

## Micromorphological Investigations on Pollen Samples of Four Yellow Flowered Taxa of *Crocus* L. (Iridaceae) from Turkey

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### ABSTRACT

In this study, yellow flowered taxa of *C. ancyrensis* (Herbert) Maw, *C. sieheanus* Barr ex Burt, *C. flavus* Weston subsp. *flavus* and *C. flavus* subsp. *dissectus* T. Baytop & Mathew were examined in the point of pollen micromorphology.

Accordingly, it was aimed that pollen micromorphological characteristics of the plant samples could be used as a taxonomic criterion considerably or not. In respect to that purpose, exine structure of pollens as regards samples was examined by scanning electron microscope (SEM). On the other hand, differences between taxa were evaluated.

As a result of that investigation, pollen exine ornamentation related to studied four taxa of *Crocus* L. genus were observed different to some extent and could be used as vice for taxonomic characteristics.

**Key Words:** *Crocus* spp, pollen micromorphology, taxonomical significance

### INTRODUCTION

*Crocus* species (about 85 species) show distribution only in northern hemisphere. Most of the *Crocus* species are found in Mediterranean basin. Their distribution extends from Portugal and Morocco on the west to Kyrgyzstan and Senyang state of Western China and Ala Tau and Tien Shan Mountains of Mongolia on the east. Most of the taxa defined are in Balkans and Turkey. Number of the taxa decreases rapidly out of these regions [1].

All of the *Crocus* species, 70 taxa show distribution in Turkey [2–4]. Due to taxon diversity, Turkey might be considered as the homeland of *Crocus*.

Erdtman [5] reported some information about pollens of Iridaceae family.

Brighton et al. [6], furthermore, informed that intra-specific variation was seen in some species and some groups have significant and steadily increasing taxonomic characteristics. However, they also noted that such studies as SEM imaging of pollen and germ testa, pollen fertility, improvement works, DNA measuring and hybridization are required.

Furness and Rudall [7] investigated “inaperturate pollen in monocotyledones”. They stated that some *Crocus* species (e.g., *Crocus sativus* and related species) have inaperturate pollen with a thin exine and spinules. Other *Crocus* species spiraperturate or polyrugoidate. Chichiricco [8], made a study in a number of *Crocus* species as regards developmental stages of the pollen wall and tapetum, together with exine morphology by light and scanning electron microscopy. Işık and Oybak Dönmez [9], investigated 29 taxa of *Crocus* genus in the point of palinology. Şık et al. [10] pointed out that high level of genetic variation detected among sampled 19 *Crocus* species. According to this study, differences were observed between classifications in the point of morphologic and molecular peculiarities.

Pollen morphology of Turkish Amaryllidaceae, Ixioliriaceae and Iridaceae was presented by Oybak Dönmez and Işık [11]. In this study, pollen morphological data for *Crocus* L. taken from their previously published [9] accounts were also included in their last report [11] to provide an overall synthesis of the pollen characteristics of the three families in Turkey.

Accordingly, in this investigation 4 taxa of *Crocus* L. genus that is naturally spreading in Turkey and flowering at spring were examined. Pollens of these taxa were investigated with scanning electron microscope (SEM). As a result, it was discussed that pollen micromorphological peculiarities could be taxonomic characters or not.

## MATERIALS AND METHODS

Information on the localities from which the plants constituting the study materials is as follows:

A. *C. ancyrensis* (Herbert) Maw: Bolu, between Abant Lake and Mudurnu, N 40°34' EO 31°15', 1050 m, 15.03.2004.

B. *C. s. sieheanus* Barr ex Burt: Adana, Pozantı, Aladağlar, N 37°39' EO 34°57', 1500 m, 27.03.2005.

C. *C. flavus* Weston subsp. *flavus*: Çanakkale, Ayvacık, N 39°42' EO 26°23', 160 m, 20.03.2005.

D. *C. flavus* Weston subsp. *dissectus* T. Baytop & Mathew: Manisa, Kırkağaç, N 39°08' EO 27°39', 240 m, 31.01.2005.

For identification of the plants, the book "Flora of Turkey" was used [2,3].

Pollen grains for SEM were fixed in acetic acid and alcohol (1:3) or treated for 60 min. with chloroform-carbon disulfide (1:1) to remove the pollenkitt [12]. The samples were then, dehydrated in an ethanol series and critical point dried with CO<sub>2</sub> [8]. They were gold coated and JEOL JSM-6060 model scanning electron microscope was used for observing their surface structures.

## Findings

Pollen types, shapes; polar and equatorial diameters; ornamentations and exine structures belong to *C. ancyrensis*, *C. s. sieheanus*, *C. flavus* subsp. *flavus* and *C. flavus* subsp. *dissectus* taxa were given in Table 1.

## RESULTS AND DISCUSSION

Pollen and seed morphology are both good characteristics for taxa differentiation at systematic botany. Especially, they can be useful and efficacious criterions for two near taxa distinction.

Erdtman [5] stated pollens of family members of Iridaceae generally 1-sulcate (rarely 2-sulcate), spirapertur(oid)ate or nonaperturatae. Erdtman [5] and Mathew [1] reported 3 aperture, (nonaperturatae, spiraperturatae and polyrugoidate) after preliminary study with *Crocus* pollens. In our samples, spiral furrows are very obvious on pollens of taxa relating *C. ancyrensis* and *C. flavus* subsp. *dissectus*. On the other hand, *C. sieheanus* and *C. flavus* subsp. *flavus* taxa have nonaperturatae type pollens. (Fig. 1, 2, 3, 4; Table 1)

Mathew [1] said a comprehensive study of pollens can give useful knowledge about evolutionary conjecture.

According to the prevalent evolutionary trends, the furrows may be considered as a primitive character, the pores as a specialization, and the colpi as intermediate between the two [13]. Thus on the basis of exine morphology, the pollen of *C. ancyrensis* and *C. flavus* subsp. *dissectus* would be classed as more primitive than the pollen of *C. sieheanus* and *C. flavus* subsp. *flavus*.

Işık and Oybak Dönmez [9] determined pollens of all *C. ancyrensis*, *C. sieheanus* and *C. flavus* species as spheroidal shaped. In this study, pollens of four taxa determined as prolate spheroidal and spheroidal shaped (Table 1). At the same time, taxa don't show correlation with pollen diameters and chromosome numbers. Generally, it is accepted that pollen diameters increase with chromosome numbers but Işık and Oybak Dönmez [9] reported some *Crocus* taxa with small pollens but more chromosomes and some of taxa with big pollens but less chromosomes, like *C. fleischeri* Gay, 2n=20, *C. kotschyanus* subsp. *cappadocicus* Mathew, 2n=10 and *C. cancellatus* Herbert subsp. *damascenus* (Herbert) Mathew, 2n=8, 10, 12 [9]. The chromosome numbers of *C. ancyrensis* as 2n=12+1B, *C. sieheanus* as 2n=16 and *C. flavus* as 2n=8 were determined [14]. According to studied taxa pollen size, *C. sieheanus* species both have the biggest pollen if the mean values considered and the most chromosomes.

Oybak Dönmez and Işık [11] stated values of pollens related to *C. ancyrensis* as 63-88 µ, *C. sieheanus* as 59-113 µ and *C. flavus* subsp. *dissectus* as 80-98 µ were given. According to current study, prolate spheroidal shaped pollens were sometimes observed so that polar and equatorial diameters were given as a first time. Some differences can be seen between diameters related to pollens given with that study and literature (Table 1). On the other hand, pollen micromorphological features of *C. flavus* subsp. *flavus* were determined with that study, firstly.

According to Işık and Oybak Dönmez [9], pollen samples exine structures of *Crocus* were found as echinate (spinulate)-microperforate. As a result of current study, exine structure was observed as scabrate relating all study materials (Fig. 1, 2, 3, 4).

In respect of literature, pollen morphological characters are also one of the data considered at taxonomical studies. In this study, pollens of *C. ancyrensis*, *C. sieheanus* and *C. flavus* species with yellow flowers at February and March, were studied in a detailed way with scanning electron microscope as a first time. According to results, pollen surface differences confirmed that they have a taxonomical importance on taxonomy of Iridaceae family. However, it appears that some of the pollen micromorphological characters like pollen type and ornamentation are useful to separate *C. ancyrensis*, *C. sieheanus*, *C. flavus* subsp. *flavus* and *C. flavus* subsp. *dissectus*.

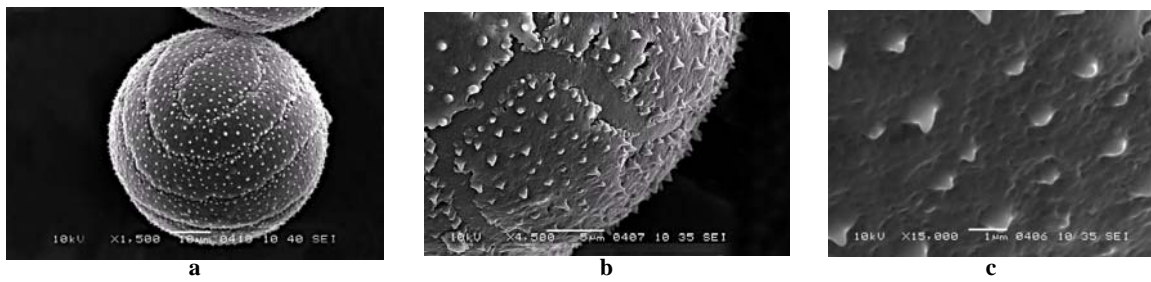
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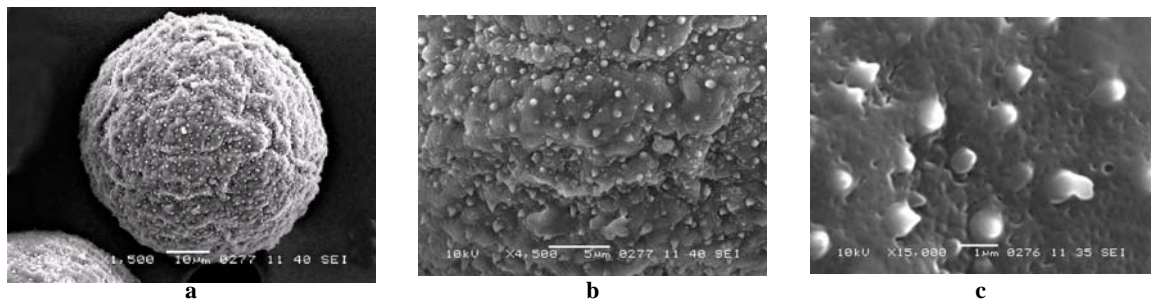
**Table 1.** Pollen micromorphological findings related to four taxa

Taxa	Pollen Type	Shape	P (µm)	E (µm)	Ornamentation	Structure
<i>C. ancyrensis</i>	Sp	M	65.1 ± 2.3	63.7 ± 2.2	Scabrate (TRSS)	Tectate
		PS	(64.3 – 70.2)	(61.9 – 69.0)		
		S	(61.9 – 66.6)	(61.9 – 66.6)		
<i>C. sieheanus</i>	Na	M	75.9 ± 16.4	74.0 ± 15.7	Scabrate (TRSS)	Tectate
		PS	(69.0 – 92.8)	(66.6 – 90.4)		
		S	(46.6 – 95.0)	(46.6 – 95.0)		
<i>C. flavus</i> subsp. <i>flavus</i>	Na	M	65.3 ± 2.0	64.3 ± 1.2	Scabrate (DS)	Tectate
		PS	(64.3 – 66.6)	(63.1 – 65.5)		
		S	(63.1 – 66.6)	(63.1 – 66.6)		
<i>C. flavus</i> subsp. <i>dissectus</i>	Sp	M	66.2 ± 2.5	65.6 ± 3.0	Scabrate (VDS)	Tectate
		PS	(63.1 – 66.9)	(59.5 – 67.1)		
		S	(61.9 – 71.4)	(61.9 – 71.4)		

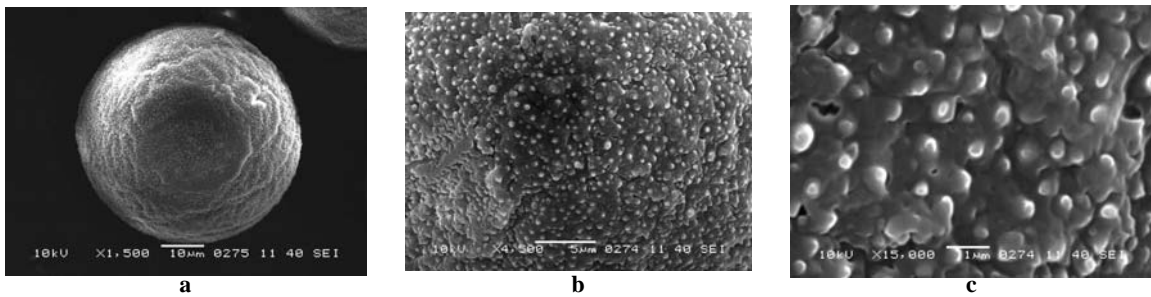
[Abbreviations: **Sp**: spiraperturate; **Na**: nonaperturate; **M**: mean value and standard deviation; **PS**: prolate spheroidal; **S**: spheroidal; **E**: equatorial diameter; **P**: polar diameter; **TRSS**: thin reticulate, sparsely spinulate; **DS**: dense spinulate; **VDS**: very dense spinulate]. (Minimum and maximum values of pollen diameters in parenthesis)



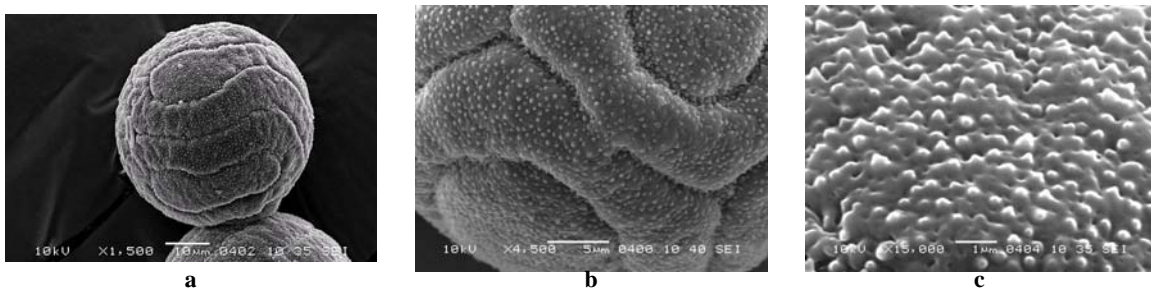
**Fig. 1.** SEM images of *C. ancyrensis*; a) general view of pollen, b) closer view of reticulation, c) exine sculpture with spinules.



**Fig. 2.** SEM images of *C. sieheanus*; a) general view of pollen, b) closer view of reticulation, c) exine sculpture with spinules.



**Fig. 3.** SEM images of *C. flavus subsp. flavus*; a) general view of pollen, b) closer view of reticulation, c) exine sculpture with spinules.



**Fig. 4.** SEM images of *C. flavus subsp. dissectus*; a) general view of pollen, b) closer view of reticulation, c) exine sculpture with very dense spinules.