

THE MORPHOLOGICAL AND ANATOMICAL STUDIES ON ENDEMIC *CROCUS BIFLORUS* MILLER SUBSP. *PULCHRICOLOR* (HERBERT) MATHEW (IRIDACEAE) IN TURKEY

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Abstract

In this study, the morphological and anatomical characteristics of *Crocus biflorus* subsp. *pulchricolor* (Iridaceae) were investigated. The subsp. *pulchricolor* has 4 leaves, 1 mm broad; bracts drying brownish. These properties are characteristics of these plants. In anatomical studies, cross-sections of the root, stem and leaves were examined. These parts photographed and compared with the other *Crocus* species and Iridaceae family species.

Introduction

The *Crocus* genus is one of the members of the family of Iridaceae. The plants in this family are herbs with rhizomes, corms or bulbs. Iridaceae is a large and diverse family of about 92 genera and 1800 species and mainly distributed in the Southern hemisphere continents (Ali & Mathew, 2000). It is represented by 37 species in Turkey (Güner *et al.*, 2000).

Different *Crocus* species have received attention by several workers, who have concentrated on the morphology (e.g., Mathew, 1984) and anatomy (Akan & Eker, 2004; Özdemir & Akyol, 2005; Özdemir *et al.*, 2004, 2006; Kerndorff & Pasche, 2003, 2004, 2006; Akan *et al.*, 2007; Satıl & Selvi, 2007; Özdemir & Kılınç, 2008).

Crocus genus is a geophyte plant. While some geophytes are used as ornamental plants, others are used in medicine and as food. In recent years there are some studies on the use of medicinal areas about geophytes (Gilani *et al.*, 2002; Seyidoğlu & Yayım, 2009; Fatima *et al.*, 2011; Sher *et al.*, 2011; Ahmad *et al.*, 2012; Deeb *et al.*, 2013).

Crocus biflorus has eighteen subspecies (Davis, 1984; Güner *et al.*, 2000, Kerndorff & Pasche, 2004; Erol *et al.*, 2009). Eight of them are endemic. In this study, morphological and anatomical characteristics of endemic *Crocus biflorus* subsp. *pulchricolor* were studied.

Materials and Methods

Plants samples were collected from Bursa Uludağ, situated in the West part of Turkey, in May 2012, Akyol 2495 (Fig. 1). Taxonomical determination of the subspecies followed Davis (1984).

Collected specimens were kept in the herbarium at Celal Bayar University. Morphological illustration of the plant taxon was made from fresh and dry specimens according to "Flora of Turkey" (Davis, 1984, Güner *et al.*, 2000). Morphological measurements were taken from the root, scape and leaf of the fresh specimens. Living materials were fixed in (70 %) alcohol for anatomical studies. An Olympus microscope was used for examination of the anatomical cross-sections after staining with Sartur Reactive (Baytop, 1981). Preparates were photographed with motorized Leica DM 300 microscope. Measurements of root, stem and leaf cell

sizes of each species were taken by using ocular-micrometer. Minimum, maximum, mean and standard deviations were determined.

Results

Morphological findings: Plant 11-12 cm length, corm tunic membranous with toothed rings at base, 1 cm diameter. Leaves usually 4, synanthous, 0.5-1 mm broad. The leaves usually exceeding the plant. Prophyll absent. Bracteole is present, subequal to bract. Throat of perianth pale yellow, glabrous or finely papillose; segments 2.2 x 0.5 cm, obtuse, deep blue violet, exterior sometimes prominently striped, veined, speckled or stained with purple, dark blue or grey. Filaments 7 mm, yellow, glabrous or finely papillose; anthers 5,5 mm, yellow, sometimes yellow with blackish basal lobes, sometimes yellow with a greyish or blackish connective. Style dividing into 3 reddish-orange expanded branches (Fig. 2).

Anatomical findings

Root: There is a single-layered epidermis covered by thick cuticle on the outer surface of root. Cortex is 4-5 layered and has parenchymatous cells. Cells of cortex are ovoid, in rectangular shapes with intercellular spaces. Endodermis is single-layered. Endodermal cells are 7, 5-12,5 x 3, 7-7, 5 µ with wall thickenings of the endodermal clear in cross-section (Table 1). Single-layered pericycle was located inside endoderm. Metaxylem is present on the median part of the cross-section. Xylem strands on the periphery of the vascular cylinder are 4-5 which reach the pericycle (Fig. 3).

Scape: The cross section of the scape show that, the cells of epidermis are covered by a thick cuticle. Epidermis is single layered and isomorphic, 5-12, 5 x 7,5-12, 5 µ in size (Table 1). 3-6 cell layered cortex cells are oval and cubic shaped 12,5-30 µ in diameter. Intercellular spaces are present in the cortex of scape. Different sized vascular bundles are located in three circles. Parenchymatic pith consists of circle shaped cell and have intercellular spaces. Pith cells are bigger when compared to with cortex cells. There are few starch grains in parenchymatic cells at cortex (Figs. 4, 5).



Fig. 1.* Natural population of *Crocus biflorus* subsp. *pulchricolor*.



Fig. 2. a: General appearance of *Crocus biflorus* subsp. *pulchricolor*, b: ovarium, c: stamen, d: pistil, e: fruit, f: leaf, g: tepal. Scale bars: 1 cm.

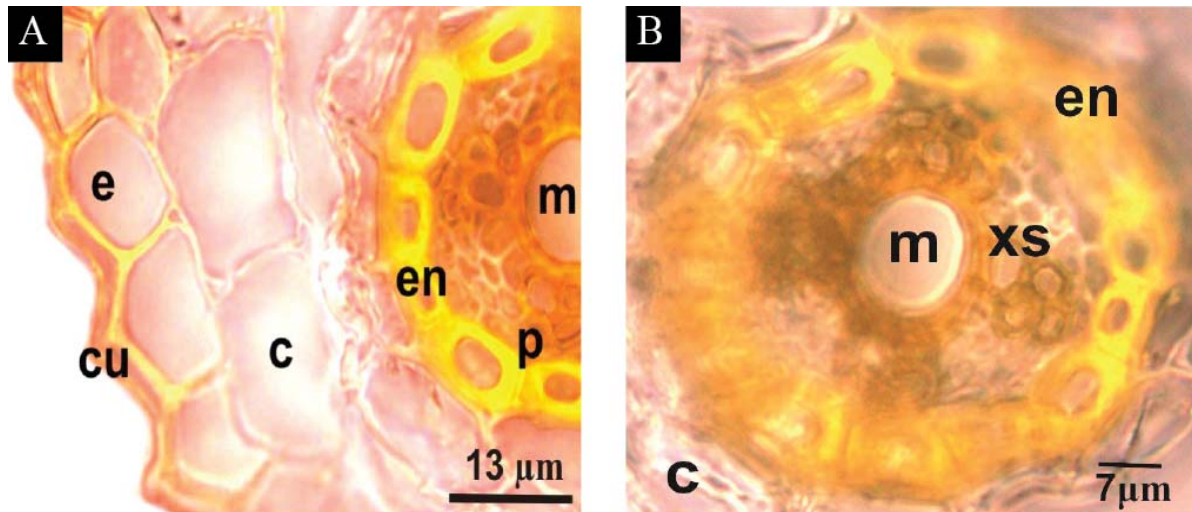


Fig. 3A,B. The cross section of root of *Crocus biflorus* subsp. *Pulchricolor*
c: cortex, **cu:** cuticle **e:**epidermis, **en:** endodermis, **m:**metaxylem, **p:** pericycle, **xs:** xylem strands.

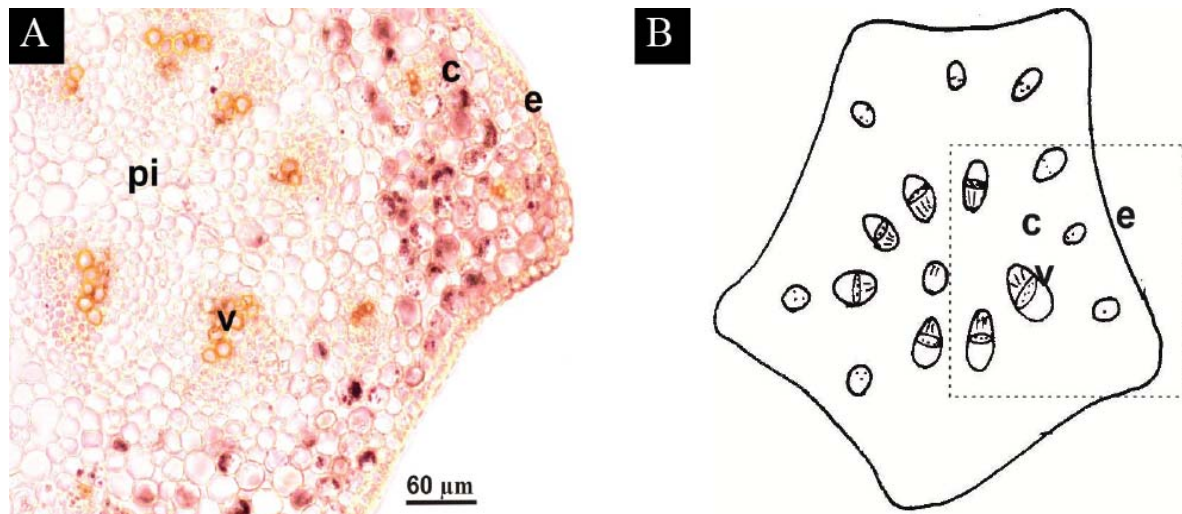


Fig. 4. A. The cross section of scape of *Crocus biflorus* subsp. *pulchricolor*; B. Enlargement of the shown area A, **c:** cortex, **e:** epidermis, **pi:** pith, **v:** vascular bundle

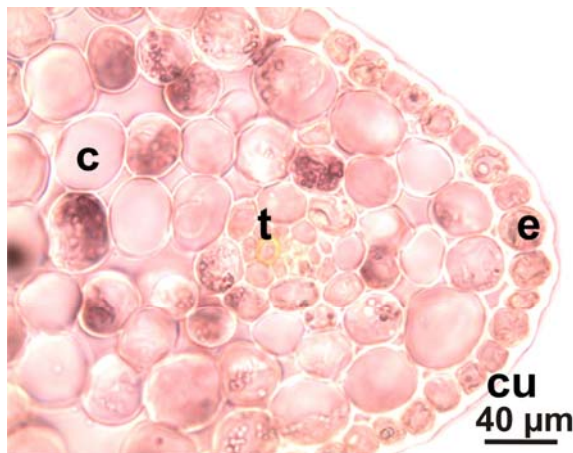


Fig. 5. The cross section of scape of *Crocus biflorus* subsp. *pulchricolor*; **c:** cortex, **cu:** cuticle, **e:** epidermis, **t:** trachea.

Leaf: Leaves of *C. biflorus* subsp. *pulchricolor* have a central rectangular keel and two lateral arms, with their margins recurved towards the keel. The characteristic pale stripe running axially along the center of the leaf is caused by the parenchymatous cells in the keel which lack chloroplast and break down to form an air space. Epidermis is single layered on abaxial and adaxial surface of leaf. Mesophyll located in the lateral arms had chloroplasts and differentiated into palisade and spongy parenchyma. Palisade parenchyma cells are 2-3 layered and rectangular shaped and $10-17,5 \times 27,5-37,5 \mu$ in sizes (Table 1). Spongy cells are present on abaxial side. Vascular bundles are located in one row in arms of keel and mostly extending around abaxial margin of the keel and towards arm margins (Fig. 6).

Table 1. Anatomical measurements of *Crocus biflorus* subsp. *pulchricolor*.

	Width (μm)		Length (μm)	
	Min-Max	Mean \pm SD	Min-Max	Mean \pm SD
Root				
Epidermis cell	10-12,5	11,5 \pm 1,2	10-15	12 \pm 1,8
Cortex cell (diameter)	15-32,5	22,5 \pm 6,8	25-30	26,2 \pm 2,1
Endodermis cell	7,5-12,5	9,2 \pm 1,8	3,7-7,5	5,2 \pm 1,2
Pericycle cell	5-7,5	5,6 \pm 1,08	2,5-5	4,2 \pm 1
Metaxylem (diameter)	12,5-15	14,1 \pm 1,03		
Scape				
Epidermis cell	5-12,5	10 \pm 1,5	7,5-12,5	10 \pm 1,5
Cortex cell (diameter)	12,5-30	22 \pm 5,7		
Trachea (diameter)	7,5-15	12,5 \pm 2,7		
Pith (diameter)	17,5-27,5	24,3 \pm 4,09		
Leaf				
Adaxial Epidermis	12,5-17,5	14,5 \pm 1,8	7,5-12,5	10,5 \pm 1,7
Abaxial Epidermis	10-15	12,5 \pm 1,5	10-12,5	7,5 \pm 12,5
Palisade parenchyma	10-17,5	15 \pm 1,7	27,5-37,5	31,2 \pm 4,1
Spongy parenchyma	7,5-15	12,5 \pm 2,73	10-25	16 \pm 5,14

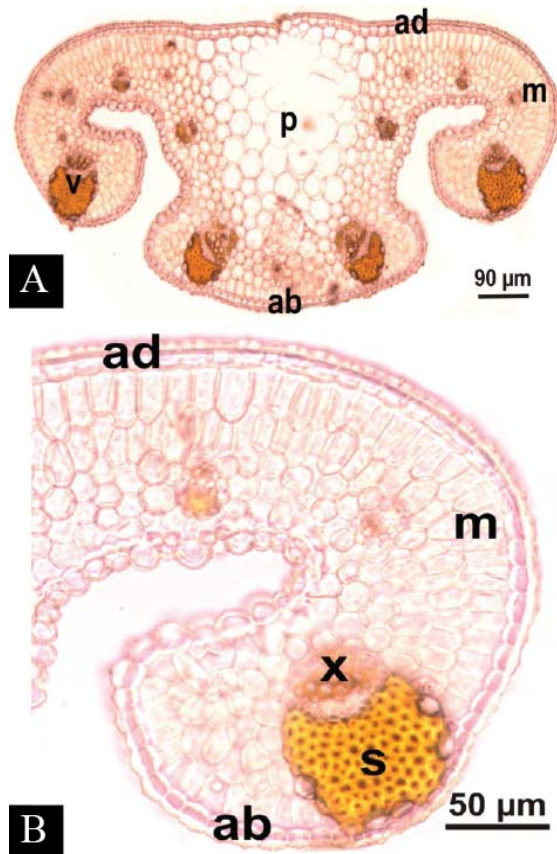


Fig. 6. A, B. The cross section of leaf of *Crocus biflorus* subsp. *pulchricolor*, **ab**: abaxial epidermis, **ad**: adaxial epidermis, **m**: mesophyll, **p**: parenchyma, **s**: sclerenchyma, **v**: vascular bundle, **x**: xylem.

Discussion

No studies on *Crocus biflorus* subsp. *pulchricolor* that is the subject of this study have been found except some taxonomically, general morphological and phytogeographical studies of these species (Kerndorff & Pasche, 2006). So the *Crocus biflorus* subsp. *pulchricolor* was investigated from morphological and anatomical view point in this study. We aimed to give detailed knowledge about morphology and anatomy of the species that have economic value *C. biflorus* subsp. *pulchricolor* have some different morphological characteristics from other subspecies of *Crocus biflorus* such as 4 pieces leaves not larger than 1mm. These properties are taxonomical characters that is used to determine the species. As regards results presented here, the morphological properties of *C. biflorus* subsp. *pulchricolor* showed some similarities and differences compared to other findings in Flora of Turkey (Davis, 1984). Morphological measurements of *C. biflorus* are given wide range in Flora of Turkey. Considering that a large number of subspecies of these species, it is important that are given measurements in this study for determining subspecies, as was done by Yousaf *et al.*, (2004).

In anatomical studies it has been determined that root of taxon is typical as monocotyledons root. Researchers have observed that in the cross section of *Lilium candidum* L., is rectangular shaped and monolayered epidermis cells (Özen *et al.*, 2012). The thickening is clear on the walls of the endodermal cells (Fukuhara & Shinwari, 1994). The walls of root endodermal cells of *C. biflorus* subsp. *pulchricolor* have completely thickening.

This feature has also been observed that, in the cross-section of root of *Crocus fleischeri* Gay, *Lilium ciliatum* P.H. Davis and (Özdemir, 2003; Özdemir *et al.*, 2004). Nawaz *et al.*, (2012) have observed thick endodermis in the cross section of root of *Asparagus* L. (Liliaceae) species. The root of *C. biflorus* subsp. *pulchricolor* has not got a pith which is then occupied by metaxylem. There are 3-4 xylem strands.

Intercellular spaces are present in the cortex of scape. Different sized vascular bundles are located in the peripheral and central parts of the stem (Table 1). These bundles are in ring position. Same features have been observed in *Crocus aeriis* Herb., *C. danfordiae* Maw and *C. fleischeri* Gay, *C. olivieri* Gay. subsp. *olivieri*, *C. cancellatus* Herbert subsp. *damascenus* (Herbert) Mathew (Özyurt, 1978; Özdemir *et al.*, 2004, 2011, Akan & Eker, 2004). But the vascular bundles in *C. pallasii* Goldb. subsp. *turcicus* Mathew (Akan & Eker, 2004) are found only in the central part of stem.

The leaves of *C. biflorus* subsp. *pulchricolor* parenchymatous cells in the keel lack chloroplast and breakdown to form air space. Rudall (1994) has also pointed out that leaves of most *Crocus* species have distinctive shape in cross section.

Rudall (1994) has also pointed out that the leaves of most *Crocus* species have a unique and distinctive shape comprising a square or rectangular central keel in cross-section. *C. biflorus* subsp. *pulchricolor* has a central rectangular keel and two lateral arms with their margins generally recurved towards the keel shape in cross section. Researchers have observed the same leaf shape in *C. pulchellus* Herbert, *C. speciosus* Bieb., *C. fleischeri* Gay, *C. chrysanthus* (Herbert) Herbert.

In the cross sections taken from leaf palisade parenchyma can be distinguished from the sponge parenchyma cells. Same features have been observed in *Lilium candidum* (Özen *et al.*, 2012).

C. biflorus subsp. *pulchricolor* has some characteristic morphological and anatomical features such as trilobed style and leaves with rectangular keel in spite of the morphological and anatomical similarity with other species of *Crocus* and members of Iridaceae.

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