

POLLEN MICROMORPHOLOGY OF FOUR TAXA OF *ANEMONE* *CORONARIA* L. FROM WESTERN TURKEY

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Abstract

The present study examined a comparison of the pollen micromorphology of four taxa of *Anemone coronaria* L. (*A. coronaria* var. *coccinea* (Jord.) Burn, *A. coronaria* var. *rosea* (Hanry) Batt, *A. coronaria* var. *cyanea*, *A. coronaria* var. *alba* Goaty & Pens) naturally distributed in western Turkey and its commercial cultivars of de Caen group. Measurements of pore width-length and exine-intine layer thicknesses were made using a micrometric ocular. In this study pore width-length and exine-intine layer thicknesses were found as decreased in all *Anemone coronaria* L. cultivars of de Caen group in respect to the varieties. The reasons for this variation could be the use of pesticides and tetraploid F₁ hybrids of *Anemone coronaria* L. cultivars of de Caen group by the producers.

Introduction

The genus *Anemone* L. *sensu stricto* belongs to Ranunculaceae and is distributed throughout the world. According to Ziman *et al.* (2008), this genus includes 15 subgenera, 23 sections, 4 subsections, 23 series and 118 species. Because of the taxon's worldwide distribution and considerable diversity in *Anemone* L. morphology, this genus has been studied for its chromosomes and genome (Heimburger 1962).

The species *Anemone coronaria* L. is known as the 'Tulip of Manisa' in Turkey. According to Davis (1965), there are four varieties of *Anemone coronaria* L. based on tepal colours in Turkey. These are *A. coronaria* var. *coccinea* (Jord.) Burn (with scarlet flowers), *A. coronaria* var. *rosea* (Hanry) Batt (with pink flowers), *A. coronaria* var. *cyanea* (with violet-blue flowers) and *A. coronaria* var. *alba* Goaty & Pens (with white flowers). The *A. coronaria* L. is a multifunctional species, though recent cultivars have been largely selected for cut-flower production. *A. coronaria* L. cultivars are classified into two major groups: 'De Caen' and 'St. Brigid'. The cultivars included in this study belong to the 'de Caen' group, which is characterized by long stems and a single flower (Laura and Allavena 2007).

The aim of this study was to investigate the pollen micromorphology of four varieties and four commercial cultivars of *Anemone coronaria* species.

Materials and Methods

Plant specimens of the four varieties of *Anemone coronaria* L. were collected from areas near the Kırkağaç-Soma highway of Manisa, a city located in Western Turkey, in March 2011. The cultivars of the same species were obtained from the commercial flower growers in the Urla area of the city of İzmir.

In this study, the *Anemone coronaria* L. cultivar group of de Caen selections (scarlet, pink, violet-blue and white cultivars) was used. Tetraploid F₁ hybrids were used as commercial forms in this study. Flora of Turkey (Davis 1965) was consulted for the taxonomic identification of the plant samples.

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Flower specimens from each group were fixed in the Carnoy's solution. The flowers were removed from the solution and then the anthers, taken from ripe floral buds with the help of a dissection needle, were mounted on a glycerine-gelatin-liquid safranin mixture (Wodehouse 1965). However, some preparations for the measurements were made according to Erdtman (1966) method. A total of 1000 pollens from each groups were used for the measurements of pore width-length and exine-intine layer thicknesses in equatorial view. These were made with the help of a Leica ICC50 HD binocular light microscope by using a Leica Digital Camera. Some pollens of the varieties, the pure group, and the cultivars were photographed using a JEOL JSM-6060 Scanning Electron Microscope (SEM) (Nepi *et al.* 1995).

Statistical analyses of the values related to all measurements in the present study were made on a SPSS 20.0 for Windows statistical program. A multiple Range Tukey Test was used for variance analyses (Tukey 1954).

Results and Discussion

In this study, the pollen type of the varieties was found to be periporate and pollen shape prolate spheroidal (Fig. 1).

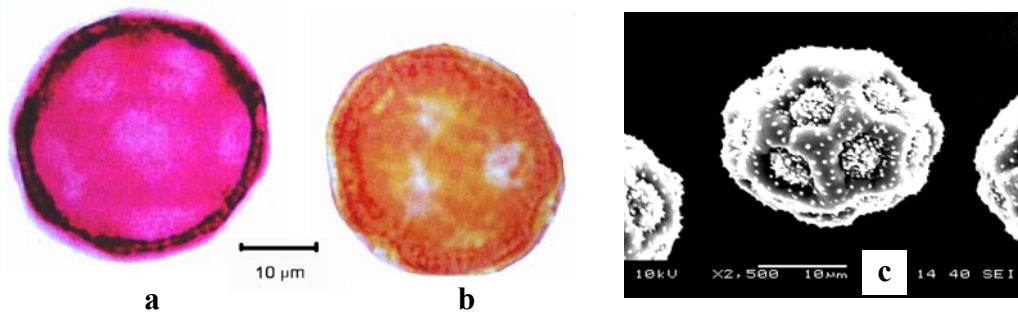


Fig. 1. The general appearance of the pollen in the pure *Anemone coronaria* var. *coccinea* (Jord.) Burn. (a) Wodehouse Method is used. (b) Erdtman method is used. (c) Scanning electron microscope is used.

In addition, pollen structure was determined as tectate and sculpture as scabrate. Pores with unclear annulus, clear operculum and spinules were seen (Fig. 2a). *A. coronaria* var. *coccinea* and *A. coronaria* var. *cyanea* pollens were found with 14 pores and *A. coronaria* var. *purpurea* and *A. coronaria* var. *alba* pollens with 16 pores.

The results of the exine and intine measurements of the pollen grains are shown in Table 1. As compared to the pure groups, the measurements of the exine and intine layers were generally lower in the commercial cultivars. Such a decrease in the exine measurements of the de Caen cultivar groups pollens except for *Anemone coronaria* pink cultivars group was statistically significant as compared to the pure groups ($p < 0.05$). Meanwhile, the decrease that occurred in the intine measurements of the de Caen cultivar group pollens as compared to the pure group except for *Anemone coronaria* pink cultivars and violet-blue cultivars groups was found to have a statistical significance ($p < 0.05$).

According to Kamenetsky and Okubo (2013), the species of the genus *Anemone* L. are cultivated in greenhouses for the domestic consumption of cut flowers. It is known that some diseases are easily caused by the greenhouse conditions. Several fungicides are used to prevent the fungi *Pythium*, *Botrytis* and powdery mildew causing diseases in *Anemone*. In addition, several

aphid species and red spider species are considered to be the major pests of *Anemone*. Some insecticides were also used against these pathogens. According to the breeding of *Anemone coronaria* L. cultivars, to bring forward the flowering, tubers can be vernalised before planting (Ohkawa 1987). Laura and Allavena (2007) reported that one of the processes after vernalising is disinfection with fungicides.

Table 1. Exine and intine measurements (μm) of the pollen grains.

Groups	Equatorial view	
	Exine (μm)	Intine (μm)
<i>A. coronaria</i> var. <i>coccinea</i> (Pure)	2.470 \pm 0.115 ^b	1.728 \pm 0.087 ^b
<i>A. coronaria</i> scarlet cultivars of de Caen group	1.605 \pm 0.109 ^a	1.301 \pm 0.057 ^a
<i>A. coronaria</i> var. <i>rosea</i> (Pure)	2.233 \pm 0.074	1.461 \pm 0.075
<i>A. coronaria</i> pink cultivars of de Caen group	1.958 \pm 0.117	1.228 \pm 0.077
<i>A. coronaria</i> var. <i>cyanea</i> (Pure)	1.953 \pm 0.085 ^f	1.693 \pm 0.066
<i>A. coronaria</i> violet-blue cultivars of de Caen group	1.361 \pm 0.058 ^e	1.453 \pm 0.044
<i>A. coronaria</i> var. <i>alba</i> (Pure)	2.431 \pm 0.087 ^h	2.204 \pm 0.047 ^h
<i>A. coronaria</i> white cultivars of de Caen group	1.548 \pm 0.091 ^g	1.714 \pm 0.095 ^g

The Tables shows that the differences among “a” and *A. coronaria* var. *coccinea* pure group, “b” and *A. coronaria* scarlet cultivars “e” and *A. coronaria* var. *cyanea* pure group, “f” and *A. coronaria* violet-blue cultivars, “g” and *A. coronaria* var. *alba* pure group, “h” and *A. coronaria* white cultivars are statistically significant ($p < 0.05$).

Table 2. Measurements of pores (μm) in equatorial view.

Groups	Equatorial view	
	Pore width (μm)	Pore length (μm)
<i>A. coronaria</i> var. <i>coccinea</i> (Pure)	4.901 \pm 0.190 ^b	6.190 \pm 0.329 ^b
<i>A. coronaria</i> scarlet cultivars of de Caen group	3.457 \pm 0.201 ^a	4.147 \pm 0.210 ^a
<i>A. coronaria</i> var. <i>rosea</i> (Pure)	6.167 \pm 0.322 ^d	6.975 \pm 0.338 ^d
<i>A. coronaria</i> pink cultivars of de Caen group	3.848 \pm 0.121 ^c	5.026 \pm 0.221 ^c
<i>A. coronaria</i> var. <i>cyanea</i> (Pure)	4.305 \pm 0.226 ^f	5.205 \pm 0.279
<i>A. coronaria</i> violet-blue cultivars of de Caen group	3.010 \pm 0.118 ^e	4.709 \pm 0.198
<i>A. coronaria</i> var. <i>alba</i> (Pure)	5.097 \pm 0.278 ^h	7.163 \pm 0.239 ^h
<i>A. coronaria</i> white cultivars of de Caen group	3.186 \pm 0.181 ^g	4.357 \pm 0.215 ^g

The Tables shows that the differences among “a” and *A. coronaria* var. *coccinea* pure group, “b” and *A. coronaria* scarlet cultivars, “c” and *A. coronaria* var. *rosea* pure group, “d” and *A. coronaria* pink cultivars, “e” and *A. coronaria* var. *cyanea* pure group, “f” and *A. coronaria* violet-blue cultivars, “g” and *A. coronaria* var. *alba* pure group, “h” and *A. coronaria* white cultivars are statistically significant ($p < 0.05$).

It was thought that the reason for the reduction in the exine and intine layers of the pollens belonging to commercial cultivars could be attributed to the use of agricultural chemicals to produce a lot of flowers and rapid growth. It was reported that different fungicides and activator treatments had significant effects on the germination, the tube growth and the morphology of pollens. All fungicides reduced the percentage of pollen germination, length of germ-tube elongation and morphological features of almond (*Prunus dulcis*) pollen (Zarrabi and Imani 2011). Öztürk Çalı and Candan (2010) stated that the ACT-2, a K-vitamin group activator treatments reduced the exine-intine layer thickness and width-length measurements of tomato (*Lycopersicon esculentum* Mill.) pollen. In another study, Equation Pro (22.5% famoxadone + 30% cymoxanil) fungicide caused morphological deformity and thinner wall layers in the pollen of tomato

(*Lycopersicon esculentum* Mill.) (Öztürk Çalı 2009). Additionally, the germination and the tube length of olive pollen were reduced when three insecticides (Dimethoate, deltamethrine and oleoparathion) were applied *in vitro* and *in vivo* to olive pollen culture media. In contrast, applications of the insecticide oleoparathion most severely reduced pollen germination, retarded germination, ruptured pollen tubes and damaged stigmatic surfaces *in vivo* in the same study (Mehri *et al.* 2007). According to Öztürk Çalı (2008), the fungicide fosefly-AI caused non-viable pollens types, such as wrinkled pollens or pollens with abnormal shapes. These were discovered in the *Lycopersicon esculentum* Mill. pollen. Moreover, pollen viability level decreased as the dosage increased in the same study.

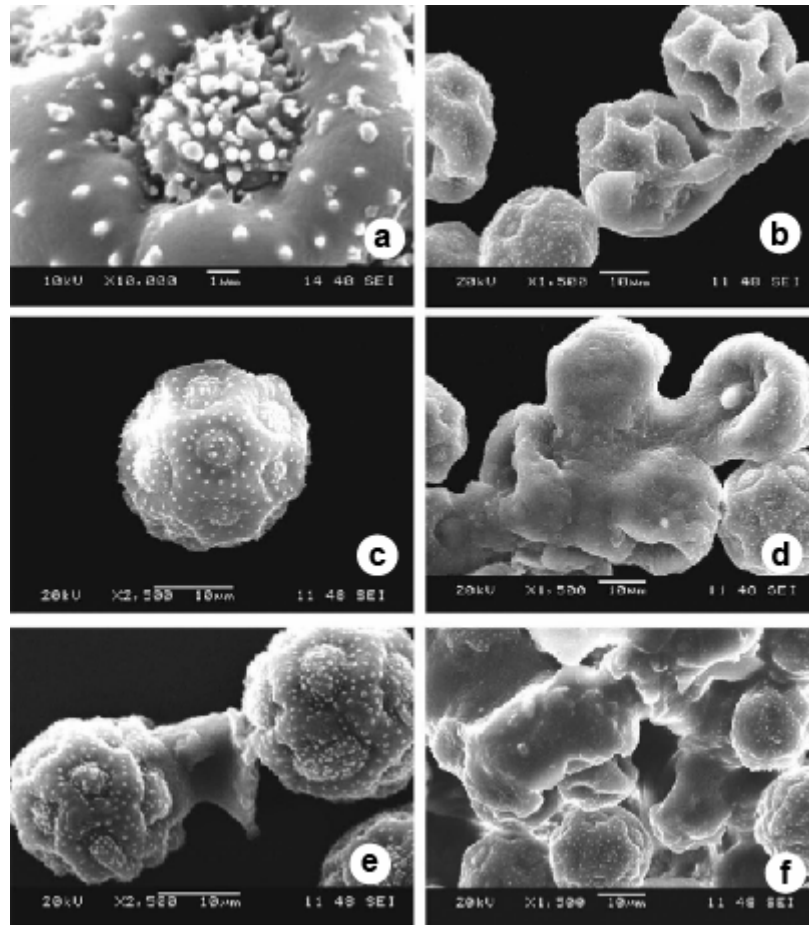


Fig. 2. SEM photographs. (a) The general appearance of the pollen in the pure *Anemone coronaria* var. *coccinea* (Jord.) Burn. with the spinules on the exine of the pollen. (b) Disappearance of the spinules on the surface layers of the pollens in the commercial *A. coronaria* red cultivars (c) Anomaly in the structure and the shape of pores in the commercial *Anemone coronaria* pink cultivars pollens (d) Disappearance of the spinules and the pores on the surface layers of the commercial *Anemone coronaria* violet-blue cultivars pollens. (e) Some changes on the shape of pores on the surface layers of the commercial *Anemone coronaria* white cultivars pollens. (f) Disappearance of the spinules and the pores on the surface layers of the commercial *Anemone coronaria* white cultivars pollen.

Many studies showed detrimental effects of hybridization on the pollen viability in the commercial cultivars (Bures *et al.* 2010, Gay 1957). No study has been carried out on *Anemone* for knowing the effects of hybridization on the exine and intine layer. The present paper was the first study which examined the effects of hybridization on the measurements of the exine and intine layers of *Anemone coronaria*.

When the results were evaluated with regards to width and length of pores in the equatorial view, it was generally seen that there was a decrease in the pore width and pore length values (Table 2). This decrease in the pore width of all the de Caen cultivar groups pollens was statistically significant as compared to the pure groups ($p < 0.05$). Besides, the decrease that occurred in the pore length of the de Caen cultivar group pollens except for *Anemone coronaria* violet-blue cultivars as compared to the pure groups was found to have a statistical significance ($p < 0.05$).

Several studies have been carried out on the detrimental effects of pesticides on the length-width measurements of pollens in the equatorial and polar views (Öztürk Çalı and Candan 2009, Öztürk Çalı 2008). Another reason could be the hybrid nature of the *Anemone* L. varieties. Although there are many studies on the effects of hybridization on the pollen morphology of plants (Datta *et al.* 2006, Henderson 1972), there is no publication about the influence of hybridization on the pore measurements of the pollens in the commercial cultivars. In this study, it was found for the first time that there was a decrease in the pore measurements of the commercial cultivars pollens due to hybridization.

In the study, it was also determined that there were some changes on the surface layers of the de Caen commercial species of *Anemone* L. pollens. The spinules on the exine of the pollens observed in the varieties had disappeared on the commercial cultivars pollens. In addition to this, formation of a rare perforation formation of the structure was observed on the exine of the pollens regarding the cultivars (Fig. 2). Moreover, some anomalies were seen on the structure and shape of the pores in the commercial cultivars of de Caen group pollens. Ravi and Nair (1986) studied three species of *Gloriosa* L. and their three hybrids and observed that the pollen exine patterns in the hybrids were different from the patterns of the parent plants. Öztürk Çalı and Candan (2009) also stated that the fungicide Agri Fos 400 (400 g/l mono and di-potassium phosphanate) caused changes on the surface layer of pollen in the *Lycopersicon esculentum* Mill.

It was concluded that there were noteworthy differences in the exine-intine and pore width-length measurements of the pollens in the de Caen cultivar groups as compared to the pure groups. The reasons for differences, might be the pesticides used to increase the growth during the commercial cultivation of the forms using of the tetraploid F_1 hybrid *Anemone* L. forms to produce more beautiful and big flowers than the pure ones.

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