mm) | 0.5-1.5 | Not recorded |
| Anther length (mm) | 0.6-1 | Not recorded |
| Capsule diameter (mm) | 3-10 | 8-10 |
| Capsule colour | Brown to bluish | Bluish |



Fig 2. General appearance of *Bellevalia paradoxa*

in method was used for transverse sections of the roots, scapes and leaves. The material was embedded in paraffin wax, sectioned at 8-12 µm thickness with a rotary microtome (Leica RM2125RT), stained in safranin-fast green solution and permanently mounted using Canada Balsam. Moreover, surface sections of the leaves were manually taken. The sections were examined and photographed using a binocular light microscope (Leica DM1000) with a microscopy camera (Leica DFC280).

Results

Morphological properties (Fig. 2)

Bulbs globose-ovoid, about (1-) 1.5-3 cm diameter, tunics membranous, pale to dark brown. Leaves 2-3 (-6), longer or shorter than scape, to (6-) 10-30 (-45) cm in length, (2-) 3-10 (-20) mm in width, ± linear-lanceolate, green, margins smooth. Scapes single or rarely 2-3, 8-30 (-45) cm in height (including raceme), often tinged purplish.

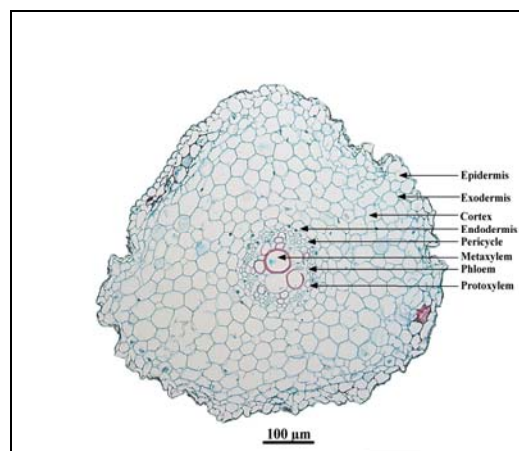


Fig 3. The transverse section of the root of *Bellevalia paradoxa* (10x10)

Raceme many flowered, 10-25 (-45), broadly ovoid to ellipsoid, spike-like, 1.5-8 x 1-3 cm, elongating to 12 cm and lax in fruit, rachis green to purplish. Bracts very small, shorter than pedicels. Pedicels a bit longer than the perianth in flower, 3-8 mm, to 14 mm in fruit. Perianth (3-) 5-6.5 (-7) mm, dark dull violet-brown or blue-violet flowers, urceolate; tube 2.5-6 mm long, lobes 0.5-1.5 mm long, outer lobes triangular. Anthers 0.6-1 mm, yellow. Capsules 3-10 mm diameter, orbicular to obovate, brown to bluish.

Habitat: Alpine pasture, fields, rock ledges, wet meadows, 500-3000 m.

Phytogeographic region: Irano-Turanian element.

Distribution: East and Northeast Anatolia: Ağrı, Artvin, Bitlis, Erzurum, Hakkari, Kars, Muş, Siirt, Tunceli, Van.

Specimens examined: A9 Erzurum: Allahuekber Mountains, Leg. Ümmü Gülsüm, Det. N. Özhatay & B. Koçak, AEF 19726! Kars: Arpaçay, Kurt Kale, between Koçköy and Bardaklı, 1750 m, 13.06.1984, H. Ocakverdi, ANK 1802! Kars: Sarıkamış, Yağbasan plateau, 2550 m,

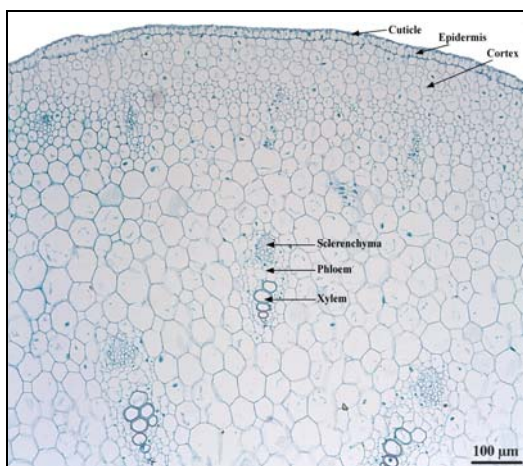


Fig 4. The transverse section of the scape of *Bellevalia paradoxa* (10x10)

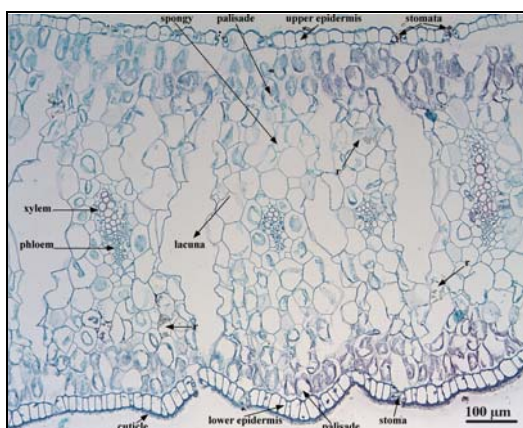


Fig 5. The transverse section of the leaf of *Bellevalia paradoxa*, r: raphide crystals (10x10)

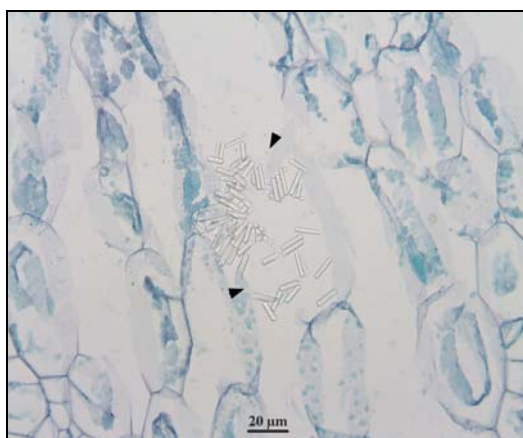


Fig 6. Raphide crystals (arrowheads) in spongy parenchyma in the leaf of *Bellevalia paradoxa* (40x10)

25.06.1973, K. Karamanoğlu, N. and M. Tanker & M. Koyuncu, AEF 4235! Kars: Kısır Mountain, 3000 m, 03.07.1957, Hedge et Davis 30534 (cited in *Flora of Turkey* as *B. paradoxa*), ANK! Kars: Horasan to Karaorgan, 14 miles (22 km) from Horasan, 2000 m,

13.06.1957, Hedge et Davis 29506 (cited in *Flora of Turkey* as *B. pycnantha*), ANK! B7 Tunceli: Ovacık to Tunceli, Ovacık valley, 1050 m, MKoyuncu 15030! B9 Bitlis: Adilcevaz to Süphan Mountain, Süte plateau, 1900 m, 04.06.1993, Y. Altan 4793, GAZI! Van: Bahçesaray, Vari Krapet pass, 2900-3100 m, 17.06.2000, E. Yurdakulol & N. Demirkuş, ANK!

Anatomical properties

Root anatomy

The epidermis is made up of a single layer of flattened cells on the outermost surface. 1-2-layered exodermis is located under the epidermis. The cortex covers a large area with 6-8 layers of mainly polygonal (rarely orbicular) parenchymatous cells. The single layered endodermis is composed of thick walled cells. The direction of these thicknesses is towards the pericycle, which consists of single layered and thin walled cells. There are 7 protoxylem ridges between the phloem strands. The central vascular cylinder is occupied by 3-4 large metaxylems (Fig. 3).

Scape anatomy

On the outside is the monolayered epidermis, which comprises a single layer of almost square (sometimes rectangular) cells and is covered by a thick cuticle. There are no hairs on the epidermis. The multilayered cortex consists of orbicular or hexagonal parenchymatic cells. Vascular bundles are numerous and scattered throughout the cortex. They are also surrounded by a sclerenchymatic bundle sheath (Fig. 4).

Leaf anatomy

The upper and lower epidermis are uniseriate. While the upper epidermis is composed of almost square cells, the lower epidermis comprises rectangular ones, often the length longer than the width. The upper epidermis is covered by a thinner cuticle than the lower epidermis. The leaf is amphistomatic and equifacial. Beneath both the surfaces of the mesophyll is present 2-3 layered palisade parenchyma (Fig. 5). There are abundant raphide crystals in spongy parenchyma located between the palisade (Fig. 6). Their dimensions are 6-20 (-30) µm long and 1.2-4 µm wide. Vascular bundles are arranged in a single row with small and large bundles alternating. There are large xylem elements in an anticlinally elongated column in larger bundles. Lacunae are present between vascular bundles. The midrib do not constitute a projecting part. Margins are tapering with 3-layered cells and with thickened walls (Fig. 7). Stomata are anomocytic, formerly known as Ranunculaceous type. They are found on the surfaces of both sides, but more abundant on the upper surface (Fig. 8). They also lie at the same level as the epidermal cells.

Discussion

Observations and measurements of some morphological characters of *Bellevalia paradoxa* (with its synonym *B. pycnantha*) are presented for the first time in this study (Table 1). The results (e.g. raceme and capsule shape, perianth and anther colour) obtained from morphological investigations are mainly congruent with the description reported in *Flora of Turkey* (Wendelbo, 1984). However,

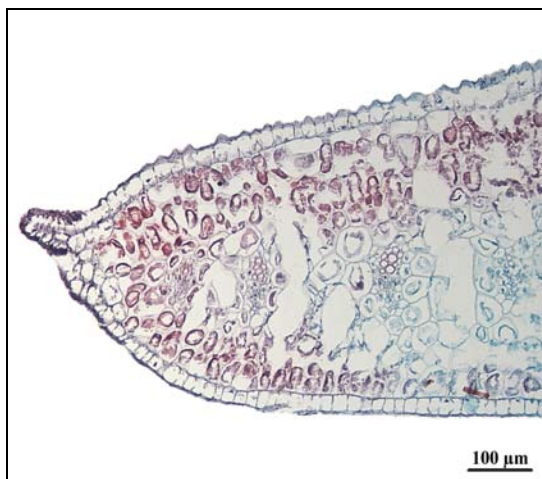


Fig 7. The transverse section of the leaf margin of *Bellevalia paradoxa* (10x10)

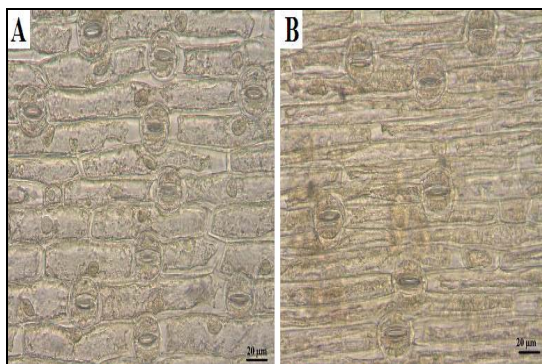


Fig 8. The surface section of the leaf of *Bellevalia paradoxa*. A, The stomata from the upper epidermis; B, the stomata from the lower epidermis (40x10)

the morphological features show some differences from those given in the *Flora*. The dimensions of some characters of the plant such as leaves, scapes, flowers, pedices and capsules seem to be more variable than previously (Table 1). In the study, the investigations on root, scape and leaf anatomy of *B. paradoxa* are first reported. The number of strands of protoxylem in roots of *Bellevalia* varies one species to another. In the root of *B. paradoxa* xylem has 7 sets of protoxylem, but the root of *Bellevalia gracilis* Feinbrun comprises 8 to 9 protoxylem arms (Kandemir et al., 2000). Thus, this character can be used to distinguish the species. Anatomical features of scapes of *B. paradoxa* are resemble to the general characteristics of monocotyledons (Cutter, 1971). Lynch et al. (2006) observed lacunae between vascular bundles in leaves of *B. glauca* Kunth, *B. nivalis* Boiss. & Kotschy and *B. romana* Sweet, except *B. flexuosa* Boiss. Furthermore, marginal cells were three-layered and thick-walled. We observed same features in the leaves of *B. paradoxa*. Our results also revealed that there were 2 to 3 layered palisade parenchyma in the mesophyll of *B. paradoxa* However, Kandemir et al. (2000) determined only 2 layered palisade parenchyma in the mesophyll of *B. gracilis*. The distribution and shape of calcium oxalate crystals in plant tissues including leaves, stems, roots, seeds and floral structures may be represented as useful taxonomical characters in monocotyledons (Prychid and

Rudal, 1999). Needle-like calcium oxalate crystals, raphides, occur in spongy parenchyma cells in only the leaves of *B. paradoxa*. Kandemir et al., 2000 observed them not only in leaves but also in the stem cortex of *B. gracilis*. Stomata are present on upper and lower surfaces of *B. paradoxa*, but they are more abundant on the upper surface. Zarinkamar (2006) has observed that the stomata were present on both surfaces of all the species examined in the Liliaceae, with the exception of *Polygonatum orientale* Desf., where they are absent from the upper surface. He also reported that in some genera such as *Asparagus*, *Bellevalia* (*B. fominii* Woronow) and *Muscaria* stomata frequencies on the upper surface are greater than those of the lower surface whereas in *Allium* and *Asphodeline* they are more on the lower surface.

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References

- Bothmer RV, Wendelbo P (1981) Cytological and morphological variation *Bellevalia*. Nord J Bot 1:4-11
- Cowley J, Ozhatay N, Mathew B (1994) New species of Alliaceae and Hyacinthaceae from Turkey. Kew Bulletin 49:481-489
- Cutter EG (1971) Plant anatomy: Experiment and interpretation, part 2, Organs. Addison-Wesley Publishing Company, London
- Feinbrun N (1938-1940) A monographic study on the genus *Bellevalia* Lapeyr. Palestine J Bot Jer Ser 1:42-54, 131-142, 336-409
- Govaerts R (1996) *World checklist of seed plants*. Antwerp, Belgium, Continental publishing 2:85-86
- Johnson MAT (2003) Polyploidy and karyotype variation in Turkish *Bellevalia* (Hyacinthaceae). Bot J Linn Soc 143:87-98
- Kandemir N, Ergen Akcin O, Cansaran A (2000) A morphological and anatomical investigation on some geophytes distributed in the vicinity of Amasya. The Herb J Syst Bot 7(2):127-147
- Kaviani B (2008) Micromorphological, morphological and anatomical investigation of the *Lilium ledebourii* (Baker) Bioss. (Liliaceae) indigenous to Iran. Aust J Crop 1(1):6-10
- Lynch AH, Rudall PJ, Cutler DF (2006). Leaf anatomy and systematics of *Hyacinthaceae*. Kew Bulletin 61:145-159
- Ozhatay N (2000) *Bellevalia* Lapeyr. In: Guner A, Ozhatay N, Ekim T, Baser KHC (eds) *Flora of Turkey and the East Aegean Islands* (Second Supplement). Edinburgh University Press, Edinburgh 11:240-241
- Persson K, Wendelbo P (1979) *Bellevalia hyacinthoides*, a new name for *Strangweia spicata* (Liliaceae). Botaniska Notiser 132:65-70
- Persson K (2006) One new and one emended species of *Bellevalia* (Hyacinthaceae) from Turkey. Bot J Linn Soc 150(2):253-260

- Prychid CJ, Rudal PJ (1999) Calcium oxalate crystals in monocotyledons: A review of their structure and systematics. *Ann Bot* 84:725-739
- Rohman MM, Suzuki T, Fujita M (2009) Identification of a glutathione S-transferase inhibitor in onion bulb (*Allium cepa* L.). *Aust J Crop* 3(1):28-36
- Vaikos NP, Markandeya SK, Pai RM (1989) Floral anatomy of the Liliaceae: Tribe Convallarieae. *Proc Indian Acad Sci* 99(2):91-95
- Wendelbo P (1980) Notes on *Hyacinthus* and *Bellevalia* (Liliaceae) in Turkey and Iran. *Notes RBG Edinburgh* 38:423-434
- Wendelbo P (1984) *Bellevalia* Lapeyr. In: Davis PH (ed) *Flora of Turkey and the East Aegean Islands*. Edinburgh: Edinburgh University Press, Edinburgh 8:264-274
- Wendelbo P (1985) 16. *Bellevalia* Lapeyr. In: Townsend CC, Guest E (eds) *Flora of Iraq*. Baghdad, Ministry of Agriculture 8:113-127
- Zarinkamar F (2006) Density, size and distribution of somata in different monocotyledons. *Pakistan J Biol Sci* 9(9):1650-1659