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COMPARATIVE LEAF ANATOMY OF SOME ENDEMIC *CROCUS* L. TAXA FROM TURKEY

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Key words: *Crocus*, Endemic, Leaf anatomy, Relationships

Abstract

Relationships among 14 *Crocus* L. taxa such as, *C. ancyrensis* (Herbert) Maw, *C. baytopiorum* Mathew, *C. biflorus* Miller ssp. *crewei* (Hook.) Mathew, *C. biflorus* ssp. *isauricus* (Siehe ex Bowles) Mathew, *C. biflorus* ssp. *nubigena* (Herbert) Mathew, *C. biflorus* ssp. *pseudonubigena* Mathew, *C. cancellatus* Herbert ssp. *cancellatus*, *C. cancellatus* ssp. *damascenus* (Herb ert) Mathew, *C. cancellatus* ssp. *lycius* Mathew, *C. cancellatus* ssp. *mazziaricus* (Herbert) Mathew, *C. cancellatus* ssp. *pamphylicus* Mathew, *C. pestalozzae* Boiss., *C. reticulatus* Steven ex Adams ssp. *hittiticus* (T. Baytop & Mathew) Mathew and *C. sieheanus* Barr ex Burtt collected from different two locations of Turkey have been investigated using 11 anatomic leaves characters. These taxa are endemic (except ssp. *damascenus*) and rare in Turkey. The anatomical variations in the cross sections of the leaf parts of the taxa were ascertained by statistical methods. It appears that the length and breadth measurements of palisade and spongy parenchyma cells, trachea diameter and cuticle thickness are important leaf anatomical characters that show variation in relation to altitudes.

Introduction

Genus *Crocus* (Iridaceae) comprises approximately 88 species distributed in the Mediterranean region. The taxa are distributed both in Turkey and in South Western Europe, South-Western parts of Asia and Western part of China (Alavi-Kia *et al*. 2008, Petersen *et al*. 2008, Kandemir 2009). Turkey has many endemic and rare *Crocus* taxa. Seventy two taxa are distributed in Turkey and 35 of them are endemic (Mathew 1984, 1988; 2000, Kerndorff and Pasche 2004, Özhatay *et al*. 2009). When the diversity of the taxa is taken into consideration, Turkey may be considered as the homeland of *Crocus* taxa. The investigated taxa are placed to *Reticulati* series (*C. ancyrensis, C. cancellatus, C. reticulatus* and *C. sieheanus*) and *Biflori* series (*C. biflorus* and *C. pestalozzae*) of *Nudiscapus* sectio and *Verni* series (*C. baytopiorum*) of *Crocus* sectio.

Crocus taxa have an important place among the geophyte plants. Because of this beatiful flowers, they are used as ornamental plants in the balconies, terraces and roof gardens (specially, *C. baytopiorum*, ssp. *mazziaricus*, ssp. *lycius* and ssp. *damascenus*). People in some regions of Anatolia make a local cheese called "herbed cheese" and "*Crocus* pilaf" from *Crocus* species (specially ssp. *damascenus*) (Baytop 1984). The extract of *Crocus* taxa has antitumor, antimutagenic and cytototic activities (Nair *et al*. 1991, Abdullaev 2003). Therefore, they are used for Behçet and gut diseases, treatment of joint pains and cancer research, recently.

The leaf anatomy of *Crocus*, leaf anatomy and phylogeny of Iridaceae family, the comparative morpho-anatomical, phylogenetic relationships and genetic diversity studies on the genus *Crocus* have been investigated by Rudall and Mathew (1990), Rudall and Goldblatt (1991), Pulido *et al*. (2004), Kandemir (2009, 2010), Petersen *et al*. (2008) and Alavi-Kia *et al*. (2008). Rudall and Mathew (1990) reported that the leaves of most *Crocus* species have a unique and distinctive shape in cross section and have a square and rectangular keel in the center and two

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lateral arms. Moreover, some researchers found that the anatomic characters of leaves are important taxonomically (Rudall and Mathew 1990, Kandemir 2009, 2010).

Ekim *et al.* (2000) placed these taxa into endangered categories Lc (least concern, *C. ancyrensis*, ssp. *isauricus*, ssp. *nubigena* and ssp. *pseudonubigena*, ssp. *cancellatus*), nt (near threatened, ssp. *lycius*) and VU (vulnerable, ssp. *crewei*, ssp. *pamphylicus*, *C. pestalozzae*, ssp. *hittiticus*, *C. sieheanus* and *C. baytopiorum*).

The aim of this paper, is to determine the degree of relationship among 14 *Crocus* taxa and based on the leaf anatomic characters by statistical methods, and to determine the intra and inter-specific variations.

Materials and Methods

The taxa in this study were collected from two different locations of Turkey in spring and autumn between 2008 and 2010 and sampling locations are given in Table 1. Taxonomic description of the taxa were made according to Mathew (1984). For anatomical studies, fresh plant

Table 1. The localities from where *Crocus* taxa were collected in Turkey."E" indicates endemic.

Taxon	Localities
C. ancyrensis (E)	Amasya: Kırklar Mountain, open areas, 710 m., 23 March 2008, Kandemir, 500.
	Amasya: Merzifon, Bakırçay valley, open areas, 1300 m., 2 April 2008, Kandemir, 501.
C. baytopiorum (E)	Denizli: Honaz Mountain, National Park stony areas, 2450 m., 2 April 2010, Çelik, 502.
	Denizli: Bozdağ, Tavas, Nikfer 2050 m., 28 April 2010, Çelik, 503.
C. biflorus ssp. *crewei*	Denizli: Honaz Mountain, National Park stony areas, 2520 m., 2 April 2010, Çelik, 504.
	Denizli: Bozdağ, Tavas, Nikfer, 2030 m., 28 April 2010, Çelik, 505.
C. biflorus ssp. *isauricus* (E)	Antalya: Termessos Park, Güllük Mountain, mezarlık district, stony areas, 950 m., 28 February 2009, Kandemir, 506.
	Gaziantep: Yeşilce Village-Sof Mountain, *Quercus* forest, 1100-1200 m., 18 March 2009, Kandemir, 507.
C. biflorus ssp. *nubigena* (E)	Balıkesir: Sındırgı, Kocabey village, open areas, 700 m., 4 February 2010, Kandemir, 508.
	Muğla: Göktepe, open areas, 1850 m., 20 March 2010, Kandemir, 509.
C. biflorus ssp. *pseudonubigena* (E)	Maraş: Maraş district, shrub areas, 800 m., 29 October 2009, Kandemir, 510.
	Gaziantep: Yeşilce Village-Sof Mountain, *Quercus* forest, 1000-1100 m., 4 November 2009, Kandemir, 511.
C. cancellatus ssp. *cancellatus* (E)	Gaziantep: Gaziantep University Campus, shrub areas, 600 m., 15 October 2009, Kandemir, 512.
	Gaziantep: Sofalıcı Village, stony areas, 1200-1300 m., 7 November 2009, Kandemir, 513.
C. cancellatus ssp. *damascenus*	Şanlıurfa: Direkli Hills, Huzurevi around, rocky areas, 700 m., 24 October 2008, Kandemir, 514.
	Gaziantep: Nur Mountain, Gaziantep to Fevzipaşa, rocky areas, 1650 m., 10 November 2008, Kandemir, 515.
C. cancellatus ssp. *lycius* (E)	Muğla: Muğla to Fethiye, rocky slopes areas, 340 m., 17 October 2008, Kandemir, 516.
	Antalya: Kaş to Akçay, Sarnıç meadow areas, 1400 m., 25 October 2008, Kandemir, 517.
C. cancellatus ssp. *mazziaricus*	Balıkesir: Savaştepe, Kozören village, open areas, 500 m., 5 December 2010, Kandemir, 518.
	Denizli: Honaz Mountain, stony areas, 900 m., 10 December 2010, Çelik, 519.

(Contd)

(Contd)

C. cancellatus ssp. *pamphylicus* (E)	Mersin: Anamur-Kaş Yaylası, stony areas, 1605 m., 28 October 2008, Yıdıztugay, 520.
	Antalya: Akseki-Alacabey, stony areas, 900-1000 m., 10 November 2010, Kandemir, 521.
C. pestalozzae (E)	İstanbul: Ümraniye, Çekmeköy district, moist heath and meadowland areas, 80 m., 22 February 2008, Kandemir, 522.
	Kırklareli: Kıyıköy-Saray, meadowland areas and rocky slopes, 100-130 m., 25 February 2008, Kandemir, 523.
C. reticulatus ssp. *hittiticus* (E)	İçel: Silifke to Gülnar, Kandil passage, slopes areas with sparce scrub, 900 m., 23 March 2008, Kandemir, 524.
	İçel: Erdemli to Güzeloluk, open rocky areas, 1750 m., 7 April 2008, Kandemir, 525.
C. sieheanus (E)	Amasya: Ziyaret-Durucasu Village, open and forest areas, 700 m., 28 March 2009, Kandemir, 526.
	Karaman: West section of Güzeller, forest areas, 1400 m., 29 April 2009, Kandemir, 527.

samples were preserved in 70% alcohol solution. Parafin method was used for preparing cross sections of the leaf parts (Algan 1981). The cross section of the plant samples collected from two different localities were taken by microtome and the anatomical measurements were made. The binocular microscope with drawing tube was used for drawings (samples). For the stasistical analysis, 11 characters of the leaves were used. The importance of difference between the leaf anatomic measurements of taxa at different altitudes and similarities were evaluated by using t-test**.** Mean and standard deviation values of the leaf anatomic measuments of taxa are given in Tables 2 and 3.

Results and Discussion

Generally, the leaves of *Crocus* taxa consisted of two lateral arms triangular (ssp. *isauricus*, ssp. *nubigena*, ssp. *pseudonubigena*, ssp. *cancellatus*, ssp. *pamphylicus*, *C. pestalozzae* and *C. sieheanus*) (Fig. 1d-g, k, l and n) or rectangular (*C. ancyrensis*, *C. baytopiorum*, ssp. *crewei***,** ssp. *damascenus*, ssp. *mazziaricus*, ssp. *lycius*, and ssp. *hittiticus*) (Fig. 1a-c, h-j and m) keel in the median region. The margins of arms are usually recurved (*C. baytopiorum*, ssp. *crewei*, ssp. *nubigena*, ssp. *damascenus*, ssp. *pseudonubigena*, ssp. *cancellatus*, ssp. *pamphylicus*, *C. pestalozzae* and *C. sieheanus*) towards the keel (Kandemir 2009). The keel filled with large parenchyma cells, called lacuna. The abaxial side of arms have 2-4 protrusions (ssp. *pseudonubigena*, ssp. *isauricus* and ssp. *nubigena*) and 4-6 protrusions (ssp. *crewei*). Micropapillae were conspicuous on the cuticle of arms (*C. ancyrensis*, *C. baytopiorum*, ssp. *crewei***,** ssp. *isauricus*, ssp. *nubigena*, ssp. *lycius*, ssp. *mazziaricus*, and ssp. *hittiticus*, *C. pestalozzae* and *C. sieheanus*). But, micropapillae were evidently conspicuous on the cuticle of arms of ssp. *cancellatus*, ssp. *pamphylicus* and ssp. *damascenus*. All of the taxa stoma cells were on the groove parts of keel and anomocytic shaped. They were in sunken position between epidermis cells with micropapillae (*C. ancyrensis*, ssp. *isauricus*, ssp. *nubigena*, ssp. *pseudonubigena*, ssp. *cancellatus*, *C. pestalozzae* and *C. sieheanus*). While epidermis cells on groove part of leaves of *C. baytopiorum*, ssp. *nubigena*, ssp. *pseudonubigena*, ssp. *damascenus*, ssp. *pamphylicus* and *C. pestalozzae* had straight sinuous walls, epidermis cells on groove parts of leaves of ssp. *crewei***,** ssp. *isauricus*, ssp. *cancellatus* and ssp. *hittiticus* had sinuous. Mesophyll consisted of palisade and spongy parenchyma. The palisade parenchyma was 2 layered (*C. ancyrensis*, ssp. *crewei*, ssp. *isauricus*, ssp. *nubigena*, ssp. *lycius*, ssp. *mazziaricus*, *C. pestalozzae* and *C. sieheanus*), 1-2 layered (*C. baytopiorum*, ssp. *pseudonubigena*, spp. *pamphylicus* and ssp.

Table 2. t-test values based on 11 leaf anatomical characters of the investigated *Crocus* taxa. All dimentions are in µm. ± standard deviation.

		Upper epidermis		Lower epidermis		Palisade cells		Spongy cells				
Taxa	Cuticle thickness	Length	Breadth	Length	Breadth	Length	Breadth	Length	Breadth	Trachea diameter	Protrusion number	t and p values
1. *C. ancyrensis*	*15.6 ± 1.34	22.4 ± 1.71	19.8 ± 1.31	18.3 ± 1.15	17.9 ± 0.99	20.7 ± 2.62	16.8 ± 1.31	19.4 ± 0.84	19.1 ± 0.87	10.0 ± 1.15	1.0 ± 0.0	t = –3.564,
	**17 ± 1.1	17.0 ± 2.94	15.4 ± 2.16	12.2 ± 1.95	11.9 ± 1.85	22.2 ± 2.09	17.5 ± 2.06	18.6 ± 2.27	17.2 ± 1.31	13.1 ± 1.10	1.0 ± 0.0	p < 0.05
2. *C. baytopiorum*	7.0 ± 1.50	24.2 ± 3.08	17.4 ± 5.85	17.1 ± 3.14	18.6 ± 3.30	23.0 ± 6.32	12.7 ± 3.43	17.9 ± 3.24	14.0 ± 3.77	8.6 ± 1.71	1.4 ± 0.51	t=2.392,
	9.8 ± 1.31	26.5 ± 2.14	23.4 ± 2.11	19.4 ± 3.56	18.7 ± 2.31	25.0 ± 4.08	13.3 ± 3.12	16.3 ± 3.97	14.0 ± 3.59	12.4 ±2.22	1.5 ± 0.52	p<0.05
3. *C. biflorus*	17.1 ± 3.31	18.5 ± 2.01	14.1 ± 3.84	20.5 ± 1.35	12.6 ± 2.41	38.0 ± 5.50	16.8 ± 2.20	20.8 ± 5.22	24.0 ± 4.59	8.0 ± 2.58	4.8 ± 0.91	t = 0.162,
ssp. *crewei*	18.0 ± 2.21	18.4 ± 1.95	13.1 ± 2.42	19.6 ± 1.07	12.7 ± 2.49	40,5 ± 4.83	18.5 ± 5.29	20.0 ± 1.56	24.0 ± 3.94	8.4 ± 2.01	4.8 ± 0.91	p > 0.01
4. ssp. *isauricus*	17.9 ± 2.18	18.7 ± 1.70	18.0 ± 1.88	17.9 ± 2.33	17.6 ± 2.22	30.0 ± 8.18	12.3 ± 2.21	26.6 ± 3.97	19.2 ± 0.91	12.3 ± 2.21	3.6 ± 0.51	t = –1.331,
	19.8 ± 2.14	20.6 ± 2.63	19.8 ± 3.85	17.6 ± 2.36	18.5 ± 2.01	31.0 ± 7.57	12.8 ± 2.44	23.0 ± 2.0	17.7 ± 2.21	13.0 ± 2.26	3.5 ± 0.52	p > 0.05
5. ssp. *nubigena*	8.9 ± 1.37	17.7 ± 1.15	14.3 ± 1.41	11.3 ± 1.05	11.2 ± 1.13	20.2 ± 1.31	9.3 ± 0.82	19.1 ± 1.10	20.2 ± 0.91	8.9 ± 1.10	3.4 ± 0.51	t = –4.472,
	13.2 ± 1.75	19.8 ± 1.93	18.1 ± 1.59	12.0 ± 1.24	13.7 ± 1.41	27.3 ± 2.11	12.2 ± 0.78	16.4 ± 1.07	20.1 ± 0.87	13.9 ± 1.28	3.5 ± 0.52	p < 0.01
6. ssp. *pseudonubigena*	10.9 ± 1.44	17.6 ± 1.95	18.3 ± 1.70	13.6 ± 1.26	12.9 ± 2.18	31.0 ± 2.90	11.6 ± 1.26	27.1 ± 1.79	23.5 ± 1.35	10.4 ± 1.71	2.4 ± 0.51	t = 1.181,
	12.9 ± 0.87	14.9 ± 2.18	17.3 ± 1.94	11.5 ± 1.17	13.3 ± 1.33	32.1 ± 1.85	15 ± 2.05	18.9 ± 1.10	19.4 ± 0.96	12.5 ± 2.06	2.5 ± 0.52	p > 0.05
7. *C. cancellatus*	12.2 ± 1.31	26.0 ± 3.77	23.7 ± 3.52	19.6 ± 1.17	21.1 ± 2.76	28.8 ± 5.26	31.2 ± 5.90	34.2 ± 4.26	32.2 ± 4.26	21.6 ± 1.83	1.4 ± 0.51	t = 0.165,
ssp. *cancellatus*	13.8 ± 1.68	24.3 ± 3.94	23.9 ± 3.78	15.2 ± 2.09	14.4 ± 2.95	30.2 ± 5.20	3.4 ± 4.99	27.4 ± 5.16	28.8 ± 6.47	24.0 ± 2.49	1.3 ± 0.48	p > 0.05
8. ssp. *damascenus*	6.6 ± 1.26	16.1 ± 0.87	18.3 ± 1.88	18.4 ± 3.23	20.5 ± 1.17	20.1 ± 1.10	7.7 ± 0.94	28.2 ± 1.75	27.7 ± 1.88	7.7 ± 1.15	1.3 ± 0.48	t = 2.040,
	8.3 ± 1.25	14.3 ± 0.82	14.0 ± 0.81	13.9 ± 0.87	13.7 ± 0.67	21.2 ± 0.91	9.4 ± 0.69	27.6 ± 2.22	23.5 ± 2.63	10.4 ± 1.17	1.4 ± 0.51	p < 0.05
9. ssp. *lycius*	10.6 ± 1.17	28.3 ± 1.56	25.2 ± 1.75	27.0 ± 0.94	23.8 ± 0.78	25.6 ± 0.96	9.8 ± 0.78	22.8 ± 2.20	19.6 ± 1.57	10.6 ± 1.26	1.0 ± 0.0	t = –1.998,
	11.8 ± 1.22	30.9 ± 1.91	23.2 ± 2.04	28.6 ± 1.57	22.3 ± 1.49	27.8 ± 1.68	10.1 ± 0.87	20.4 ± 1.26	19.8 ± 1.75	13.4 ± 2.27	1.0 ± 0.0	p < 0.05
10. ssp. *maziaricus*	10.7 ± 1.15	17.1 ± 0.87	15.4 ± 0.69	8.2 ± 0.78	9.2 ± 0.78	27.1 ± 1.44	13.0 ± 0.81	17.1 ± 1.37	19.1 ± 0.87	8.7 ± 1.15	1.6 ± 0.51	t = 0.812,
	12.5 ± 1.43	19.8 ± 1.54	16.8 ± 1.91	9.8 ± 1.31	11.3 ±1.41	28.4 ± 1.26	13.9 ± 0.78	13.8 ± 1.31	15.3 ± 1.05	9.2 ± 0.78	1.6 ± 0.51	p > 0.05
11. ssp. *pamphylicus*	9.2 ± 0.78	13.4 ± 1.77	13.5 ± 2.32	13.5 ± 1.90	12.3 ± 2.05	24.0 ± 3.12	22.8 ± 3.29	23.8 ± 3.19	23.6 ± 3.05	9.1 ± 0.87	2 ± 0.48	t = 0.178,
	11.1 ± 0.87	15.1 ± 0.99	14.4 ± 1.34	13.9 ± 1.66	13.8 ± 2.39	25.2 ± 2.20	22.6 ± 2.50	20.2 ± 2.39	21.2 ± 2.14	10.4 ± 1.17	2 ± 0.48	p > 0.05
12. *C. pestalozzae*	9.6 ± 1.57	22.9 ± 1.96	21.6 ± 1.50	22.2 ± 2.14	22.8 ± 1.87	40.0 ± 3.94	22.9 ± 2.33	28.4 ± 1.95	28.2 ± 1.87	12.2 ± 1.75	1.0 ± 0.0	t = 0.689,
	10.6 ± 1.64	21.9 ± 1.91	22.5 ± 2.32	21.6 ± 1.57	22.2 ± 2.09	42.9 ± 2.18	21.9 ± 2.28	28.7 ± 1.56	27.8 ± 1.87	13.4 ± 1.77	1.0 ± 0.0	p > 0.05
13. *C. reticulatus* ssp. *hittiticus*	4.8 ± 0.91	14.1 ± 1.59	10.9 ± 1.10	12.5 ± 0.70	12.9 ± 0.87	18.1 ± 1.28	10.9 ± 0.99	25.0 ± 1.93	24.2 ± 1.75	11.7 ± 1.25	1.0 ± 0.0	t = 1.739,
	7.4 ± 1.26	15.0 ± 0.94	12.6 ± 1.34	14.8 ± 0.91	15.2 ± 0.91	28.3 ± 1.33	12.9 ± 1.52	21.8 ± 1.75	22.4 ± 1.83	14.0 ± 1.15	1.0 ± 0.0	p > 0.05
14. *C. sieheanus*	12.4 ± 1.83	16.0 ± 0.94	14.1 ± 0.87	11.8 ± 1.39	11.0 ± 0.94	17.9 ± 1.44	10.0 ± 1.63	22.6 ± 3.23	20.2 ± 1.75	13.6 ± 1.42	1.5 ± 0.52	t=1.844,
	14.1±1.52	14.5 ± 1.26	12.2 ± 1.68	13.1 ± 1.66	12.2 ± 1.68	19.0 ± 2.16	10.0 ± 1.63	20.4 ± 1.57	22.9 ± 1.96	16.0 ± 1.49	1.6 ± 0.51	p > 0.05

hittiticus) and 3-4 layered (ssp. *damascenus*). The spongy parenchyma was 2-3 layered (ssp. *crewei*, ssp. *pseudonubigena*, ssp. *hittiticus* and *C. sieheanus*), 3 layered (*C. baytopiorum* and ssp. *isauricus*) and 3-5 layered (*C. ancyrensis*, ssp. *nubigena*, ssp. *damascenus*, ssp. *lycius*, ssp. *mazziaricus*, ssp. *pamphylicus* and *C. pestalozzae*). In ssp. *cancellatus*, mesophyll composed of only 3-5 layered and oval shaped parenchyma cells. There were four large vascular bundles and different number of small vascular bundles in leaves. Two of large bundles were at the corners of the keel and the other two are at the end of arms. The small bundles were between the arms and the keel. The bundle sheath consisted of sclerenchyma cells at the phloem pole of the large bundles. There were sclerenchyma cells either at the phloem poles (*C. ancyrensis*, *C. baytopiorum*, ssp. *crewei*, ssp. *nubigena*, ssp. *cancellatus*, ssp. *damascenus*, ssp. *lycius*, ssp. *mazziaricus*, ssp. *pamphylicus* and *C. sieheanus*) or the phloem and xylem poles (ssp. *isauricus*, ssp. *pseudonubigena*, *C. pestalozzae* and ssp. *hittiticus*) of large and small bundles. All of these anatomic characters have taxanomic significance.

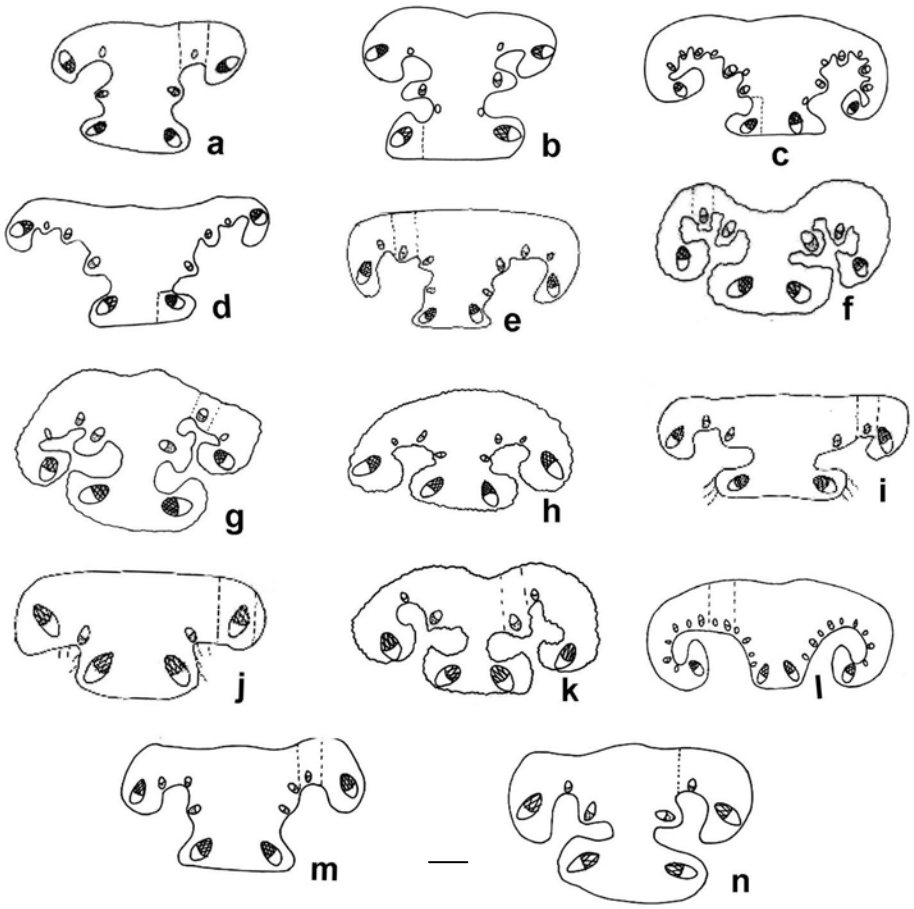

Fig. 1. The general drawing of the *Crocus* leaves: a. *C. ancyrensis*, b. *C. baytopiorum*, c. *C. biflorus* ssp. *crewei*, d. *C. biflorus* ssp. *isauricus*, e. *C. biflorus* ssp. *nubigena*, f. *C. biflorus* ssp. *pseudonubigena*, **g**. *C. cancellatus* ssp. *cancellatus*, h. *C. cancellatus* ssp. *damascenus*, i. *C. cancellatus* ssp. *mazziaricus*, j. *C. cancellatus* ssp. *lycius*, k. *C. cancellatus* ssp. *pamphylicus*, l. *C. pestalozzae*, m. *C. reticulatus* ssp. *hittiticus*, n. *C. sieheanus* (Bar = 400 µm).

They show considerable variation in morphologic characters, since *C. biflorus* and *C. cancellatus* are complex and wide geographically distributed taxa. Thus, there are problems in taxonomic of these two taxa. Although there is high homoplosy between both morphologic and anatomic characters, leaf anatomy provides some significant data for Iridaceae, especially at the tribus levels (Rudall 1994). Moreover, anatomical and palynological data can be used to infer phylogenetic relations among the *Crocus* taxa (Almida *et al*. 2009). While the ssp. *maziaricus*, ssp. *lycius* and ssp. *damascenus*, ssp. *cancellatus* as morphologic are closer to each other, the ssp. *pamphyllicus* is a different subspecies. According to the general leaf anatomy, it is seen that ssp. *pamphylicus* and ssp. *cancellatus* are similar to each other and ssp. *maziaricus* and ssp. *lycius* are also similar (Kandemir *et al*. 2011). Statistically, we have found that there are differences and similarities among subspecies of *C. cancellatus* respect to the leaf anatomic characters. But, ssp. *damascenus* are significantly different from other subspecies of *C. cancellatus* relating to the general leaf anatomy. It is concluded that there are similarities in anatomic characters of ssp. *damascenus* and *C. pallasi* ssp. *turcicus* by Akan and Eker (2004). However, the vascular bundles of ssp. *damascenus* are more abundant and regular. In this study, it is also seen that this subspecies is similar to *C. pallasi* ssp. *turcicus*. The two subspecies are relatively isolated taxonomically. It is considered that these the two subspecies are closely related.

On the other hand, although there are anatomic and morphologic differences between the subspecies of *C. biflorus*, it is seen that ssp. *isauricus*, ssp. *crewei* and ssp. *nubigena* (except ssp. *pseudonubigena*) are closer subspecies to each other relating to leaf anatomic characters. According to the statistical results, there are important correlations among ssp. *crewei*, ssp. *isauricus* and ssp. *nubigena* which are subspecies of *C. biflorus* at the level of $p > 0.05$ (Table 3). This similar phenomenon has been found in polen morphology of subspecies of *C. biflorus* (except ssp. *nubigena*) by Işık and Dönmez (2006). Despite the ssp. *pseudonubigena* is a subspecies of *C. biflorus* and ssp. *cancellatus* is a subspecies of *C. cancellatus*, they are close subspecies to each other according to their leaf anatomic structure. These similarities between two subspecies are

Table 3. Correlation based on t- test between investigated 14 *Crocus* taxa.

Taxon	Mean difference	t value	p values	Signi-ficance	Taxon	Mean difference	t value	p value	Signi-ficance
3 - 4	0.30	0.209	0.839 p > 0.05	NS	6 - 7	–5.17	–2.75	0.020 p > 0.01	NS
3 - 5	0.50	0.953	0.345 p > 0.05	NS	6 - 8	0.49	0.31	0.763 p > 0.05	NS
4 - 5	1.70	0.643	0.655 p > 0.01	NS	7 - 8	5.66	2.28	0.045 p < 0.05	*
3 - 12	–3.17	–2.06	0.065 p > 0.05	NS	8 - 9	4.97	2.23	0.040 p < 0.05	*
3 - 6	1.29	3.960	0.037 p < 0.05	*	8 - 10	5.47	3.68	0.039 p < 0.05	*
4 - 6	1.07	3.925	0.039 p < 0.05	*	8 - 11	6.64	4.89	0.001 p < 0.01	**
5 - 6	1.18	–3.144	0.010 p < 0.05	*	7 - 11	0.98	0.53	0.607 p > 0.05	NS
1 - 13	1.27	0.66	0.51 p > 0.05	NS	9 - 10	0.94	0.23	0.849 p > 0.05	NS
1 - 14	1.03	0.63	0.54 p > 0.05	NS	13 - 14	–0.23	0.23	0.823 p > 0.05	NS

NS, non significant; * significant at the level of 0.05; ** significant at the level of 0.01

supported by statistical results (Table 3, $p > 0.05$). Also, the soil analysis results of ssp. *pseudonubigena* showed a different subspecies of *C. biflorus* (Kandemir *et al*. 2011). So, the

taxanomic status of ssp. *pseudonubigena* and ssp. *damascenus* should be designed relating to their leaf anatomic and ecologic characters.

According to statistical analysis, it is seen that the leaf character measurements of ssp. *crewei*, ssp. *isauricus*, ssp. *pseudonubigena*, ssp. *cancellatus*, ssp. *mazziaricus*, ssp. *pamphylicus*, *C. pestalozzae*, ssp. *hittiticus* and *C. sieheanus* do not have any significant changes at different altitudes (Table 2, $p > 0.05$). However, there have seen more or less variations about the leaf anatomic measurements of *C. ancyrensis*, *C. baytopiorum*, ssp. *nubigena*, ssp. *damescanus* and ssp. *lycius* relating to the altitude (Table 2, $p < 0.05$). As shown in table 3, there are statistically important differences among 3-6, 4-6, 5-6, 7-8, 8-9, 8-10 and 8-11 taxa at levels of 0.05 and 0.01. The reason of these differences may be caused the distribution of these taxa at different ecological conditions. On the other hand, there are no statistically important differences among 3-4, 3-5, 4-5, 3-12, 1-13, 1-14, 6-7, 6-8, 7-11, 9-10 and 13-14 taxa (Table 3, $p > 0.05$). Also, ssp. *crewei* and *C. pestalozzae*, *C. ancyrensis* and *C. sieheanus*, *C. ancyrensis* and ssp. *hittiticus*, *C. sieheanus* and ssp. *hittiticus* are closer to each other based on leaf anatomic characters (Table 3, $p > 0.05$). The similarities between these taxa may be originated from their distribution at similar ecologic conditions. When the altitude increases, in the leaf anatomic measurements (specially, palisade parenchyma beadth and length, cuticle thickness and trachea diameter) of taxa are seen increased. But, in measurements of breadth and length of spongy parenchyma are seen decreased (Table 2).

As a general, there have found to have differences among the taxa in leaf anatomic characters such as the layer number and structure of mesophyll, shape of the epidermis cells, whether they have sinuous on epiderma, shape and base structure of keel, whether they have sclerenchyma in vascular bundles, number and the status of vascular bundles and protrusions number in arms. It appeared that the leaf characters mentioned may be used as important taxonomic characters of *Crocus* taxa. It is determined that palisade and spongy parenchyma length-breadth, trachea diameter and cuticle thickness are the best characters which represent the anatomic variations between these taxa relating to altitude (Table 2). There may be a xeromorphic adaptation, because, some taxa have sunkened stomata and triangular keel structure. In other anatomic characters considerable variations were not found.

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