

THE ANATOMICAL STUDIES ON TWO *BIARUM* (ARACEAE) SPECIES IN TURKEY

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In this study, anatomical properties of *Biarum marmarisense* (P. C. Boyce) P. C. Boyce and *Biarum pyrami* (Schott) Engl. var. *pyrami* were examined. The specimens were collected from Antalya province, South West of Anatolia. Degree of spathe tube margin connection, distribution of pistillodes and staminate-pistillate flower zone interstice length characters are important in *Biarum* Schott. taxonomy at specific level. The cross-sections of root, stem and leaf parts of the plant were examined and demonstrated by photographs. Although thicknesses of the cell wall of the endodermis and xylem strands are distinguishing features, raphide crystals are same feature in both species. *B. marmarisense* has observed paracytic stoma types while *B. pyrami* var. *pyrami* has anomocytic stoma types. Most of the anatomical properties were similar to the other members of the Araceae family.

Key words: anatomy, *Biarum*, geophytes, Turkey

INTRODUCTION

Araceae family of 113 genera and over 3,170 species is most diverse in the New World tropics, although also distributed in the Old World tropics and north temperate regions (Anonymous 2013, Mayo *et al.* 1997). Some members of Araceae were used as ornamentals, house plants, food, medicinal, as arrow poisons and as biopesticide (Ajaib *et al.* 2011, Anonymous 2014, Mohammed *et al.* 2006, Rajput *et al.* 2010). *Biarum* is a genus of flowering plants in the Araceae family and comprises 21 species of dwarf tuberous-stemmed herbs that occurs in semi-arid and seasonally dry areas of southern Europe, North Africa, and the Near and Middle East. The centre of diversity is the Middle East, where 75% of the species occur as endemics (Boyce 2006). *Biarum* are often found growing in rock crevices and gravelled soil composed largely of limestone (Bown 2000). *Biarum* genus has been represented by 10 taxa in Turkey (Akan and Balos 2008).

Biarum marmarisense and *Biarum pyrami* var. *pyrami* are distributed in the south of Turkey (Fig. 1). *Biarum* species is perhaps the most readily distinguishable of the autumn flowering *Biarum* species due to the globose, strongly inflated, spathe tube, large spathe and greatly attenuated spadix appendix.

The bullate leaves of the typical variety are also unusual in the genus and provide a ready means of identifying *B. pyrami* var. *pyrami* in Turkey, where no other species shares this character (Boyce 2008, Fig. 1). *B. marmarisense*, on the island of Simi in the eastern Aegean has shown that bees and queen wasps are attracted by the sweet lilac-like perfume emitted by the inflorescences (Boyce 2008).

Since the classical work by Solereder and Meyer (1928), there have been many publications on leaf and petiole anatomy of Araceae, and an extensive list of references can be found on French *et al.* (1995) and Keating (2000, 2003, 2004a). The recent extensive revision on the morphology of *Biarum* has been done by Boyce (2006, 2008). But, there is no study on *B. marmarisense* and *B. pyrami* var. *pyrami*. The aim of the present paper to provide anatomical information about these species.

MATERIAL AND METHODS

Plants samples were collected from Antalya-Finike, which is situated in the SW part of Turkey, in October 2013. Taxonomic description of taxa was done according to Davis (1984). Some plants were dried according to herbarium techniques. Fresh samples and herbarium material were used in each case for experimental analysis and morphological measurements.

Plant samples were fixed in 70% alcohol for anatomical studies. Anatomical studies were carried out on fresh samples or samples kept in alcohol. Hand-made cross-sections were prepared with razor blade from root, scape and leaves. The sections were stained by safranin/fast green dyeing method (Bozdağ *et al.* 2016). Anatomical measurements were made with the help of micrometric ocular. Results were presented by original drawings, photographs and tables. Photographs were taken with Leica DM LB microscopy.

RESULTS

Biarum pyrami var. *pyrami*

Root: The epidermis consists of elongated cells is two layered on the outer surface of the root. There is no cuticle layer on the epidermal cells. The cortex consisted of 6–8 layered parenchymatous cells has not got intercellular spaces. The thickness of the cell wall of endodermis cells is not clear, but towards metaxylem, the cell walls are observed thicker. On the inside of the endodermis and the adjacent to it is a cylinder shape of parenchyma cells called the pericycle. This is one cell wide and consists of thin-walled cells. Root xylem forms 4–5 strands which point toward the pericycle. There are one or more metaxylem on the midpoint of the vascular cylinder (Fig. 2A, B).

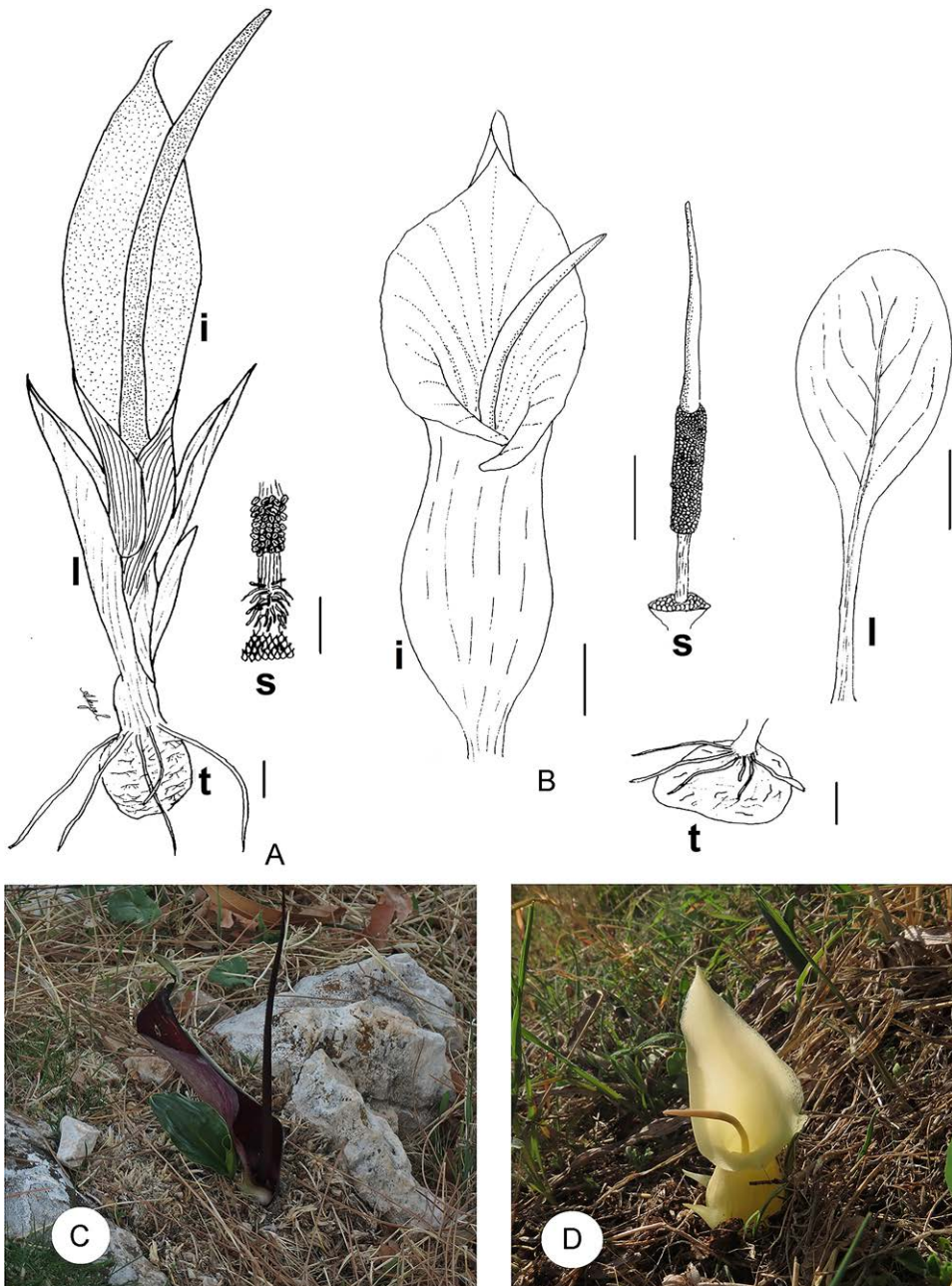


Fig. 1. Details and general appearance in natural habitat of *Biarum pyrami* var. *pyrami* (A, C) and *B. marmarisense* (B, D) (s = spadix, I = inflorescence, l = leaves, t = tuber. Scale bars: 1 cm)

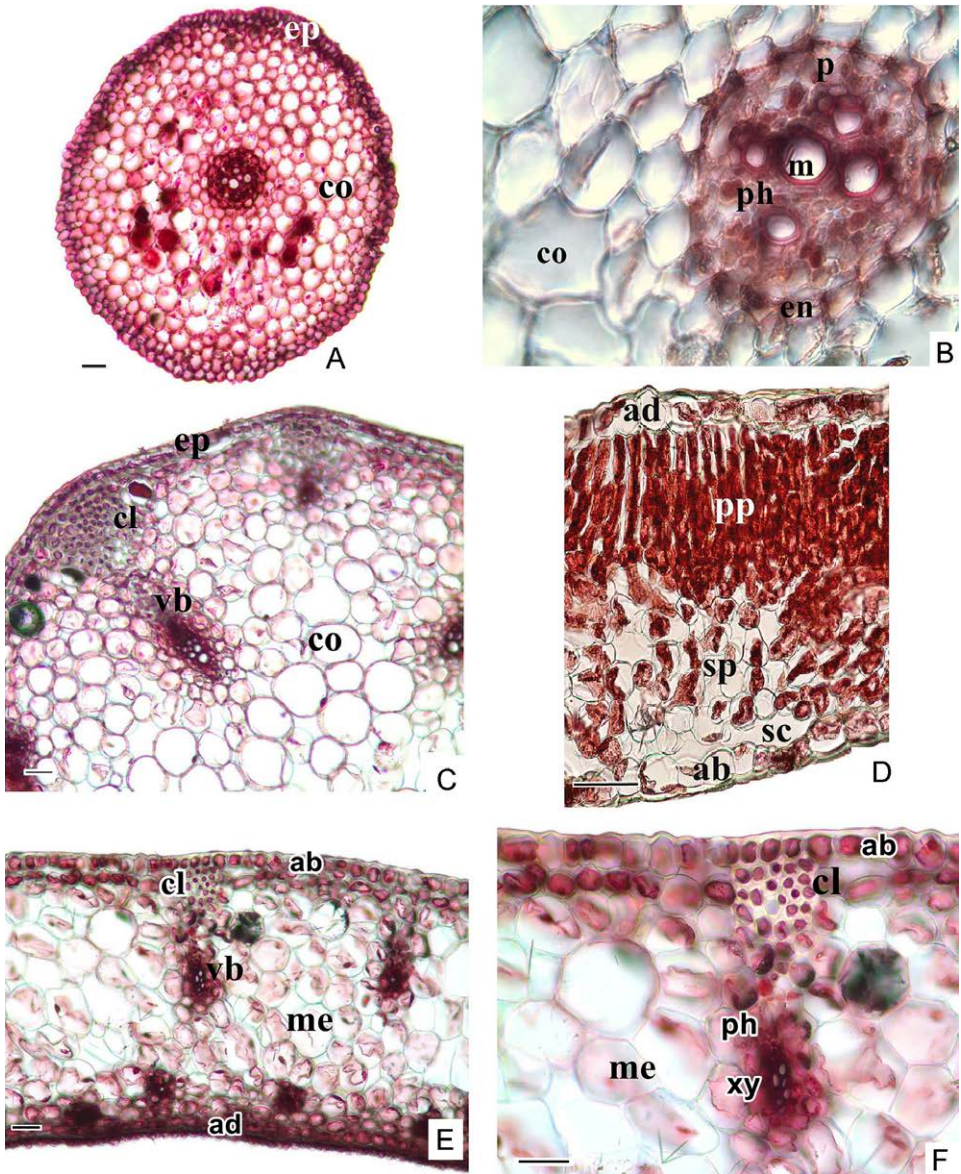


Fig. 2. The cross-section of root (A, B), scape (C), leaf (D), spathe (E, F) of *Biarum pyrami* var. *pyrami* (ab = abaxial epidermis, ad = adaxial epidermis, cl = collenchyma, co = cortex, en = endodermis, ep = epidermis, m = metaxylem, me = mesophyll, p = pericycle, ph = phloem, pp = palisade parenchyma, sc = substomatal cavity, sp = spongy parenchyma, vb = vascular bundle, xy = xylem). Scale bars: 50 μ m)

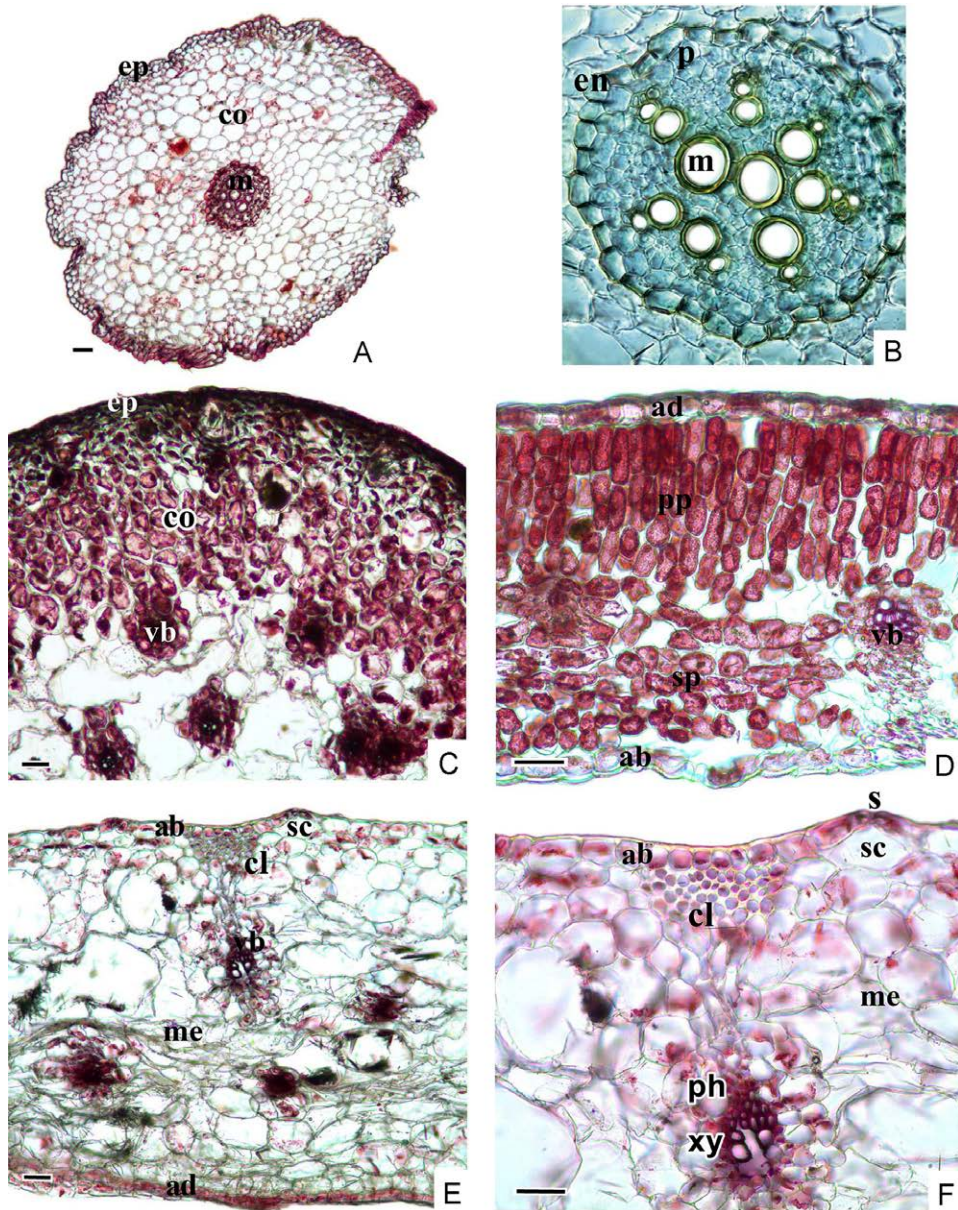


Fig. 3. The root of (A, B), scape (C), leaf (D) and spathe (E, F) cross-section of *Biarum marmarisense* (ab = abaxial epidermis, ad = adaxial epidermis, cl = chlorenchyma, co = cortex, en = endodermis, ep = epidermis, m = metaxylem, me = mesophyll, p = pericycle, ph = phloem, pp = palisade parenchyma, s = stomata, sc = stomatal cavity, sp = spongy parenchyma, vb = vascular bundle, xy = xylem). Scale bars: 50 μ m)

Table 1
Anatomical measurements of *Biarum* species (SD = standard deviation)

	Width (µm)		Length (µm)	
	min.–max.	mean±SD	min.–max.	mean±SD
<i>B. pyrami</i> var. <i>pyrami</i>				
Root, epidermis cell	13.60–27.20	18.20±4.37	10.88–19.04	15.54±2.89
Root, cortex cell (diam.)	32.64–59.84	44.20±5.70		
Root, trachea (diam.)	9.52–16.32	12.58±2.36		
Scape, epidermis cell	21.76–38.08	28.75±5.90	10.88–24.48	16.32±4.01
Scape, pith cell (diam.)	32.13–107.10	61.96±9.78		
Scape, trachea (diam.)	9.52–21.76	14.96±4.41		
Leaf, adaxial cuticle	2.72–5.44	4.08±1.07		
Leaf, adaxial epidermis	13.60–21.76	18.49±3.01	16.32–27.20	23.39±4.00
Leaf, abaxial epidermis	13.60–21.76	18.49±3.02	16.32–24.48	20.12±2.48
Leaf, palisade parenchyma	10.88–19.04	14.96±2.51	21.76–35.36	28.10±4.32
Leaf, spongy parenchyma	10.88–27.20	19.04±5.26		
Leaf, trachea	5.44–16.32	10.88±3.56		
Leaf, abaxial cuticle	2.72–6.80	4.76±1.42		
<i>B. marmarisense</i>				
Root, epidermis cell	10.88–21.76	16.59±3.25	13.60–27.20	20.94±4.56
Root, cortex cell (diam.)	19.04–68.00	38.89±8.17		
Root, trachea (diam.)	10.88–29.92	17.95±3.44		
Scape, epidermis cell	10.71–32.13	17.24±4.15	10.71–53.55	26.77±5.78
Scape, pith cell (diam.)	42.84–117.8	70.60±9.64		
Scape, trachea (diam.)	10.71–64.26	29.77±6.73		
Leaf, adaxial cuticle	2.72–5.44	3.70±1.01		
Leaf, adaxial epidermis	19.04–43.52	27.74±4.48	16.32–29.92	22.30±4.30
Leaf, abaxial epidermis	10.88–24.48	17.95±4.30	13.60–21.76	17.40±2.70
Leaf, palisade parenchyma	16.32–27.20	21.76±3.43	40.80–73.44	55.76±5.57
Leaf, spongy parenchyma	19.04–40.80	28.10±4.40		
Leaf, trachea	5.44–13.60	9.61±3.01		
Leaf, abaxial cuticle	2.72–8.16	5.44±1.82		

Scape: The epidermis consists of elongated cells is a single layer of cell covering the entire of the scape. The presence of thick cuticle layer on the outer surface of the epidermal cells can be distinguished. The cortex composed of thin-walled and circular parenchyma cells with intercellular spaces

is 8–10 layered under epidermis. A 10–12 collateral vascular bundles, which have thick-walled cells are arranged in a ring. The 8–9 layered collenchyma is present under the epidermis at the corners of scape. The xylem is observed in a larger area than the phloem (Fig. 2C). There are pith cells in the centre of scape. These cells are 32.13–107.10 μm in diameter (Table 1).

Leaf: The epidermis is a single layer of cells covering the abaxial and adaxial surfaces, where there is a thick cuticle layer. This layer is 2.72–6.80 μm . Two regions of the mesophyll are distinguishable, the uppermost consists of barrel-shaped palisade parenchyma cells that are in 2–3 rows and the lower region is consisting of loosely arranged spongy parenchyma cells are multi-layered with abundant air spaces between them (Fig. 2D). The raphides are seen in the mesophyll (Fig. 4A, B). The vascular bundles are arranged regularly in the centre of the mesophyll.

Spath: Thick cuticle is present on adaxial and abaxial surfaces of the leaf. The abaxial epidermis is perforated by the stomata under which there is large substomatal cavity. Single layered hypodermis is situated on the inside of the epidermis. The collenchymatous cells are present in the adaxial zones (Fig. 2E, F). Mesophyll layer is composed of homogeneous cells and also raphide crystals are observed there (Fig. 4A, B).

Biarum marmarisense

Root: The epidermis is two layered on the outer surface of the root. There is very thin or no cuticle layer on the epidermal cells. Cortex is composed of 10–12 layered parenchymatous cells and these cells become smaller towards to endodermis and epidermis. The cell wall is on the cortex-side of the endodermis is suberised. There is pericycle layer, which is formed thin-walled cells in a single row under the endodermis. Six–eight xylem strands are present on the periphery of the vascular cylinder of *B. marmarisense* (Fig. 3A, B).

Scape: There is a cuticular layer on the epidermal cells of the cross-section of the scape. The cortex, which is composed of multi-layered parenchyma cells adjacent to a single-layered epidermis, has some aerenchyma cells in which there are raphide crystals and tannin. Vascular bundles become compactly arranged towards to the pith (Fig. 3C).

Leaf: A thick cuticle layer covers the outer surface of the leaves. Abaxial cuticle is thicker than adaxial cuticle with 2.72–8.16 μm in size (Table 1). Leaf is bifacial, consisting of palisade and spongy parenchyma. Palisade parenchyma is 3–4 layered and arranged very compactly. Spongy parenchymatic region is very narrow. Palisade parenchyma is 5–6 layered and covers almost equal area to spongy parenchyma (Fig. 3C, D, E). Raphide crystals are observed intensively in aerenchyma (Fig. 4C, D).

Spathe: There is a thick cuticle on both surfaces of the spathe. Towards to abaxial side, the collenchyma is situated on vascular bundles (Fig. 3E, F). Mesophyll is homogeneous and raphide crystals are observed intensively (Fig. 4D).

DISCUSSION AND CONCLUSIONS

The anatomical analysis given in this work provides the first detailed description of *Biarum pyrami* var. *pyrami* and *B. marmarisense*, which is compa-

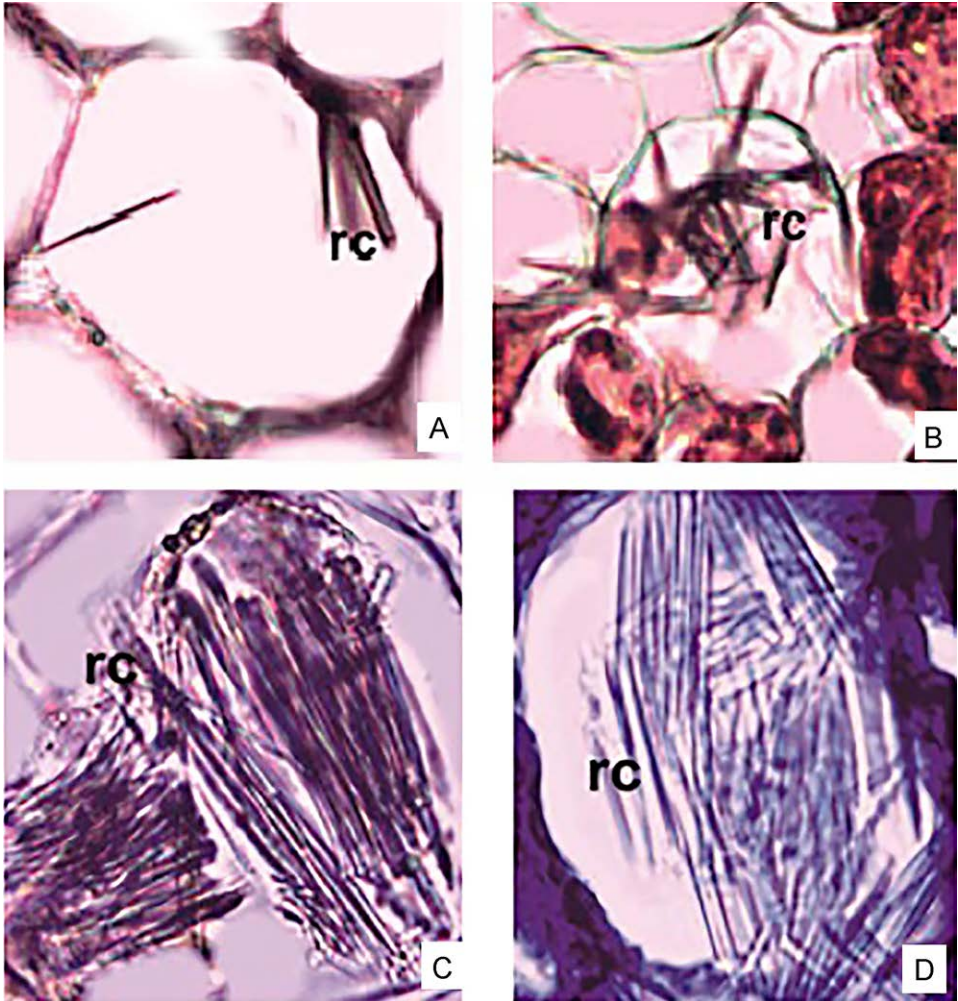


Fig. 4. Crystals in cross-section of *Biarum pyrami* (A, B) and *B. marmarisense* (C, D) (rc = raphide crystals)

rable with findings of some other examined Araceae members (Higaki *et al.* 1984, Mantovani and Pereira 2005, Mastroberti and Mariath 2003, Bercu and Făgăraş 2010).

Characteristically, the primary endodermis consists of slightly suberised cells possessing, at places, passing cells opposite xylem (Kroemer 1903). The present study showed that the thicknesses of the cell wall of the endodermis cells are not the same with each taxon; at *Biarum marmarisense* the cell walls are observed thicker on outwards, while at *B. pyrami* var. *pyrami* towards to the centre. Similarly Bercu and Făgăraş (2010) reported one-layered with slightly suberised cell walls in cross-section of root of *Spathiphyllum wallisii* Regel. *B. pyrami* var. *pyrami* has 4–5 xylem strands, while *B. marmarisense* has 6–8 xylem strands. Bercu and Făgăraş (2002) and Batanouny (1992) have observed that the vascular system is quite well developed numbering in more than six other Araceae species, i.e. *Monstera deliciosa* Liebm. and *Spathiphyllum* Schott spp.

There is a hypodermis layer on the inside of the epidermis of scape of *B. pyrami* var. *pyrami*. The cross-section of the scape of *B. marmarisense* has large intercellular spaces in which there are some raphide crystals. Researchers have observed druse and raphide crystals in mesophyll of Araceae before. Also, they have noted that other crystals can be found in this layer (Genua and Hillson 1985, Keating 2003, 2004b, Mayo *et al.* 1997). Mantovani and Pereira (2005) have concluded that raphides of calcium oxalate crystals are found with low frequency, but druses are found very frequently. The raphides occur rarely and only on the spongy parenchyma while the druses are frequent in all mesophyll and in both surfaces in *Anthurium* Schott species. The presence, morphology and distribution of crystals within species are very important because it is under genetic control (Franceschi and Nakata 2005).

Epidermal cells in leaves of *Biarum* species are short, randomised, but the vascular bundles are elongated and distributed parallel to the longitudinal direction of the body as in *Biarum* species. This provision is generally found in leaves of monocotyledons (Metcalf 1963). However, Mayo (1986) showed randomly distributed small cells in the epidermis of the spathe species of *Philodendron* Schott (Araceae). The number and distribution of cells subsidiaries of stomata are vary significantly in Araceae (French *et al.* 1995). In case of some plant species leaf anatomy and leaf epidermal anatomy can help identification therefore it has got great importance sometimes (Hameed *et al.* 2002, Munsif *et al.* 2007). In this study paracytic stoma types have been observed in *B. marmarisense*, while *B. pyrami* var. *pyrami* has anomocytic stoma types.

The cross section of spathe of mesophyll is uniform, showing no differentiation between palisade and spongy parenchyma of spathe of both species. The mesophyll cells have compact arrangement of *Biarum* species. The same feature has been observed in *Anthurium* species (Mantovani *et al.* 2010). Ac-

According to Keating (2000), the expression of collenchyma appears to be complex in this family. That is, its position in an organ is reliable as an indicator of some suprageneric categories. On the adaxial surface of the palisade parenchyma is usually interrupted by a collenchymatous tissue in both species. The same feature is observed among other members of the Araceae family (Mantovani and Pereira 2005, Bercu and Făgăraş 2010). Batanouny (1992), Fahn (1990) and Bercu (2009) have concluded that it is a characteristic feature that for Araceae species both epidermis continuity is broken by the presence of stomata with large substomatal cavities. Druse crystals are seen in some of the mesophyll cells. This substomatal cavity has been seen in both *Biarum* taxa.

As a result, this work points out that *B. pyrami* var. *pyrami* is compared with *B. marmarisense*. However, further research with the other Araceae species as in a recently done study may illuminate whether any taxonomic implication is present or not within the genus *Biarum* or in the family. We expect these findings to contribute to the further phylogenetic and taxonomic studies into the *Biarum* species.

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