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Leaf Anatomy of Some Endemic *Crocus* L. (Iridaceae) Taxa from the West Anatolia

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Abstract: Certain anatomical and micromorphological features of six endemic *Crocus* taxa (*C. fleischeri* J. Gay, *C. gargaricus* Herb. subsp. *herbertii* B. Mathew, *C. wattiorum* (B. Mathew) B.F. Mathew, *C. antalyensis* B. F. Mathew, *C. olivieri* J. Gay subsp. *istanbulensis* Mathew ve *C. candidus* Clarke) from the Flora of Turkey were observed in this study in which most of the observed features were reported for the first time. In the anatomy section, general outlook of the crosssections of the leaves is defined with photographs. In addition, arms, keel, papillae, mesophyll features and vascular bundles of the leaves were examined. In the micromorphology section, the leaf surface features of the studied taxa were inspected and epicuticular waxes and shapes of the epidermis cells were determined.

Key words: *Crocus*, leaf anatomy, SEM, iridaceae, micromorphology

INTRODUCTION

There are almost 70 taxa of the genus *Crocus* in Turkey (Mathew, 1984; Davis, 1988; Güner, 2000). Considering this number, the native land of the genus *Crocus* is most probably Turkey. Two or three new *Crocus* taxa from Turkey are described every year, but just a few of them have been investigated by micromorphological aspects (Davis *et al.*, 1984; Mathew, 1982).

In this project, leaf anatomy in cross sections- and the morphological variability of the epicuticular wax layer were investigated in some *Crocus* species.

The leaves of the *Crocus* species have a unique shape in cross section (Rudall and Mathew, 1990). They hold keel at the center of the leaf and two arms next to the keel. They keep this structure with some exceptions. General outline of cross sections, differences and characteristics of leaf blade have been given in some studies on *Crocus* leaves. (Collins, 1937; Erol and Küçüker, 2005; Karasawa, 1942; Rudall, 1990; Rudall and Mathew, 1990; Shorina, 1975). Rudall and Mathew (1990) has an important place among these studies and put forth anatomical data belong to the leaf for consideration as: general outline of cross sections, the existence of papillae, the presence of anticlinal walls of the epidermis cells as situate or smooth, the thickness of the sclerenchyma layer on vascular bundles and the extension of sclerenchyma out of lamina.

Excluding the study of Rudall (1994), any study regarding the epicuticular wax layer has not been known in current literature. The morphological structure of the epicuticular wax layer was analyzed on the leaves of the researched taxa and named according to Barthlott *et al.* (1998).

MATERIALS AND METHODS

Species examined (arranged according to Mathew, 1982): Section Nudiscapus, Series Reticulati (*Crocus gargaricus* Herb. subsp. *herbertii* B. Mathew ISTF 39931); Series Biflori (*Crocus wattiorum* (B. Mathew) B. F. Mathew ISTF 39932); Series Flavi (*Crocus antalyensis* B. F. Mathew ISTF 39933, *Crocus olivieri* J. Gay subsp. *istanbulensis* B. Mathew ISTF 39934, *Crocus candidus* Clarke ISTF 39935); Series Intertexti (*Crocus fleischeri* J. Gay ISTF 39930).

The examined taxa were collected from the field during flowering time. The living material was fixed in FAA and stored in 70% alcohol. For light microscope observation, material was prepared as hand-cut. Cross sections were stained with 0.1% Safranin solution in distilled water (Prasad, 1986) (1% stock solution was diluted according to the need). Semi-permanent slides were prepared with 20% Glycerine. These slides with cover-slips were photographed with the Micros Austria Photo Microscope.

A diagram was drawn in order to evaluate the cross sections of the leaf lamina. The general outline of cross sections of leaves identified as revolute by using ptyxis terminology (Cullen, 1978; Stearn, 1983).

For micromorphological observation, preferably fully developed and uncontaminated leaf samples were mounted on aluminum stubs using double-sided adhesive tape, coated with gold. The specimens were examined with Jeol 5200 SEM.

RESULTS AND DISCUSSION

Figure 1 shows for the evaluation of the cross-sections of *Crocus* leaves. Differences between the observed taxa are given in Table 1. SEM data are summarized in Table 2.

Anatomy:

Leaf surface: Epidermis cells are generally rectangular or elliptical, in the Series Biflori they get narrow at ends and named rhombicus. Stomata are on the abaxial surface. They are located under the arms and lateral sides of the keel. The type of stomata is anomocytic. Generally, the anticlinal walls of the epidermis cells are smooth on the abaxial surface. However *C. candidus* has sinuous anticlinal walls (Fig. 2).

Leaf blade: *Crocus* leaf consists of two arms and a keel in the middle of the arms (Fig. 1 and 3). The general outline of the leaf cross section of genus *Crocus* is revolute in shape with some exceptions (Rudall and Mathew, 1990): *C. carpetanus* Boiss. and Reut.-terete-; *C. scharojanii* Rupr, *C. scardicus* Kosanin arms length equal to keel-.

The leaf type was called trifacial by Velenovsky (1907). This type was also called physiological bifacial by Troll (1939).

The arms can be recurved towards the keel, connected to the keel with a wide angle and ribbed (*C. wattiorum*). Some taxa also have flexible arms and they touch the base of keel (*C. gargaricus* subsp. *herbertii*) (Fig. 3). The base of the keel is either narrow or wide.

The abaxial sides of arm ends and corners of keel might have hair *C. graveolens* Boiss. and Reuter has adaxial hair (Rudall and Mathew, 1990). Some of the observed taxa have hairs which indicated Table 1.

Mesophyll: The parenchyma cells which form the mesophyll have thin walls and are broken down in the middle of the leaf. This area is called lacuna in Fig. 1 and this hollow forms the characteristics white stripe of *Crocus* leaves.

The rest of the leaf is green. Two types of parenchyma cells are present: The palisade layer which is located on the upper side of the arms and occasionally also at the base of the keel as one or two lined and spongy layers in shape of an oval or ellipse. The leaf looks as if it is bifacial with two types of parenchyma and the shape of leaf blade in cross sections; however, it is homologous to unifacial leaves (Rudall, 1990; Rudall and Mathew, 1990).

Vascular bundles: The vascular bundles of the *Crocus* leaf are quite specific. When leaf cross sections were examined, four major vascular bundles appear. Two of these are located diagonally on the corners of the keel. The others are on the end of arms and the phloem poles appear vertically towards the keel. These are the largest and the most prominent bundles and called the largest vascular bundles (Fig. 1). Large vascular bundles were occasionally seen on the line of the connection of the arms and the keel, the curls of the arms and in the middle of the largest vascular bundles in the keel. There are same areas that jutt out to give the characteristics shape to the leaves especially in the area of large vascular bundles. There is a thick, ring shaped sclerenchyma layer on the largest and large vascular bundles. Despite the different leaf shapes, *Romulea* Maratti (terete) and *Gladiolus* L. (ensiform) also have a ring shaped sclerenchyma layer on large vascular bundles. This condition is particular to subfamily Crocoidea (Erol and Kucuker, 2003; Rudall, 1990).

Small and smallest vascular bundles are on the arms and sides of the keel and occur alternately, depending on the leaf width (Fig. 1).

The important taxonomical structures of *Crocus* leaf

anatomy: General outline of leaf cross section: The general outlines of leaf cross sections of observed taxa are revolute. The length of arms and curving degrees differ amongst the species. Some taxa (*Crocus candidus* and *Crocus olivieri* subsp. *istanbulensis*) are typically revolute; however, some of them (*Crocus wattiorum*) are less distinguishably revolute (Fig. 3). The presence of papillae: Papillae of *C. gargaricus* subsp. *herbertii* are finger like, whereas others' are domelike. Anticlinal walls of epidermis cells being situate or smooth: The anticlinal walls are observed as situate when the leaf surface of *C. candidus* is examined under SEM (Table 2). Thickness of sclerenchyma layer and its jutting areas towards lamina on the vascular bundles: Extension of the sclerenchyma layer at the xylem pole up to the epidermal cells is not seen in any observed taxa. However, in the cross sections

Table 1: The characteristics of the leaf cross sections

| | <i>C. fleischeri</i> | <i>C. gargaricus</i> subsp. <i>herbertii</i> | <i>C. wattiorum</i> | <i>C. antalyensis</i> | <i>C. olivieri</i> subsp. <i>istanbulensis</i> | <i>C. candidus</i> |
|-------------------|--|---|--|---|---|--|
| Leaf shape | | | | | | |
| General outlook | Revolute | Revolute | Revolute | Revolute | Revolute | Revolute |
| Arms | Thick short, ends vertical, reach half of the carina | Long, curled, flexible | Thick, short, connect to the carina with a wide angle, | Connect to carina with a right angle, tips parallel to the carina | Thin long, tips reach to the base of carina | Thin long, ends reach to the base of carina |
| Keel | Corners oval, juttred out | Base wide, corners acute and reach arms | Base extremely wide, corners circular | Square | Corners hairy, base narrow | Corners±acute, base narrow |
| Mesophyll | | | | | | |
| Parenchyma | | | | | | |
| Spongy | Oval | Oval | Oval elliptical | Oval | Oval | Oval-elliptical |
| Palisade | Double lined, narrow-long rectangular | Double lined, short rectangular | Single lined short, rectangular | Double lined, short rectangular | Double lined, short rectangular | Double lined, inferior line smaller, short rectangular |
| Vascular bundles | Typical | Typical | Large bundles jut out | Typical | Typical | Typical |
| Epidermis | | | | | | |
| Shape | Single lined±square | Single lined±square, occasionally sinuous | Single lined ±square | Single lined ±square | Single lined ±square | Single lined ±square |
| Papillae | Dome | Finger-like | Dome | Dome | Dome | Dome |
| Hair | Absent | Absent | Absent | Absent | On the arm ends and the comers of the carina | on the corners of the carina |

Table 2: The characteristics of the leaf surfaces

| Taxa | Shape of epidermal cells Adaxial surface | Wax characteristic |
|--|---|---|
| <i>C. fleischeri</i> | Short, rectangular | Thickenigs around the guardian cells |
| <i>C. gargaricus</i> subsp. <i>herbertii</i> | Long rectangular | Uniform |
| <i>C. wattiorum</i> | Rombicus | Platelets near the anticlinal walls, other surface irregular striati on |
| <i>C. olivieri</i> subsp. <i>istanbulensis</i> | Long rectangular | Uniform |
| <i>C. antalyensis</i> | Narrow and long rectangular | Uniform |
| <i>C. candidus</i> | Long rectangular, anticlinal walls sinuat | Uniform |

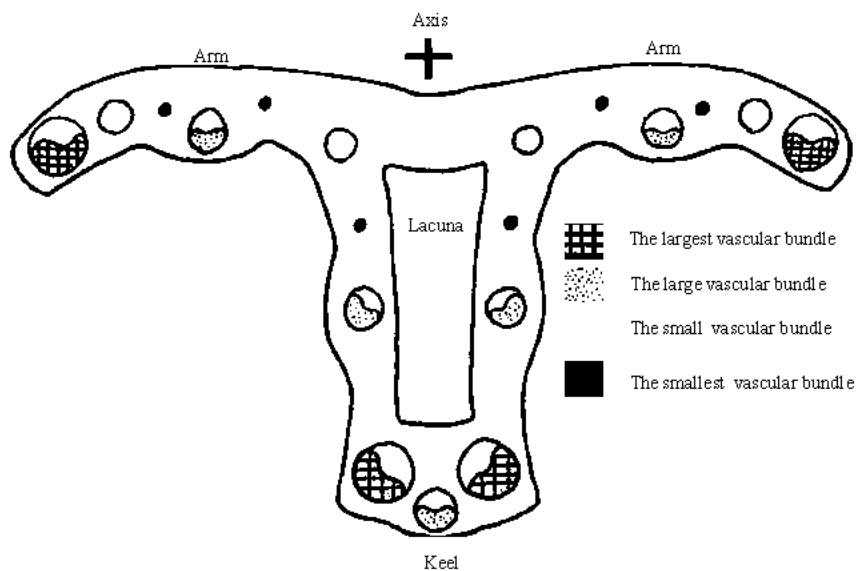
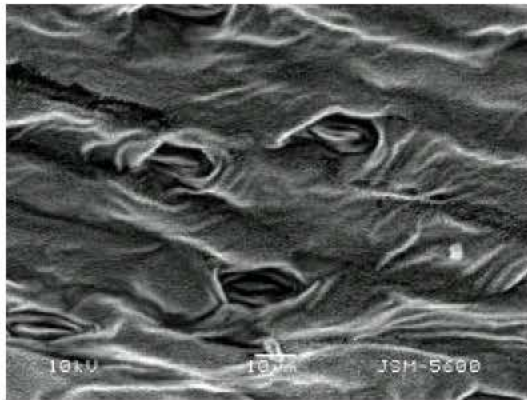
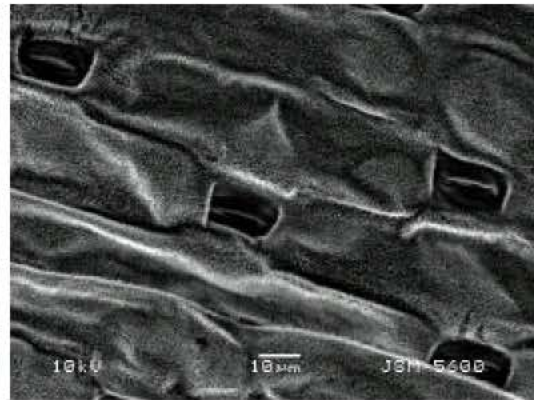


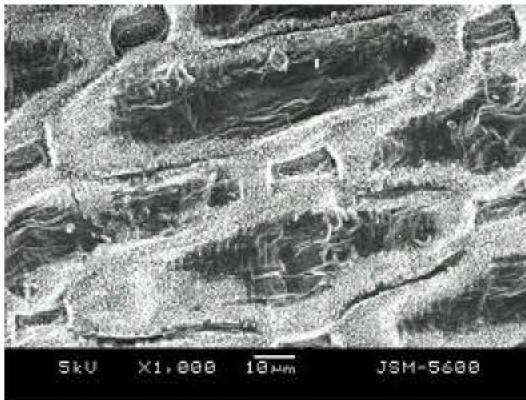
Fig. 1: The diagram of the leaf cross section and types of the vascular bundles



C. fleischeri



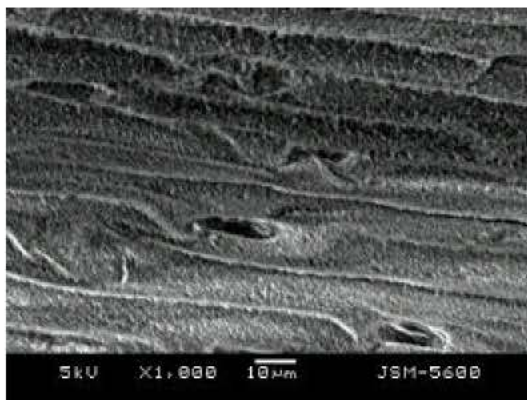
C. gargaricus subsp. *herbertii*



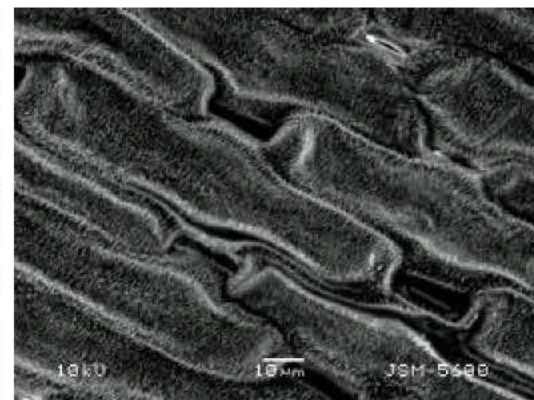
C. wattiorum



C. olivieri subsp. *istanbulensis*



C. antalyensis



C. candidus

Fig. 2: The adaxial leaf surfaces

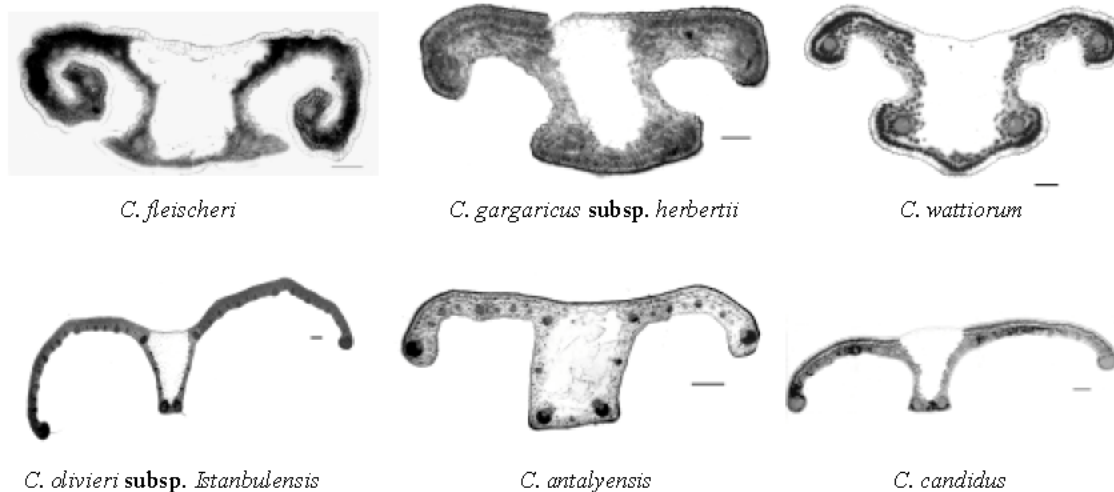


Fig. 3: The general outline of the leaf cross sections. Scale bars: above 100 μ m, below 200 μ m

of *C. wattiorum*, the sclerenchyma layer on phloem poles of large vascular bundles, which are located on arms and keel, are quite thick and the leaf juts out in this region.

The shape of the keel and the position of the arms, the shape of spongy-palisade parenchyma cells and the number of lines are distinguished and important systematically anatomic characteristics as identified by the author shown at Table 1.

In addition to these, a diagram showing systematically anatomic data and the general outline of leaf cross sections, arms, keel and vascularization are described as permanent characteristics. This diagram is also recommended for use as a diagnostic key for the identification of *Crocus* species.

Micromorphology: There are no other studies on leaf surface except the SEM photograph of *C. speciosus* M.Bieb. presented by Rudall (1994).

When the surfaces of *Crocus* leaves were investigated under scanning electron microscope (SEM), it was observed that the adaxial sides of leaves were uniform. Minor morphological differences were detected on the abaxial sides of leaves (Table 2). The wax layer was described as *Convallaria* Type (parallel oriented platelets) according to Barthlott (1998).

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