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## Statistical Comparative Leaf Anatomy of Some *Crocus* L. Taxa

<sup>1</sup>A. Y. Özdemir, <sup>2</sup>A. Özdemir and <sup>3</sup>C. Özdemir

<sup>1</sup>Department of Mathematic, Faculty of Arts and Sciences,  
Boğaziçi University, 80815 Bebek, Istanbul, Turkey

<sup>2</sup>Department of Mathematics,

<sup>3</sup>Department of Biology, Faculty of Arts and Sciences,  
Celal Bayar University, Manisa, Turkey

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**Abstract:** The aim of this study is to describe and compare statistically the anatomical characters of mature leaves of *Crocus fleischeri* Gay, *C. danfordiae* Maw, *C. chrysanthus* (Herbert) Herbert, *C. pulchellus* Herbert, *C. flavus* Weston subsp. *flavus*, *C. pallasii* Goldb. subsp. *pallasii*, *C. speciosus* Bieb. subsp. *speciosus* Mathew, *C. speciosus* Bieb. subsp. *ilgazensis* Mathew, *C. speciosus* subsp. *xantholaimos* Mathew, *C. olivieri* subsp. *olivieri*. The anatomical variations in ten *Crocus* taxa have been investigated by means of numerical methods (Analysis of variance and Pearson correlation). By the analysis of the investigated taxa from eight leaf anatomy related characters, it has been determined that palisade cell height and spongy cell width are the best character pairs which represent the variations in them. It has been also found that the results from numerical analysis of the leaf anatomy characters can provide additional evidences which correspond to the anatomy for the recognition of the taxa.

**Key words:** *Crocus*, leaf, anatomy, statistical analysis

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### INTRODUCTION

The *Crocus* genus belongs to Iridaceae which had a large and diverse family of about 92 genera and 1800 species and is mainly distributed in the Southern hemisphere (Ali and Mathew, 2000). It is represented by 37 species in Turkey (Guner *et al.*, 2000). Many species of the family Iridaceae are grown in parks and gardens as ornamental plants due to their beautiful flowers (Baytop, 1984). Some *Crocus* species were used for making dye, perfume and medicaments since 1600 B.C. (Rudall and Mathew, 1990). The saffron *Crocus* (*Crocus sativus* L.) was the first to be cultivated and has been grown for economic purposes since ancient times. Abdullaev (2003) pointed out that the saffron could be useful in cancer chemoprevention in near future. Different studies on some *Crocus* species has been found in the literature (Mathew and Brighton, 1977; Dainauskaite *et al.*, 2001; Halevy, 1990; Miroslavov *et al.*, 2000; Loskutov *et al.*, 2000). But there have been a few studies on the morphology and anatomy of *Crocus* species in Turkey (Ozdemir *et al.*, 2004; Ozyurt, 1978; Akan and Eker, 2004; Akan *et al.*, 2007; Ozdemir and Akyol, 2005). Recently, some researchers have reported that the extract of *Crocus* has antitumor, antimetagenic and cytotoxic activities and inhibits nucleic acid synthesis in human malignant cells (Nair *et al.*, 1991; Abdullaev, 2003; Loskutov *et al.*, 2000; Fatehi *et al.*, 2003). The leaves of most *Crocus*

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**Corresponding Author:** Canan Özdemir, 273. Sokak, No. 2/1, Ozgün Sitesi B/Blok,  
Kat:7 Daire: 13. 35030, Bornova, Izmir, Türkiye

Table 1: The investigated samples and their voucher specimens

Code	Taxon	Locality	Time period	Herbarium No.
A	<i>C. fleischeri</i> (E)	Manisa; Maldan district 450 m	06.02.2003 02.03.2003	Akyol 475
B	<i>C. danfordiae</i> (E)	Manisa; Spil mountain 1240 m	02.02.2003 05.04.2003	Alçitepe 034
C	<i>C. chrysanthus</i>	Manisa: Maldan district in Yunt Mountain region, open hillsides in short turf, 450 m	08.02.2002 01.03.2002	Akyol 083
D	<i>C. pulchellus</i>	Manisa:Maldan district in Yunt Mountain region, forest area 400 m	17.10.2002	Akyol 024
E	<i>C. flavus</i> subsp. <i>flavus</i>	Salihli-Bahçecik district forest, 600 m	31.01.2003	Baran 014
F	<i>C. pallasii</i> subsp. <i>pallasii</i>	Manisa-Spil Mountain Atalanı area, 1200 m	10.10.2004	Akyol 1475
G	<i>C. speciosus</i> subsp. <i>speciosus</i>	Samsun-Kocadağ 1310 m	28.09.1997 24.11.1998 25.09.1999	Özdemir 030
H	<i>C. speciosus</i> subsp. <i>ilgazensis</i> (E)	Trabzon-Zigana dağı, 2010 m	25.10.1997	Özdemir 031
		Trabzon-Karadağ, 2000 m	25.10.1997	Özdemir 032
		Sinop-Dranaz dağı, 1350 m	20.09.1997	Özdemir 033
		Sinop-İsfendiyar dağı, 1455 m	11.10.1997	
			20.09.1997	Özdemir 034
I	<i>C. speciosus</i> subsp. <i>xantholaimos</i>	Amasya-Akdağ, 1800 m	27.09.1997	Özdemir 035
		Çankırı-İlgaz dağı, 1850 m	18.10.1998	
			18.10.1997	Özdemir 036
J	<i>C. oliveri</i>	Kırklareli forest area 600 m	26.03.2006	Özdemir 096

E. Endemic

species have a unique and distinctive shape in cross section. We aimed to give detailed knowledge about the anatomical characteristics of leaves of ten *Crocus* taxa in Turkey.

## MATERIALS AND METHODS

Plant samples were collected from natural populations between 1997-2006 (Table 1). Taxonomic description of the plant was made according to Davis (1984) and Mathew (1982). The experimental studies were conducted at Celal Bayar University, Faculty of Arts and Sciences Department of Biology. Anatomical works were carried out on the fresh samples preserved in 70% alcohol. Paraffin method was used for preparing cross sections of the tissues (Algan, 1981). Transverse sections, 15-20  $\mu$ m were made using a sliding microtome and stained with Safranin-Fast Green. Microscopic examinations were made on an Olympus BX50 microscope. Micrometric ocular was used for the anatomical measurements. For the numerical analysis, 8 characters of the leaves were selected. This selection was based on the variations of the leaf anatomical data. Characters were coded as 1, 2, 3, 4, 5, 6, 7, 8 and the taxa were coded as A, B, C, D, E, F, G, H, I, J. Significance of the differences between the taxa and characters were evaluated by analysis of variance (regression analysis) and pearson correlation.

## RESULT AND DISCUSSION

### *C. fleischeri*

The leaves had a central rectangular keel and two lateral arms, with their margins recurved towards the keel with a pale stripe running axially along the center of the leaf formed by the parenchymatous chlorophyllous cells. Both adaxial and abaxial surface except the groove parts of leaf had the cuticle. Epidermis cells, except the groove parts of abaxial surface are with straight walls. The epidermal cells on the groove parts had walls with papillae. Stoma

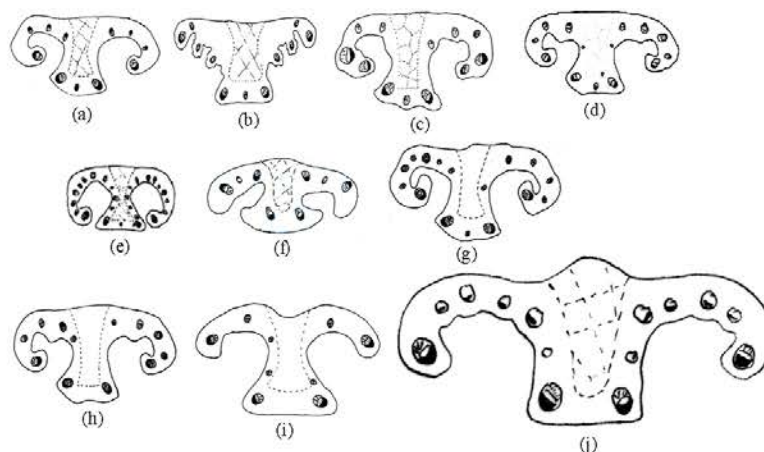


Fig. 1: General drawing of the *Crocus* leaves. (a) *C. fleischeri*, (b) *C. danfordiae*, (c) *C. chrysanthus*, (d) *C. pulchellus*, (e) *C. flavus* subsp. *flavus*, (f) *C. pallasii* subsp. *pallasii*, (g) *C. speciosus* subsp. *speciosus*, (h) *C. speciosus* subsp. *ilgazensis*, (i) *C. speciosus* subsp. *xantholaimos* and (j) *C. olivieri* subsp. *olivieri* (scale bar: 500  $\mu\text{m}$ )

cells are present only on groove part of leaf. These cells are in sunken position between epidermis cells with papillae. Palisade parenchyma cells are 1-2 layered. Spongy cells are present on abaxial side. Vascular bundles are located in one row in arms of keel and extending round abaxial margin of keel, but not across adaxial side. Major bundles occur at angles of keel and towards arm margins (Fig. 1, 2a).

#### *C. danfordiae*

Leaves of *C. danfordiae* had outline with central keel and two inflexed arms. The large central area of keel had thin walled cells which usually break down to form air space. The abaxial side of arms had two major protrusions. The epidermal cells of this protrusion had white papillae. Epidermis is cuticled except the grooved parts, which is single layered with flat-ovoidal cells. In the grooved parts the epidermal cells are papillate. Stomata is usually absent except in the grooved parts. Palisade parenchyma is 1-2 layered with spongy cells on periphery of vascular bundles. Vascular bundles are in one row in margins of arms and keel. Major bundles occur at angles of keel and arms, small bundles are located between the major vascular bundles (Fig. 1, 2b).

#### *C. chrysanthus*

The leaves had central rectangular keel and two lateral arm, with their margins recurved towards the keel. The characteristic pale stripe runs axially along the centre of the leaf. Stoma cells are in sunken position between epidermis cells with papillae. Adaxial surface and abaxial outer edge of keel except the groove parts of these surfaces are without stomata. Epidermal cells are 4-sided and with straight walls except in the edges of groove parts of abaxial surface. Epidermal cells on groove part of abaxial surface of leaf keel had walls with papillae. Vascular bundles are located in one row in arms of keel and extending around abaxial margin of keel, but not across adaxial side. Major bundles occur at angles of keel and towards arm margins (Fig. 1, 2c).

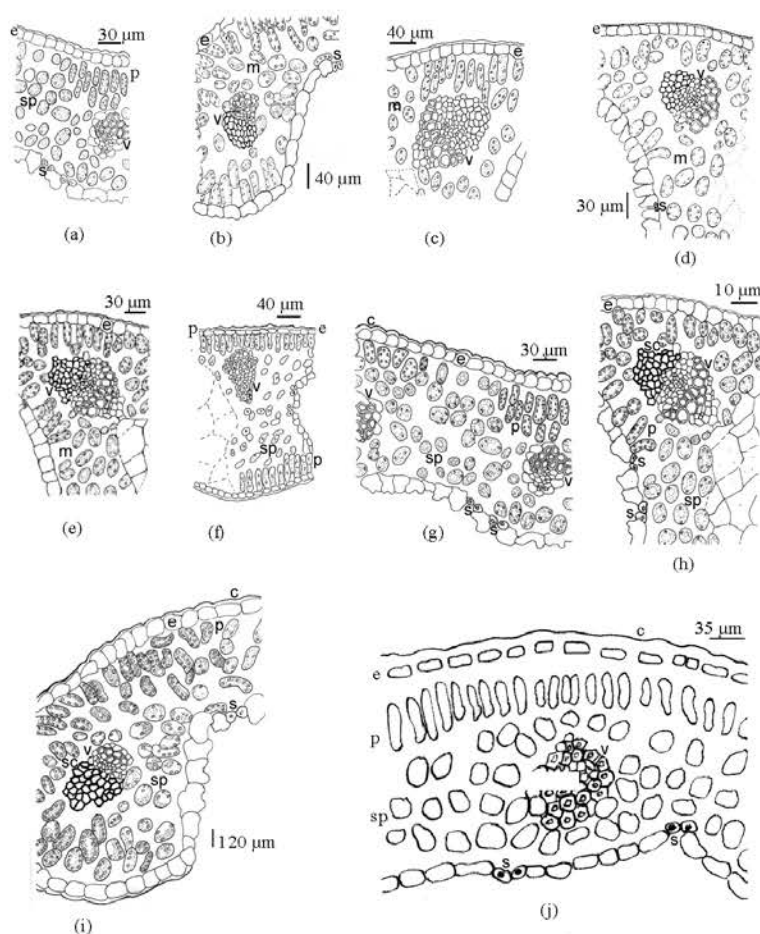


Fig. 2: Cross-sections of the *Crocus* leaves. (a) *C. fleischeri*, (b) *C. danfordiae*, (c) *C. chrysanthus*, (d) *C. pulchellus*, (e) *C. flavus* subsp. *flavus*, (f) *C. pallasii* subsp. *pallasii*, (g) *C. speciosus* subsp. *speciosus*, (h) *C. speciosus* subsp. *ilgazensis*, (i) *C. speciosus* subsp. *xantholaimos* and (j) *C. olivieri* subsp. *olivieri*, e: Epidermis, m: Mesophyll, c: Cuticle, p: Palisade parenchyma, s: Spongy parenchyma, v: Vascular bundle, sc: Sclerenchyma

### *C. pulchellus*

Leaves had central rectangular keel and two lateral arms, with their margins recurved towards the keel. The characteristic pale stripe runs axially along the center of the leaf is formed by the parenchymatous cells in the keel, which lack chloroplasts and break down to create an air space. Adaxial surface and abaxial outer edge of keel except the groove parts of these surfaces are without stomata. The cuticle is present on abaxial and adaxial surface. Epidermal cells are 4-sided. These cells except the ones on the groove parts of abaxial surface are with straight walls. It is difficult to distinguish the cells of palisade parenchyma from the cells of spongy parenchyma on the mesophyll of leaf. Because the mesophyll cells are more or less uniform in shape. Vascular bundles are located in one row

in arms of keel and extending around abaxial margin of keel, but not across adaxial side. Major bundles occur at angles of keel and towards arm margins. Stoma cells are present on the groove parts of keel. These cells are in sunken position between epidermis cells with papillae (Fig. 1, 2d).

***C. flavus* Weston subsp. *flavus***

The leaveshad central triangular keel and two long lateral arms with their margins recurved towards the keel. The characteristic pale stripe runs axially along the center of the leaf is formed by the parenchymatous cells in the keel, which lack chloroplasts and break down to create an air space. Both adaxial and abaxial surfaces, except the parts in the groove had a thick cuticle. The epidermal cells are slightly furnished with papillae on the groove parts of the arms; stomata are present on these grooved parts. Mesophyll cells are more or less uniform in shape. Vascular bundles are located in one row and below the abaxial epidermis. The bundle sheath consists of sclerenchymatic cells at the phloem pole of major bundles. Abaxial epidermis had stomata while the adaxial epidermis had no stomata (Fig. 1, 2e).

***C. pallasii* Goldb. subsp. *pallasii***

The leaf had central slightly square keel and two lateral arms with recurved towards the keel. There is a pale stripe lying axially along the center of the leaf. The leaveshad the cuticle except groove part. The epidermal cells had cuticle with papillae. Adaxial epidermis is thicker than abaxial epidermis. Stoma cells are present only on the groove parts of leaf and in sunken position between epidermis cells. Palisade parenchyma cells are single layered. Vascular bundles are located in one row in arms of keel and extending around abaxial margin of keel, but not across adaxial side. There are major bundles occur at angles of keel and towards arm margins (Fig. 1, 2f).

***C. speciosus* Bieb. subsp. *speciosus***

The leaves had central rectangular keel and two lateral arms, with their margins recurved towards the keel with a pale stripe running axially along the center of the leaf. Both adaxial and abaxial surface except the groove parts of leaf had the cuticle. Epidermal cells are 4-sided, except the ones on the groove parts of abaxial surface are with straight walls. Stoma cells are present only on the groove parts of leaf. These cells are in sunken position between epidermis cells with papillae. Palisade parenchyma cells are 1-2 layered. Spongy cells are present on abaxial side. Vascular bundles are located in one row in arms of keel and extending around abaxial margin of keel, but not across adaxial side. Major bundles occur at angles of keel and towards arm margins (Fig. 1, 2g).

***C. speciosus* subsp. *ilgazensis***

The leaves had central slightly square keel. Margins of lateral arms recurved towards the keel with a pale stripe running axially along the center of the leaf. Both adaxial and abaxial surface except the groove parts of leaf had the cuticle. The epidermal cells on groove part had walls with papillae. Stoma cells are in sunken position between epidermis cells with papillae. Palisade parenchyma cells are 1-2 layered. Spongy cells are present on abaxial side. Vascular bundles are located in one row in arms of keel and extending around abaxial margin of keel, but not across adaxial side. Major bundles occur at angles of keel and towards arm margins (Fig. 1, 2h).

***C. speciosus* subsp. *xantholaimos***

The leaves had central nearly triangular keel and two lateral arms. The margins of the lateral arms are recurved towards the keel. The keel of leaf is filled with large parenchymatous cells. Stoma cells are present on the groove parts of keel. These cells are in sunken position between epidermis cells with papillae. Vascular bundles are located in one row in arms of keel and extending around abaxial margin of keel (Fig. 1, 2i).

***C. olivieri* Gay subsp. *olivieri***

Leaves had central and nearly rectangular keel and two lateral arms. The tips of the lateral arms are recurved towards the keel. The keel of leaf is filled with large parenchymatous cells that lacked chloroplasts and were broken down in places so as to form air space. Mesophyll is located in the lateral arms had chloroplasts and differentiated into palisade and spongy parenchyma. It was somehow difficult to distinguish the palisade cells from the others in the mesophyll. Vascular bundles are arranged in one row along the arms of keel and they are closer to the abaxial side. Major bundles occur at the angles of keel and at the tips of arms. The cuticle on adaxial epidermis is thicker than the cuticle on the abaxial. The outer surface of epidermis is straight everywhere except the groove part of leaf surface. Only the groove parts of the leaf surface had stomata. Stoma cells are in sunken position between epidermis cells with conspicuous micropapillae (Fig. 1, 2j).

**Statistical Analysis**

The anatomical measurements of the investigated taxa were shown in Table 2. Significance of the differences between the *Crocus* taxa was evaluated by analysis of variance (regression analysis) and Pearson correlation (correlation). And the statistical analysis of the results were given Table 3-6.

The differences among the investigated taxa are shown as A-C, A-E, A-F, B-C, B-F, B-J, C-E, C-F, D-H, G-I and J-A in Table 3 and 5, are significant at levels of 0.01 and 0.05. Furthermore, a significant difference has been found at levels of 0.05 between E and B according to Table 3 based of Pearson correlation method (correlation). According to Table 4 and 6, there are important correlations among the anatomical characters of the leaves of the investigated taxa, shown as (Table 2) 1-4, 1-6, 4-5, 4-6 and 7-8 at levels of 0.01 and 0.05.

**Table 2: Anatomical measurements of the *Crocus* taxa**

Taxon	Measurements ( $\mu\text{m}$ )							
	Cuticle thickness	Upper epidermis width	Upper epidermis length	Lower epidermis width	Lower epidermis length	Palisade length	Palisade width	Spongy height
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
<i>A. C. fleischeri</i>	7.9±0.73	8.0±0.72	10.0±0.11	23.0±0.28	20.0±0.19	15.0±0.11	30.0±0.81	18.0±0.21
<i>B. C. danfordiae</i>	6.0±0.66	7.0±0.74	15.0±0.17	28.0±0.32	23.0±0.26	20.0±0.22	40.0±0.45	22.0±0.32
<i>C. C. chrysanthus</i>	1.5±0.87	12.0±0.94	6.0±0.72	23.0±0.32	12.0±0.91	12.0±0.19	35.0±0.51	25.0±0.45
<i>D. C. pulchellus</i>	2.5±0.36	15.0±0.13	18.0±0.19	13.0±0.24	12.0±0.17	8.0±0.10	15.0±0.18	18.0±0.17
<i>E. C. flavus</i> subsp. <i>flavus</i>	8.0±0.73	13.0±0.16	14.0±0.12	17.0±0.19	12.0±0.13	10.0±0.10	30.0±0.21	20.0±0.33
<i>F. C. pallasii</i> subsp. <i>pallasii</i>	1.8±0.16	9.5±1.90	7.4±0.81	11.0±0.97	7.9±0.86	10.0±0.21	29.0±0.21	20.0±0.45
<i>G. C. speciosus</i> subsp. <i>speciosus</i>	6.0±0.73	18.0±0.19	15.0±0.18	22.0±0.31	20.0±0.19	5.0±0.19	14.0±0.61	8.0±0.81
<i>H. C. speciosus</i> subsp. <i>ilgazenis</i>	6.0±0.73	18.0±0.19	15.0±0.18	22.0±0.31	20.0±0.19	5.0±0.19	14.0±0.61	8.0±0.81
<i>I. C. speciosus</i> subsp. <i>xantholaimos</i>	2.5±0.36	20.0±0.27	18.0±0.21	25.0±0.21	15.0±0.11	10.0±0.90	18.0±0.78	13.0±0.34
<i>J. C. olivieri</i> subsp. <i>olivieri</i>	7.0±0.56	18.0±0.14	11.0±0.18	25.0±0.28	15.0±0.12	16.0±0.81	45.0±0.36	25.0±0.48

Values are in Mean±SD. SD: Standard deviation; 1-8: Character codes

Table 3: Pearson correlation (correlation) based on anatomical characters of the leaves of the investigated taxa

Taxa	A	B	C	D	E	F	G	H	I
B	0.985 0.000								
C	0.891 0.003**	0.871 0.005**							
D	0.340 0.410	0.259 0.536	0.214 0.611						
E	0.800 0.017*	0.821 0.013*	0.924 0.001**	-0.092 0.828					
F	0.790 0.020*	0.806 0.016*	0.940 0.001**	-0.111 0.794	0.955 0.000				
G	0.581 0.131	0.513 0.193	0.577 0.135	0.146 0.730	0.602 0.114	0.448 0.266			
H	0.431 0.287	0.358 0.384	0.208 0.621	0.756 0.030*	0.055 0.898	-0.096 0.821	0.625 0.097		
I	0.481 0.227	0.433 0.284	0.494 0.214	0.241 0.566	0.460 0.251	0.334 0.419	0.893 0.003**	0.682 0.063	
J	0.861 0.006**	0.859 0.006**	0.968 0.000	0.089 0.834	0.954 0.000	0.953 0.000	0.599 0.117	0.151 0.721	0.461 0.250

\*Significant at the level of 0.05; \*\*Significant at the level of 0.01

Table 4: Pearson correlation (correlation) based on 8 anatomical characters of the leaves of the investigated taxa

Characters No.	1	2	3	4	5	6	7
2	0.125 0.731						
3	-0.177 0.625	0.386 0.270					
4	0.774 0.009**	0.188 0.604	-0.028 0.939				
5	0.576 0.082	-0.071 0.845	0.242 0.501	0.802 0.005**			
6	0.636 0.048*	-0.364 0.301	-0.327 0.356	0.711 0.021*	0.602 0.065		
7	-0.421 0.225	-0.357 0.311	-0.146 0.687	-0.358 0.310	-0.224 0.534	0.164 0.650	
8	-0.180 0.619	-0.383 0.275	-0.493 0.147	-0.199 0.581	-0.317 0.373	0.372 0.290	0.846 0.002**

\*Significant at the level of 0.05; \*\*Significant at the level of 0.01

Table 5: Correlation between 10 investigated *Crocus* taxa (regression analysis)

Taxon	MS	F-value	Probability	Significance
A-C	386.32/16.76	23.05	0.003	**
A-E	311.62/29.21	10.67	0.017	*
A-F	304.06/30.47	9.98	0.020	*
A-J	361.18/17.21	17.24	0.006	**
B-C	644.02/34.16	18.85	0.005	**
B-F	563.15/50.62	11.12	0.016	*
B-J	640.07/37.80	16.93	0.006	**
C-E	724.31/20.78	34.86	0.001	**
C-F	750.60/16.39	45.78	0.001	**
D-H	193.71/24.19	8.01	0.030	*
D-E	583.74/47.19	12.37	0.013	*
E-H	1.010/56.50	0.02	0.898	NS
G-H	122.13/31.68	3.85	0.097	NS
G-I	248.80/10.57	23.54	0.003	**
H-I	152.48/29.25	5.21	0.063	NS

MS: Mean square; NS: Not significant; \*p<0.05; \*\*p<0.01

Rudall (1994) has also pointed out that leaves of most *Crocus* species had a unique and distinctive shape in cross section, comprising a central square or rectangular keel and two lateral arms. The present investigations are in agreement with Rudall's (1994) findings, except



Table 6: The results of analysis of variance among the 10 investigated taxa (regression analysis)

Taxon	MS	F-value	Probability	Significance
2-Jan	4.09/32.37	0.13	0.731	NS
4-Jan	157.62/13.18	11.96	0.009	**
5-Jan	87.20/21.98	3.97	0.082	NS
6-Jan	106.28/19.60	5.42	0.048	*
4-Mar	0.11/18.09	0.01	0.939	NS
5-Apr	342.40/23.75	14.42	0.005	**
6-Apr	269.34/32.88	8.19	0.021	*
6-May	79.12/17.39	4.55	0.065	NS
8-Jul	710.09/35.19	20.18	0.002	**

MS: Mean square; NS: Not significant, \* $p < 0.05$ , \*\* $p < 0.01$

that the leaves of *C. flavus* subsp. *flavus* and *C. speciosus* subsp. *xantholaimos*. These taxa had central slightly triangular keel different from the types mentioned above. These differences may be caused by these taxa localized more arid region. Because triangular keel which exists in the leaves of *C. flavus* subsp. *flavus* and *C. speciosus* subsp. *xantholaimos* cause deeper groove parts in the leaf surface. Therefore stoma cells of these taxa are in sunken position between epidermis. It is observed that the leaves have a pale stripe running axially along the centre of the leaf. This is a common feature in the genus (Rudall and Mathew, 1990). The results of the study show that there is a single layered palisade parenchyma in mesophyll of *C. pallasii* subsp. *pallasii* and *C. olivieri* subsp. *olivieri* while there is a 2-3 layered palisade parenchyma in mesophyll of the other *Crocus* taxa. Akan and Eker (2004) pointed out that there is 4-5-layered palisade parenchyma in mesophyll of *Crocus cancellatus* subsp. *damascenus* and *C. pallasii* subsp. *turcicus*.

As shown in Table 3 and 5, there are important correlations between *C. speciosus* subsp. *xantholaimos* and *C. speciosus* subsp. *speciosus* which are subspecies of *C. speciosus*. On the other hand, there are no important differences between subsp. *speciosus* subsp. *ylgazensis* and subsp. *xantholaimos* which are subspecies of *C. speciosus*.

It is mentioned that there are close relationships between *C. danfordia* and *C. chrysanthus* (Davis, 1984). Also, according to the statistical results derived, there is a considerable relation between the two taxa, at the level of  $p < 0.01$ . The results of the statistical analysis were presented in Table 4 and 6. It was found that there were statistically important differences between element 1-4, 1-6, 4-5, 4-6 and 7-8 at levels of 0.01 and 0.05. By the analysis of investigated taxa from eight leaf anatomy related characters, it has been determined that palisade cell height and spongy cell width are the best characters pairs which represents the variations in them. It has been also found that the results from numerical analysis of the leaf anatomy characters can provide distinct evidences, which are corresponding to the anatomy for recognition of the taxa.

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