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COMPARATIVE ANATOMIC AND ECOLOGIC INVESTIGATION ON SOME ENDEMIC *CROCUS* TAXA (*IRIDACEAE)* IN TURKEY

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Abstract

In the present study, anatomic and ecologic characteristics of 5 *Crocus* L. taxa (*C. baytopiorum* Mathew, *C. biflorus* Miller subsp. *crewei* (Hooker) Mathew, *C. biflorus* subsp. *isauricus* (Siehe ex Bowles) Mathew, *C. biflorus* subsp. *pseudonubigena* Mathew and *C. cancellatus* Herbert subsp. *cancellatus*) were compared. These taxa have both endemic (except subsp. *crewei*) and limited distribution in Turkey. The subsp. *isauricus* subsp. *crewei* and *C. baytopiorum* are flowering in early spring whereas subsp. *cancellatus* and subsp. *pseudonubigena* are flowering in autumn. In anatomical studies, the cross-sections of the stem and leaves of these taxa were examined. Moreover, arms, keel, micropapillae, mesophyll, stomata types, shape of the epidermis cells, crystal types and vascular bundles properties of the leaves were investigated. The number and the status of vascular bundles in stem, the status of mesophyll layer in leaves of these taxa were asserted as important taxonomic characters. The chemical and physical analysis of the soil samples were also determined.

Introduction

Turkey is a rich country in geophyte plant species. Most of these plants have economical and medical importance (Koyuncu, 1994). However their future is under threat because of some activities in their distribution areas such as fires, dam constructions, grazing, fertiliser applications, forest exploitation, agriculture and use of pesticides. *Crocus* L. genus have an important place among the geophytes. The *Crocus* taxa are the most attractive ornamental plants, they are cultivated to design the rock gardens, floral parks, lawns, balconies, terraces and roof gardens (specially *C. baytopiorum*). In all 72 species of genus *Crocus* are distributed in Turkey (Mathew, 1984; 2000; Kerndorff & Pasche, 2004; Özhatay *et al*., 2009). Most of these plants bloom either in the early days of the Spring or in autumn. The distribution of taxa investigated in this study are very limited. Specially agriculture, forest exploitation, grazing and gathering are threatening the life of the subsp. *isauricus*, subsp. *pseudonubigena* and subsp. *cancellatus* as they are distributed very close to the population areas. According to IUCN endangered categories, these taxa have been reported in the category of VU (vulnerable, subsp. *crewei*, *C. baytopiorum*) and LR Lc=least concern, subsp. *isauricus*, subsp. *pseudonubigena* and subsp. *cancellatus* (Ekim *et al.,* 2000).

The leaves of most *Crocus* species have a unique and distinctive shape in cross-section with a square and rectangular keel in the center and two lateral arms (Rudall & Mathew, 1990). The central part of the keel consist of large thin walled parenchyma cells which usually break down to form air space. It was found that the major leaf anatomical variations are; the existence of micropapillae on cuticle, the presence of anticlinal walls of the epidermis cell as sinuous or smooth, the thickness of the sclerenchyma layer on vascular bundles, the extension of sclerenchyma out of lamina, the crystal and stoma types of leaves, mesophyll type, the presence of protrusions of arms and keel in cross-section of leaves are taxonomically important (Rudall & Mathew, 1990; Satıl & Selvi 2007; Kandemir, 2009; 2010; 2011).

The aim of this study was to present a comparetive account on the anatomical and ecological characters of the five *Crocus* taxa, as well as enlighten the the taxanomical importance of the anatomical characters of the leaves and stem in these taxa.

Materials and Methods

The investigated taxa were collected from different locations of Turkey in spring and autumn between 2009 and 2010 and sampling locations were shown in Table 1. Taxonomic description of the samples was given according to Mathew (1984). Fresh plant samples were fixed in 70% alcohol. Paraffin method was used for preparing cross-sections of the stem and leaves (Algan, 1981). The binocular microscope with drawing tube was used for longitunal and cross-sections drawing. Analysis of soil samples were made in the soil analyses Laboratory of Southeastern Anatolian Project Institution of Soil-Water resources and Agricultural Research Directorate. Soil texture, total salinity, calcium carbonate ($CaCO_3$) and pH were determined following the standard methods (Kaçar, 1996). Nitrogen, phosphorus, potassium and organic matter and microelements contents of the soil samples were analyzed by micro-Kjeldahl apparatus, ammonium-molybdate-stannous chloride, flame photometer and the Walkley-Black and DTPA (Diethylenetriaminepentaacetic acid) + $CaCl_2$ (Calcium chloride) + TEAL Triethanolomine) methods, respectively (Kaçar, 1996).

Results

Anatomic characters of taxa

Stem anatomy

***Crocus baytopiorum*:** Epidermis cells are isodiametric shaped and 20-25 µm in diameter. Cuticle is thin. Cortex consists parenchyma cells which are 5-7 layered, oval shaped, 40-55 µm diameter. There are five big and small vascular bundles in the middle of stem (Fig. 1a). Nine small vascular bundles are located in the periphery part of the stem.

Table 1. The localities of *Crocus* taxa in Turkey from where the samples were collected.

Taxon	Localities
Crocus baytopiorum (E)	-Denizli: Honaz Mountain, National Park stony areas, 2450 m., 2 April 2010, Çelik, 458. -Denizli: Bozdağ, Tavas, Nikfer, 2050 m., 28 April 2011, Çelik, 457.
Crocus biflorus subsp. *crewei*	-Denizli: Honaz Mountain, National Park stony areas, 2520 m., 2 April 2010, Çelik, 456. -Denizli: Bozdağ, Tavas, Nikfer, 2030 m., 28 April 2011, Çelik, 455.
Crocus biflorus subsp. *isauricus* (E)	-Antalya: Termessos Park, Güllük Mountain, graveyard district, stony areas, 950 m., 28 February 2010, Kandemir, 454. -Gaziantep: Yeşilce Village-Sof Mountain, Quercus forest, 1100-1200 m., 18 March 2010, Kandemir, 453. -Antalya: Manavgat-Akseki, Fersin Village, rocky areas, 800 m., 5 March 2009, Kandemir, 452.
Crocus biflorus subsp. *pseudonubigena* (E)	-Maraş: Maraş district, shrub areas, 800 m., 29 October 2009, Kandemir, 451. -Gaziantep: Yeşilce Village-Sof Mountain, Quercus forest, 1000-1100 m., 4 November 2009, Kandemir, 450. -Gaziantep: Yonas, 25 km. E. of Gaziantep, rocky areas, 1000 m., 5 November 2009, Kandemir, 449.
Crocus cancellatus subsp. *cancellatus* (E)	-Gaziantep: Gaziantep University Campus, shrub areas, 600 m., 15 October 2009, Kandemir, 448. -Gaziantep: Sofalıcı Village, stony areas, 1200-1300 m., 7 November 2009, Kandemir, 447.

E: Endemic

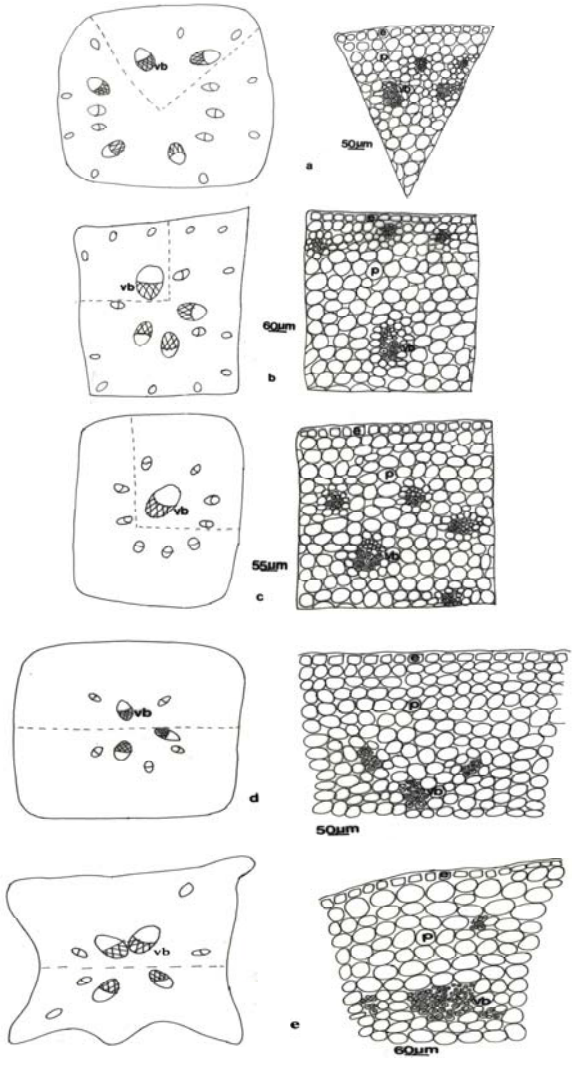

Fig. 1. Cross-sections of the *Crocus* stems: a-*C. baytopiorum*, b-*C. biflorus* subsp. *crewei*, c-*C. biflorus* subsp. *isauricus*, d-*C. biflorus* subsp. *pseudonubigena*, e-*C. cancellatus* subsp. *cancellatus*: e-epidermis, p-parenchyma, vb-vascular bundles.

***Crocus biflorus* Miller subsp. *crewei*:** Epidermis cells are 20-30 µm diameter and square shaped. The cuticle is thick. Cortex is 5-8 layered, parenchymatic, 40-60 µm and oval or hexagonal shaped. There are four big and three small vascular bundles in the middle of stem. The small vascular bundles are 12 in number. They are located in the periphery part of the stem (Fig. 1b).

***Crocus biflorus* subsp. *isauricus*:** Cuticle is thick. Epiderma consists of square shaped cells, 25-30 µm diameter. Cortex is parenchymatic. These cells are 5-8 layered, oval shaped, 40-55 µm diameter. One big and nine small vascular bundles are in the middle of stem. There are not vascular bundles in the periphery of the stem (Fig. 1c).

Crocus biflorus subsp. pseudonubigena: Epidermis cells are 15-20 µm and square shaped. The cuticle layer is thick. Cortex is 6-8 layered, oval shaped and 25-40 µm diameter. There are three big and five small vascular bundles in the middle of stem, but no vascular bundles in the periphery of the stem (Fig. 1d).

Crocus cancellatus* subsp. *cancellatus: Epidermis is isodiametric shaped and 15-20 µm diameter. Cuticle is thick. Cortex has parenchyma cells, which are 5-7 layered, oval shaped, 30-50 µm diameter. There are four big and three small vascular bundles in the middle of stem (Fig. 1e). Two-three small vascular bundles are located in the periphery part of the stem.

Leaf Anatomy

***C. baytopiorum*:** Leaves consist of two lateral arms and a slight rectangular keel in the central. Margins of arms are slightly recurved towards the keel. The abaxial side of arms has 1-2 protrusions. The base of the keel is extremely wide (Fig. 2a, Table 2). Micropapillae are conspicuous on the cuticle. Epidermis cells on the groove of leaf keel have straight-sinuous walls. They are rectangular shaped. Stomata are anomocytic and only on the lower surface. Mesophyll is bifacial type. Palisade parenchyma is 1-2 layered. Spongy parenchyma is 3-layered (Fig. 3a) and oval or elliptical shaped. Two of the

large bundles are in keel corner. The other two are at the end of the arms. Small vascular bundles are between the arms and the keel. Sclerenchyma cells are found only in the phloem poles of the large and the small bundles. The bundle sheath consists of sclerenchyma cells at the phloem pole of the large bundles. There were dense crystalline granules only on the lower surface (Fig. 4a) but absent on the upper surface of the leaf (Fig. 4b).

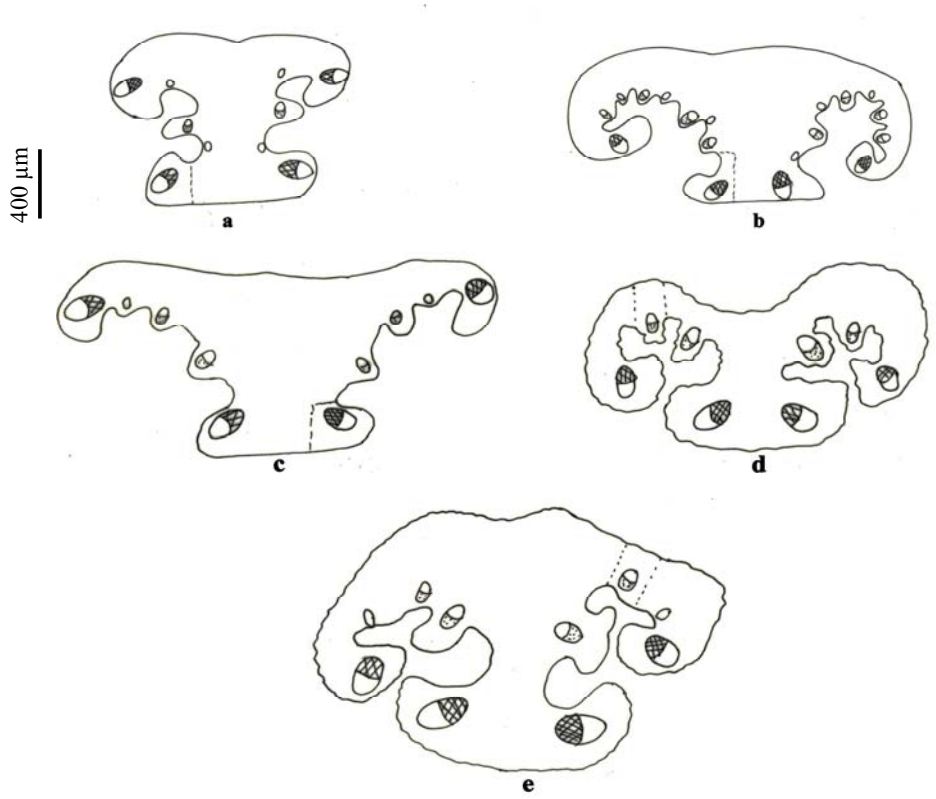

Fig. 2. Drawing of the *Crocus* leaves: a-*C. baytopiorum*, b-*C. biflorus* subsp. *crewei*, c-*C. biflorus* subsp. *isauricus*, d-*C. biflorus* subsp. *pseudonubigena*, e-*C. cancellatus* subsp. *cancellatus*.

***C. biflorus* subsp. *crewei*:** The leaves have rectangular keel and two lateral arms. Margins of arms recurved towards the keel. The base of the keel was narrow. The abaxial side of arms has 4-6 protrusions (Fig. 2b, Table 2). Epidermis cells are square shaped. Anticlinal walls of them were sinuous on groove parts of the keel. Both upper and lower epidermis have thick cuticle. Micropapillae are conspicuous on the cuticle of arms and upper epidermis. But, they are slight-conspicuous on the cuticle of lower epidermis. The anticlinal walls of the epidermal cells are smooth on the lower and upper surfaces of the leaf. The upper epidermal cells are bigger than the lower epidermal cells. The stomata are anomocytic, only on the lower surface. Palisade parenchyma is 2-layered, with dense chloroplasts. Spongy parenchyma is 2-3-layered and elliptical shaped (Fig. 3b). The largest bundles occur at the corners of the keel and at the tips of the arms. Large and small bundles are present on the sides of the keel. Sclerenchyma cells exist only in the phloem poles of large and small bundles. The bundle sheath consists of sclerenchyma cells at the phloem pole of large bundles. There were dense square shaped styloid and crystalline granules on the lower surface of leaf (Fig. 4c). However, on the upper surface of leaf crystals are absent (Fig. 4 d). The square shaped styloid crystals were observed in corm tunics.

***C. biflorus* subsp. *isauricus*:** There were 2 lateral arms and a nearly rectangular keel. Margins of arms are slightly recurved towards the keel. The abaxial side of arms has 3-4 protrusions (Fig. 2c, Table 2). The base of the keel is narrow. Both epidermal cells are square shaped. Anticlinal walls of them are sinuous on groove part of abaxial keel. Micropapillae are conspicuous on the cuticle of arms. However, micropapillae are slightly conspicous on the cuticle of upper and lower epidermis. The lower epidermal cells are bigger than the upper. Stoma cells are present only on the groove parts of the leaf. Mesophyll is differentiated into palisade and spongy parenchyma (Fig. 3c). The palisade parenchyma is 2-(3)-layered, with a big cell and dense chloroplast. The spongy parenchyma is 3-layered, elliptic shaped and with rare air space. The largest bundles occur at the corners of the keel and at the tips of the arms. Large and small bundles lie on the sides of the keel. The bundle sheath consists of sclerenchyma cells at the phloem pole of the large bundles. Sclerenchyma cells exist the phloem and xylem poles of the largest, larger and smaller bundles. While big hexagonal crystals and crystalline granules are dense on the lower surface of leaf, crystalline granules are dense on the upper surface of leaf (Figs. 4e, f). There are hairs on both upper and lower surface of the leaf. Similar types of crystals are observed in corm tunics.

Table 2. Comparison of stem and leaf anatomical characters of investigated Crocus taxa.

Taxa	Micropapillae in cuticle	Anticlinal walls of epidermis	Protrusion number	Arms structure	keel	Palisade layer	Spongy layer	Vascular bundles of leaves	Crystal types of leaves	Vascular bundles at the middle of stem	Vascular bundles in the periphery of stem
Crocus baytopiorum	Conspicuous	Slight sinuous	1-2	Thick short, reach half of the carina	Corners circular, reach arms, base extremely wide	1-2 narrow-long rectangular	3 layered, oval-eliptical	tpical	Crystalline granules	Five big and small	9
Crocus biflorus subsp. *crewei*	Conspicuous	Sinuous	4-6	Thick long, curled flexible and reach to the base of carina	Corners oval, base narrow slight jutted out	2 layered, short rectangular	2-3 layered, eliptical	tpical	Squre shaped styloid and crystalline granules	Four big and three small	12
Crocus biflorus subsp. *isauricus*	Conspicuous	Sinuous	3-4	Thick short, curled flexible	Base narrow corners circular	2-(-3) layered, short rectangular	3 layered, eliptical	tpical	Hexagonal crystal and crystalline granules	One big and nine small	absent
Crocus biflorus subsp. *pseudonubigena*	Conspicuous	Sinuous	2-(-3)	Thick long, ends reach to the base of carina	Base extremely wide, corners circular, reach arms	1-2 layered, narrow-long rectangular	2-3 layered, oval-eliptical	tpical	Hexagonal crystal and crystalline granules	3 big and 5 small	absent
Crocus cancellatus subsp. *cancellatus*	Extremely conspicuous	Sinuous	2-3	Thick long, ends reach to the base of carina	Base wide, corners circular, reach arms	3-5 layered, oval	-	tpical	crystalline granules	4 big and 3 small	2-3

Table 3. Physical and chemical analysis of the soil samples.

Taxa	Texture	Salinity (%)	$CaCO_3$ (%)	pH	Organic matter %	N %	P %	K %	Ca (ppm)	Mg (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)
C. baytopiorum Honaz Mountain	sandy-loam	0.030	1.72	6.11	5.64	0.314	4.73	260.0	3981	281.00	1.870	0.560	17.64	15.83
C. baytopiorum Bozdağ	sandy-loam	0.042	1.27	6.09	4.91	0.283	3.99	249.3	3965	272.08	1.451	0.398	16.62	15.80
subsp. *crewei* Honaz Mountain	sandy-loam	0.030	1.70	8.10	6.14	1.308	8.40	164.44	3887	133.24	0.540	2.220	10.56	8.25
subsp. *crewei* Bozdağ	sandy-loam	0.042	1.26	7.10	4.98	0.291	3.85	249.17	3969	272.23	1.458	0.388	16.58	15.83
subsp. *isauricus* Güllük Mountain	loam	0.230	1.63	7.35	3.12	4.52	5.60	147	3150	145	1.580	1.72	13.7	17.6
subsp. *isauricus* Sof Mountain	clayey-loam	0.557	1.50	7.32	1.89	0.156	25.33	144.70	3250	171	2.963	2.607	15.77	45.95
subsp. *isauricus* Manavgat-Akseki	loam	0.23	2.60	7.35	4.12	4.50	5.20	134	3270	150	2.4	0.67	13.8	15.5
subsp. *pseudonubigena* Maraş District	clayey-loam	0.420	1.82	6.90	2.18	2.91	12.53	182	3544	166	1.468	1.95	12.4	36.57
subsp. *pseudonubigena* Sof Mountain	clayey-loam	0.557	1.50	7.32	1.89	0.156	25.33	144.7	3520	152	2.963	2.607	15.7	45.95
subsp. *pseudonubigena* Gaziantep-Yonas	loamy	0.293	1.20	7.70	1.74	0.121	22.06	138.8	3219	255	2.113	0.584	12.7	38.03
subsp. *cancellatus* Gaziantep University	clayey-loam	0.325	2.65	6.45	2.50	1.25	18.56	185	2886	183	2.370	2.341	14.04	54.6
subsp. *cancellatus* Gaziantep-SofalıVillage	clayey-loam	0.653	2.20	7.61	3.80	0.191	21.89	209.50	3128	138	2.781	3.049	16.67	51.59

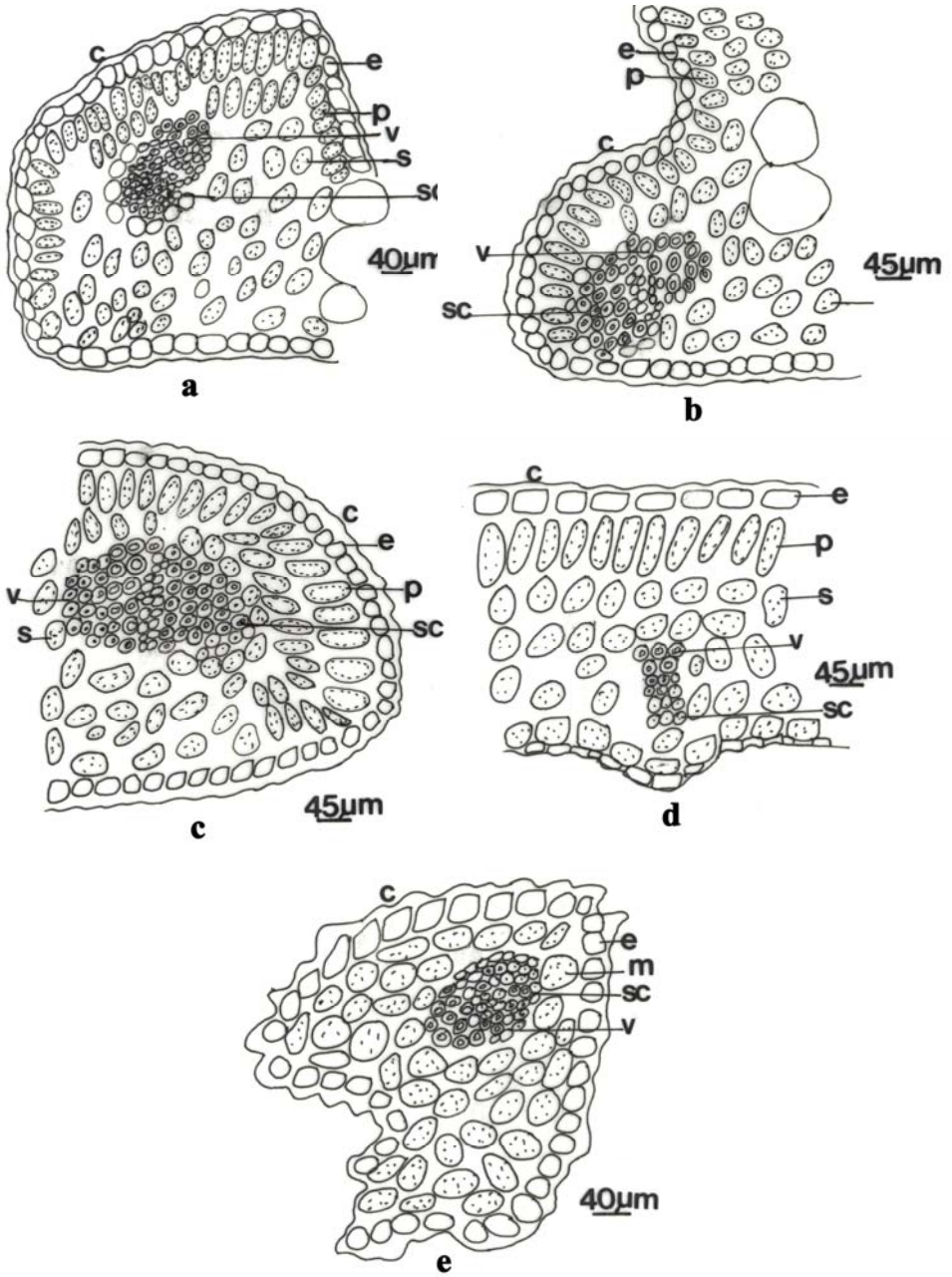

Fig. 3. Cross-sections of the *Crocus* leaves: a-*C. baytopiorum*, b-*C. biflorus* subsp. *crewei*, c- *C. biflorus* subsp. *isauricus*, d-*C. biflorus* subsp. *pseudonubigena*, e-*C. cancellatus* subsp. *cancellatus*: c-cuticle, e-epidermis, m-mesophyll, p-palisade parenchyma, s-spongy parenchyma, v-vascular bundles, sc-sclerenchyma.

C. biflorus* subsp. *pseudonubigena: Leaves have a slightly triangular keel and two flexible arms. The arms reach to the keel. The base of the keel is extremely wide. The abaksial side of arms has 2-(-3) protrusions (Fig. 2d, Table 2). The epidermal cells of protrusion are sinuous. But, other areas of leaf are straight-sinuous. Both epiderma have thick cuticle. Micropapillae on the cuticle are conspicuous. Epidermal cells are rectangular shaped. The upper epidermal cells are bigger than lower epidermal cells. Stomata are usually absent except in the groove parts. They are in sunken position between epidermal cells. Mesophyll is bifacial type (Fig. 3d). Palisade parenchyma is 1-2 layered and rich in chloroplasts. Spongy parenchyma is 2-3 layered and oval-eliptical shaped. Four large vascular bundles occur at the corners of keel and arm margins. Small bundles are located between the keel and the arm margins. There are sclerenchyma cells as caps at the phloem and xylem poles of the large bundles and only at the phloem poles of small bundles. The bundle sheath consiss of sclerenchyma cells at the phloem pole of the large bundles. The upper and lower surfaces of leaf rarely possess both crystalline granules and hexagonal crystals (Figs. 4h and g). But, dense square shaped styloid crystals are observed in corm tunics. There are hairs on both upper and lower surfaces.

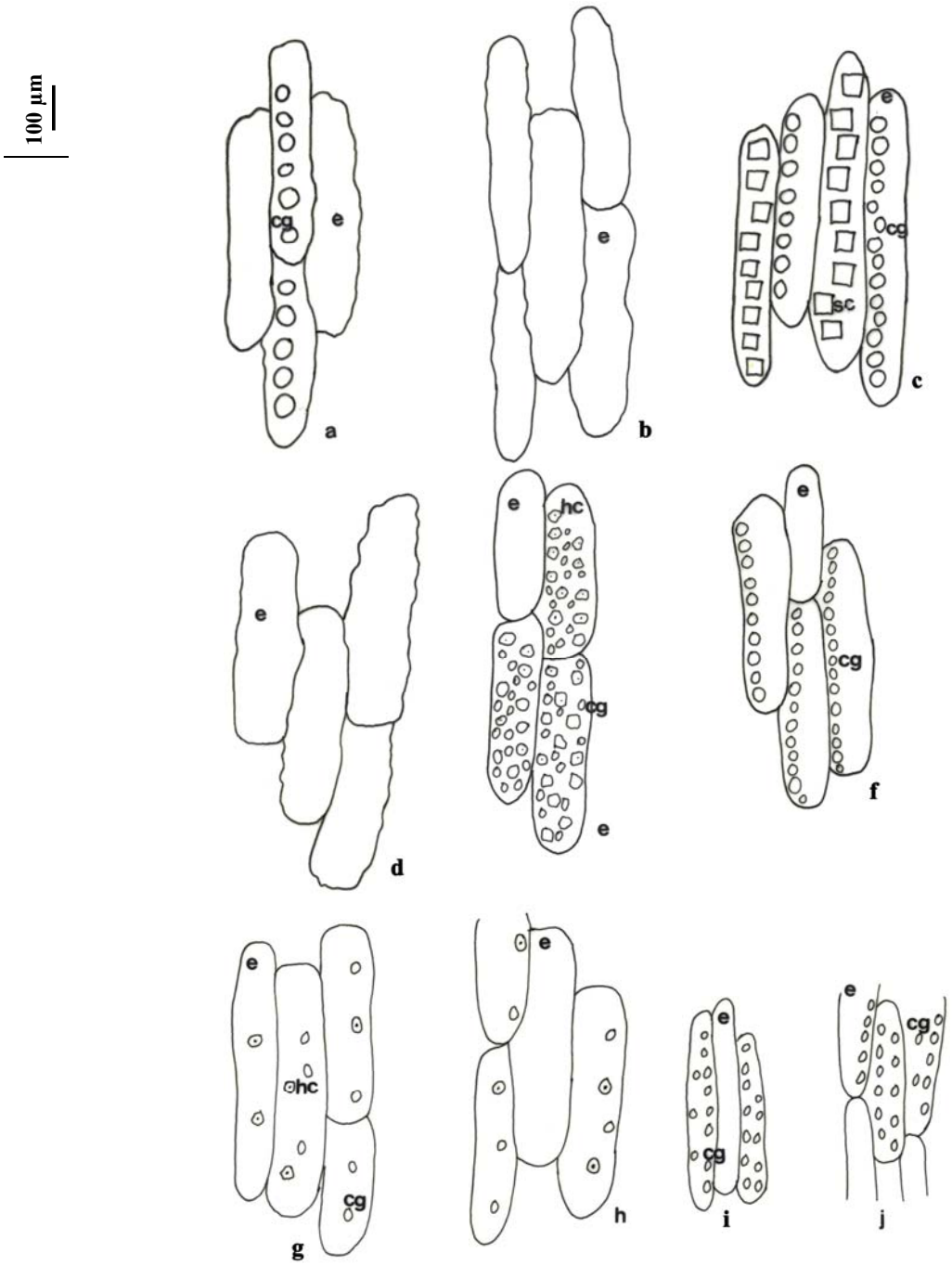

Fig. 4. The crystal types in surface view of the *Crocus* leaves: a-lower surface of *C. baytopiorum* leaf, b-upper surface of *C. baytopiorum* leaf, c-lower surface of *C. biflorus* subsp. *crewei* leaf, d-upper surface of *C. biflorus* subsp. *crewei* leaf, e-lower surface of *C. biflorus* subsp. *isauricus* leaf, f-upper surface of *C. biflorus* subsp. *isauricus* leaf, g-lower surface of *C. biflorus* subsp. *pseudonubigena* leaf, h-upper surface of *C. biflorus* subsp. *pseudonubigena* leaf, i-lower surface of *C. cancellatus* subsp. *cancellatus* leaf, j-upper surface of *C. cancellatus* subsp. *cancellatus* leaf: e-epidermis, cg-crystalline granules, sc-square shaped styloid crystal, hc-hexagonal crystal.

***C. cancellatus* subsp. *cancellatus*:** Leaves consist of slightly triangular keel and two lateral arms. Their margins are recurved towards the keel (Fig. 2e). The base of the keel is wide. The abaxial side of arms has 2-3 protrusions (Table 2). Micropapillae on the cuticle are extremely conspicuous. The upper and lower epidermal cells are big and square shaped. Anticlinal walls of epidermal cells of arms are sinuous. The stoma cells are present only on the lower surface of the leaf. They are in sunken position between epidermal cells. Mesophyll layer is composed of only parenchyma cells (Fig. 3e). They are 3-5 layered and oval shaped. Two of the large vascular bundles are located at the corner of the keel and the others are at the end of the arms. The small bundles extend

between the keel and the arm margins. There are sclerenchymatic cells only at the phloem poles of large and small vascular bundles. The bundle sheath consist of sclerenchyma cells at the phloem pole of the large bundles. The upper and lower surfaces of the leaf have dense crystalline granules (Figs. 4i and j). The corm tunics have crystalline granules and rarely square shaped styloid crystals.

Ecologic characters of taxa

***C. baytopiorum*:** This species shows a limited distribution. Soil sample were taken from two localities in Honoz Mountain and Bozdağ (Denizli). The texture of soil is sandy-loam, pH and salinity values vary between 6.09-6.11 and 0.030- 0.042%, respectively. $CaCO_3$ content lies between 1.27 and 1.72%. The organic matter, N, P and K contents are 4.91-5.64%, 0.283-0.314%, 3.99-4.73% and 249.3-260.0%, respectively. The Ca, Mg, Cu, Zn, Mn and Fe values lie between 3965-3981, 272.08-281.0, 1.451-1.870, 0.398-0.560, 16.62-17.64 and 15.80-15.83 ppm, respectively (Table 3).

***C. biflorus* subsp. *crewei*:** It is not a widely distrubuted species. The soil from two localities namely Honoz Mountain and Bozdağ (Denizli) were analysed. The soil caharacteristics are as follows: medium level alkali (7.10-8.10), non-saline (0.030-0.042%), medium level calcareous (1.26-1.70%) and sandy-loam texture. The organic matter, N, P and K contents of soil sample vary between 4.98-6.14%, 0.291-1.308%, 3.85-8.40% and 164.44-249.17%, respectively, and Ca, Mg, Cu, Zn, Mn and Fe contents lie between 3887-3969, 133.24-272.23, 0.540-1.458, 0.388-2.220, 10.56-1658 and 8.25-15.83 ppm, respectively.

***C. biflorus* subsp. *isauricus*:** Soil samples of this taxon are taken from three localities; Güllük Mountain (Antalya), Sof Mountain (Gaziantep) and Manavgat-Akseki (Antalya) (Table 3). pH values of the soils lies between 7.32 and 7.35. Salinity and $CaCO_3$ contents vary from 0.230 to 0.557 and 1.50 to 2.60 percent, respectively. The texture of the soils is loam and clayey-loam. Organic matter content varies between 1.89 and 4.12%, N between 0.156 and 4.52%, P between 5.20 and 25.33%, K between 134 and 147%. Ca, Mg, Cu, Zn, Mn, and Fe values vary between 3150-3270, 145-171, 1.580-2.963, 0.67-2.607, 13.7-15.77 and 15.5-45.95 ppm, respectively.

***C. biflorus* subsp. *pseudonubigena*:** Soil characteristics of this taxon are based on the samples taken from three different localities in Maraş, Gaziantep and Sof Mountain (Gaziantep). The pH varied from 6.90 to 7.70. The level of the $CaCO_3$ and salinity of the soils are 1.20-1.82 and 0.293-0.557 percent, respectively. They have a clayey-loam and loamy texture. The organic matter, N, P and K contents are 1.74-2.18%, 0.121-2.91%, 12.53-25-33% and 138.8-182%, whereas the Ca, Mg, Cu, Zn, Mn and Fe contents are 3219-3544, 152-255, 1.468-2.963, 0.584-2.607, 12.4-15.7 and 36.57-45-95 ppm, respectively (Table 3).

***C. cancellatus* subsp. *cancellatus*:** Soil samples are taken from two localities in Sof Mountain (Gaziantep) and Gaziantep. The texture of soil is clayey-loam (Table 3). pH and salinity values are 6.45-7.61 and 0.325-0.65 percent, respectively. The $CaCO_3$ content is 2.20-2.65%. The organic matter, N, P and K contents are 2.50-3.80%, 0.191-1.25%, 18.56-21.89% and 185-209.50%, respectively. While the Ca, Mg, Cu, values vary between 2886-3128, 138-183 and 2.370-2.781 ppm, the Zn, Mn and Fe between 2.341-3.049, 14.4-16.67 and 51.59-54.6 ppm, respectively.

Discussion

The investigated *Crocus* taxa have different number of vascular bundles in both central and peripheral parts of the stems. These properties have been observed in the cross section of the stem of *C. cancellatus* subsp. *damascenus* (Herbert) Mathew (Akan & Eker, 2004), *C. pestolazzae* Boiss. (Kandemir, 2009) and *C. reticulatus* subsp. *hittiticus* (T. Baytop and Mathew) Mathew and *C. cancellatus* subsp. *lycius* Mathew (Kandemir, 2010). But, the vascular bundles in *C. pallasii* Goldb. subsp. *turcicus* Mathew (Akan & Eker, 2004), subsp. *isauricus* and subsp. *pseudonubigena* are found only in the central part of the stem. These characters can be used as distinguishing anatomical characters in taxonomy of *Crocus* taxa, since the number and the status of vascular bundles in stem are constant among populations of same species and are different among species.

In the systematical study of the family the leaf anatomical characters are important. Although there is high homoplosy between both morphological and anatomical characters, leaf anatomy provides some significant data for *Iridaceae* (Rudall, 1994). Rudall & Mathew (1990) have pointed out that leaves of most *Crocus* taxa have a unique and distinctive morphology in cross-section. They comprise a square or rectangular shaped keel in the central and two flexible lateral arms. The central part of the keel consists of large thin walled parenchyma cells which usually break down to form air space. Although general outline of cross-section of the leaves of investigated taxa are in agreement with Rudall & Mathew (1990), the leaf characters of these taxa show some differences. These characters are as follows;

The arms of subsp. *crewei*, subsp. *pseudonubigena* and subsp. c*ancellatus* reach to the base of keel. The arms of subsp. *isauricus* and *C. baytopiorum* are slightly flexible towards the keel. The base of the keel is narrow in subsp. *crewei* and subsp. *isauricus* but, it is wide in subsp. *cancellatus*, subsp. *pesudonubigena* and *C. baytopiorum* (Table 2). The leaves of subsp. *pseudonubigena* and subsp. *cancellatus* have central slightly triangular keel. The triangular keel which exists on the leaves of subsp. *pseudonubigena* and subsp. *cancellatus* results in groove parts deeper on the leaf surface. As such, stoma cells of these species are in sunken position between epidermis. This state may be due to the fact that these species are localized in more arid region. Moreover our ecological findings support the present status (Table 3). There are protrusion in the abaxial surfaces of the arms and the sides of keel in subsp. *isauricus* (3-4), subsp. *pseudonubigena* (2-3) and subsp. *crewei* (4-6), subsp. *cancellatus* (2-3) and *C. baytopiorum* (1-2). Satıl & Selvi (2007) have observed the same characteristic in the leaf of *C. biflorus* subsp. *nubigena*.

Epidermal cells are rectangular shaped in *C. baytopiorum* subsp. *pseudonubigena* and are square shaped in subsp *crewei*, subsp. *isauricus* and subsp. *cancellatus*. Anticlinal walls of epidermis in stomatal regions are generally sinuous in subsp. *crewei*, subsp. *isauricus*, subsp. *pseudonubigena* and subsp. *cancellatus*, straight-sinuous in *C. baytopiorum*. While the upper epidermis cells are bigger than the lower epidermal cells in subsp. *crewei* and subsp. *pseudonubigena*, the lower epidermis cells are bigger than the upper epidermal cells in subsp. *isauricus*; upper and lower epidermal cells are same in subsp. *cancellatus* and *C. baytopiorum*. Micropapillae on the cuticle of arms are conspicuous in the 5 taxa investigated by us. But, micropapillae on other parts the of the cuticle are slightly conspicous in all taxa. Stomata of all taxa are anomocytic and occur only on the lower surfaces.

Mesophyll occurs palisade and spongy parenchyma in all taxa (except subsp. *cancellatus*). Palisade parenchyma is 1-2 layered in *C. baytopiorum* and subsp. *pseudonubigena*, 2-layered in subsp. *crewei*, 2-(3) layered in subsp. *isauricus*. Spongy parenchyma is 3 layered in *C. baytopiorum* and subsp. *isauricus*, 2-3 layered in subsp. *crewei* and subsp. *pseudonubigena*. The same properties have been observed in the leaves of other *Crocus* taxa (Kandemir, 2011), subsp. *hittiticus* and subsp. *lycius* (Kandemir, 2010), *C. pestolazzae* (Kandemir, 2009), *C. leichtlinii* (D. Dewar) Bowles (Akan *et al.,* 2007), *C. chrysanthus* Herbert, *C. olivieri* subsp. *istanbulensis* Mathew, *C. cancellatus* subsp. *mazziaricus* (Herbert) Mathew, subsp. *nubigena* (Herbert) Mathew, *C. pulchellus* Helbert (Satıl & Selvi, 2007) and subsp. *damascenus* (Akan & Eker, 2004). Mesophyll of subsp. *cancellatus* occurs only in parenchyma cells and these cells are 3-5 layered.

Vascular bundles of the *Crocus* taxa leaves are quite specific. Rudall & Mathew (1990) have suggested that this character can be used as important taxonomical character to classify these taxa, since sclerenchyma layer of vascular bundles of *Crocus* taxa are very distinct. The four largest vascular bundles appear. The largest and larger vascular bundles are generally at the tips of the arms and at the corner of keel. Large and small bundles are at the sides of the keel and in protrusion of arms in subsp. *crewei*, subsp. *isauricus*, and subsp. *pseudonubigena*. Small bundles are between the arm margins and the keel in subsp. *cancellatus* and *C. baytopiorum*. There are sclerenchyma cells as caps only at the phloem poles of large bundles and small bundles in *C. baytopiorum*, subsp. *crewei* and subsp. *cancellatus*. But, the sclerenchyma cells have been observed in pholem poles and xylem poles of the largest, large and small bundles in subsp. *isauricus* and only of the large bundles in subsp. *pseudonubigena*.

All taxa of *Iridaceae* family have styloids (except *Sisyrinchium*) (Franceschi & Nakata, 2005). There are dense crystalline granules on the lower surface of *C. baytopiorum* leaves. The square shaped styloid and crystalline granules are dense on the lower surface of subsp. *crewei* leaf. Also, the corm tunics of subsp. *crewei* have square shaped styloid crystals. While big hexagonal crystals and crystalline granules are dense on the lower surface of leaf and corm tunics, only crystalline granules are dense on the upper surface of subsp. *isauricus* leaf. Both upper and lower surfaces of subsp. *pseudonubigena* leaf have hexagonal crystals and crystalline granules. There are square shaped styloid crystals in corm tunics of this subspecies. The upper and lower surfaces of subsp. *cancellatus* leaf possess dense crystalline granules. The corm tunics of subsp. *cancellatus* have crystalline granules and sparsely square shaped styloid crystals. Kandemir (2010) has observed the same characteristics in the leaf and corm tunics of subsp. *lycius*. As crystal shape and distribution are constant among species, crystals in corm tunics and leaves of these taxa may be used as distinguish taxonomic character in the classification of *Crocus* taxa.

The investigated *Crocus* taxa grow on sandy-loam (*C. baytopiorum* and subsp. *crewei*), clayey-loam (subsp. *isauricus*, subsp. *pseudonubigena*, subsp. *cancellatus*) and loamy soils (subsp. *pseudonubigena* and subsp. *isauricus*) These are non-salinity, medium calcareous, neutral (subsp. *pseudonubigena*), medium alkaline (subsp. *crewei*), slightly alkaline (subsp. *isauricus*, subsp. *cancellatus*, subsp. *pseudonubigena*) and slightly acidic (*C. baytopiorum*, subsp. *cancellatus*) (Table 3). Similar observations have been recorded in other *Crocus* and *Iris* taxa by Kandemir (2009), Kandemir *et al*., (2011) and Satıl & Selvi (2007). The N, P, K and organic matter contents are high in all soil samples. Ca, Mg, Cu, Zn, Mn and Fe values of the soils samples show normal levels. Similar findings have been reported in some *Crocus* taxa by Şık & Candan (2009), Khattak & Khattak (2011). Although 21% of soils in Turkey show limited amount of nitrogen (Bosgelmez *et al.,* 2001), *Crocus* taxa in this study grow in soils with high nitrogen. This state has been also reported for other *Crocus* taxa by Kandemir (2009), Şık & Candan (2009). It is suggested that some *Crocus* taxa (*C. baytopiorum*, *C. vitellinus*, *C. graveolens*) grow even on limestone (Mathew, 1982). But, the levels of calcareousness in soils where investigated *Crocus* taxa grow are generally at medium level.

They show considerable variation in morphological characters, since *C. biflorus* and *C. cancellatus* are complex and wide geographically distributed taxa. Thus, there are problems in taxonomic of these two taxa. While the subsp. *maziaricus*, subsp. *lycius* and subsp. *damascenus*, subsp. *cancellatus* as morphologic are closer to each others, the subsp. *pamphyllicus* is a different subspecies. According to the general leaf anatomy, it is seen that subsp. *pamphylicus* and subsp. *cancellatus* are closer to each others and subsp. *maziaricus* and subsp. *lycius* are also closer (Kandemir, 2010; 2011; Satıl & Selvi, 2007). Statistically, Kandemir (2011) has suggested that there are differences and similarities among subspecies of *C. cancellatus* respect to the leaf anatomical characters. But, subsp. *damascenus* is a different subspecies of *C. cancellatus* relating to the general leaf anatomy. It is concluded that there are similarities in anatomical characters of subsp. *damascenus* and *C. pallasi* subsp. *turcicus* by Akan & Eker (2004). It is considered that these the two subspecies are closely related. Although there are anatomical and morphological differences among the subspecies of *C. biflorus*, it is seen that subsp. *isauricus*, subsp. *crewei* and subsp. *nubigena* (except subsp. *pseudonubigena*) are closer subspecies to each others. Statistically, Kandemir (2011) have reported that there are important correlations among subsp. *crewei*, subsp. *isauricus* and subsp. *nubigena* which are

subspecies of *C. biflorus*. This state has been found in pollen morphology of subspecies of *C. biflorus* (except subsp. *nubigena*) and *C. cancellatus* (except subsp. *lycius*) by Işık & Dönmez (2006). The ecological and anatomical findings in this study support the relationship among *C. biflorus* subspecies. On the other hand, the subsp. *pseudonubigena* is a different subspecies of *C. biflorus*. Despite the subsp. *pseudonubigena* is a subspecies of *C. biflorus* and subsp. *cancellatus* is a subspecies of *C. cancellatus*, they are closer subspecies to each others relating to their leaf anatomic and ecologic characters. The similarities between these 2 taxa may be originated from their distribution at similar ecologic conditions. Based on the leaf anatomic and ecologic characters, we suggested that the subsp. *pseudonubigena* and the subsp. *cancellatus* may be dependent species with close relationships. Briefly, the taxanomic status of subsp. *pseudonubigena* and subsp. *damascenus* should be designed relating to their leaf anatomic and ecologic characters. Also, the leaf anatomic characters mentioned may be used as important taxonomic characters of *Crocus* taxa. The same state is seen in some ecotix plant species by Iftikhar *et al*., (2009).

As a result, when the anatomical characteristics are taken into consideration taxonomically, different anatomical characters have been observed between the subspecies of *C. biflorus* and *C. cancellatus*. Since morphological characters of subspecies of both *C. biflorus* (especially 3 subspecies in our study) and *C. cancelatus* are very similar to each other, the risk of commiting mistakes in the classification of these subspecies may be high. Therefore, we think that the leaf anatomical characters mentioned may be used as important taxonomical characters of investigated *Crocus* taxa. However, it will be good to carry out plastid and nuclear DNA sequence studies on the subspecies of *C. biflorus* and *C. cancellatus*.

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