

# Pollen Morphology and Anatomy of Cornelian Cherry (*Cornus mas* L.) Cultivars

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**Abstract.** Morphology and ultrastructure of pollen grain were described for six cornelian cherry (*Cornus mas* L.) cultivars (Degirmendere, Erkenci Degirmendere, İri Bardak, Yuvarlak Bardak, Uzun Memeli, and Bugur) using light microscopy and both scanning and transmission electron microscopy. Pollen grains of cornelian cherry cultivars are trizonocolporate, the germinal furrow extending almost the full length of the grain axis. Pollen grain length for the studied cultivars ranged from 23.63 to 25.13  $\mu\text{m}$ . Two different pollen shapes were observed: oblate spheroidal and prolate spheroidal. Significant changes in various layers of the pollen wall are as follows: the thickness of the intine: 233.1 to 406.4 nm, exine: 510.2 to 842.7 nm, and total wall 851.4 to 1175.8 nm. Percentages of abnormal pollen grains ranged from 3.9% to 27.6% among the cultivars. Abnormal pollen grains were categorized as: 1) shrunken pollen grains of abnormal appearance with little cytoplasm; 2) pollen grains of normal appearance with little or no cytoplasm; and 3) shrunken, abnormal pollen grains of elliptical shape with a colpate-type aperture.

Cornelian cherry (*Cornus mas* L.) is one of the original fruit species of the Anatolian peninsula. They have a small or medium size tree form and the fruit is a stone fruit with one seed. Fruits are similar to sour cherries except the fruit shape, which is rather elliptical. Flowering time is early relative to various other fruit species and occurred in the middle of February. This early flowering is evident in the orchards as a result of its bright yellow flowers.

Taxonomists and paleobotanists considered the importance of pollen development and morphology in clarifying the classification and identity of many plant species, e.g., peach [*Prunus persica* (L.) Batsch], nectarine [*Prunus persica* var. *nectarina* (Ait.) Maxim.], sweet cherry [*Prunus avium* (L.) L.], European plum (*Prunus domestica* L.) (Fogle, 1977a), olive (*Olea europaea* L.) (Javady and Arzani, 2001; Lanza et al., 1996), apple (*Malus domestica* Borkh.) (Currie et al., 1997; Fogle, 1977b), and chestnut (*Castanea sativa* Mill.) (Mert and Soylu, 2007). Pollen grains are the male gametophytes and take part in the reproductive biology. Fruit set is also dependent mainly on the fertilization process in the cornelian cherry cultivars. For this reason, the viability and/or sterility of the pollen grains is of great importance in the fruit set of these cultivars (Mert and Soylu, 2006).

The features and anatomy of normal and abnormal pollen grains have shown great

variation in various fruit and plant species. Many researchers have investigated the definition and characterization of pollen grains with respect to their abnormalities (Abreu et al., 2006; De Vries and Ie, 1970; McCreight, 1984; Mert and Soylu, 2007; Radice et al., 2008). Shape, size, surface morphology, and ultrastructure of pollen grains are of great importance in characterization of the pollen

grains (Currie et al., 1997; Fogle, 1977a; Martens and Fretz, 1980; Mert and Soylu, 2007).

Structural characteristics of the pollen grains of many fruit species have been described in previous studies (Arzani et al., 2005; Currie et al., 1997; Fogle, 1977a, 1977b; Javady and Arzani, 2001; Lanza et al., 1996; Martens and Fretz, 1980; Mert and Soylu, 2007; Westwood and Challice, 1978). However, no prior literature exists on the surface morphology and ultrastructure of the pollen grain of cornelian cherry. The present study represents the first report on this subject. The aim of this study was to determine the shape, dimensions, anatomical structure, and surface morphology of the pollen grains of some cornelian cherry cultivars native to the Anatolian peninsula.

## Materials and Methods

The study was conducted on cornelian cherry cultivars Degirmendere, Erkenci Degirmendere, İri Bardak, Yuvarlak Bardak, Uzun Memeli, and Bugur.

*Dimensions of pollen grains.* Samples of pollen grains were placed on a microscope slide and fixed with a drop of glycerin before they were covered with a coverslip. An ocular micrometer was used to measure the dimensions of 50 pollen grains of each cultivar. In addition, length:width ratios were calculated, and shape indices were determined according to Erdtman (1966).

*Light microscopy and transmission electron microscopy.* Flower cluster samples were fixed in FAA solution (10% formalin, 5% glacial acetic acid, 50% ethanol, 35%

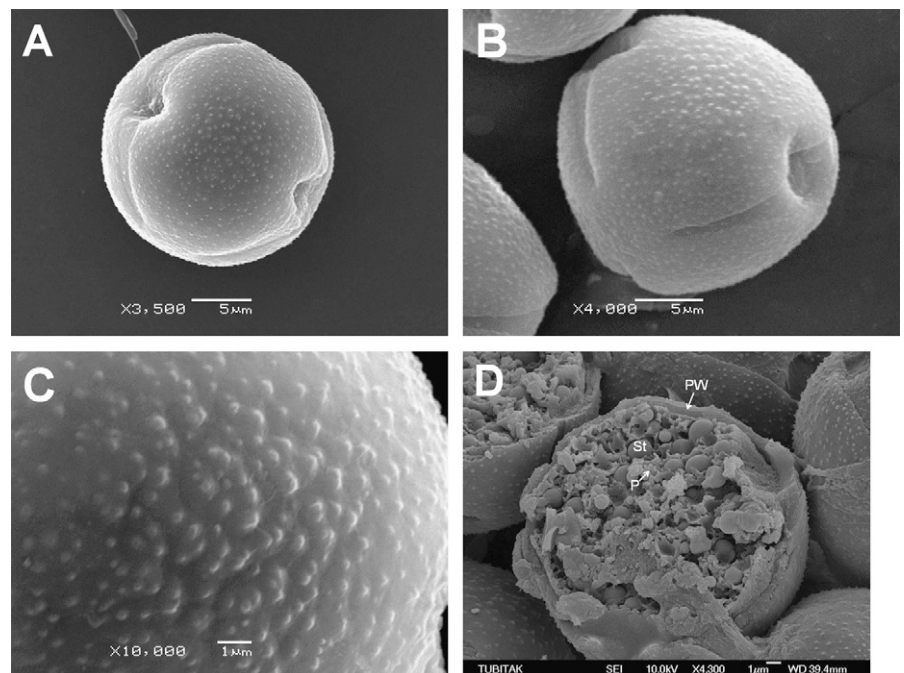


Fig. 1. Scanning electron micrograph (SEM) images of pollen grains of cornelian cherry cultivars. Equatorial view of grain from Uzun Memeli cultivar (A). Polar view of grain and surface view of grain from Erkenci Degirmendere cultivar (B–C). View of starch granules, protein bodies, and pollen wall in Degirmendere cultivar (D). P = protein bodies; PW = pollen wall; St = starch granules.

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water, by volume) and washed three times in a phosphate buffer (pH 7.2) for 20 min. The anthers were then fixed in 1% osmium tetroxide for 2 h, dehydrated in a graded ethanol series (50%, 70%, 95%, and twice with a 100%), and embedded in Spurr's epoxy resin and sectioned (1  $\mu\text{m}$ ) using an ultramicrotome (Reichert Supernova; Leica, Wien, Austria). Anther sections were stained with 1% toluidine blue and examined under a light microscope (BH-2; Olympus Optical Co., Tokyo, Japan). To study the ultrastructure of the pollen wall, anthers were thin-sectioned. Thin sections (90 to 100 nm thick) were stained with uranyl acetate and lead citrate. Sections were observed with a JSM-1220 transmission electron microscope (TEM) (Jeol, Tokyo, Japan).

Measurements of the exine and intine layers of the pollen wall were made in nanometers using a TEM with an "image measuring system." The thickness of the exine, intine, and total pollen wall was determined on 10 pollen grains at three different regions each.

**Scanning electron microscopy.** After dehydration on a silica gel drier, small quantities of pollen grains were mounted on scanning electron microscopy (SEM) stubs and coated with gold-palladium (Polaron SC7620; VG Microtech, Uckfield, U.K.) and examined with a JSM-5600 LV SEM (Jeol).

**Statistical analysis.** The data were analyzed using MSTAT-C statistical software (version 2.1; Michigan State University, East Lansing, MI), and means were compared using Duncan's multiple range test ( $P \leq 0.05$ ).

## Results and Discussion

### Pollen surface morphology and dimensions.

Pollen grains in each of the cornelian cherry cultivars examined were elliptical and trizonocolporate with subdivision of the surface area into three equal parts (Fig. 1A–B). Pollen grains have echinate exine. Some small, blunt spines were present on the pollen surface (Fig. 1C). Perveen and Qaiser (2002) reported similar results and stated that the pollen grain of *Cornaceae* has a tricolporate-type aperture. Like in various other fruit species (e.g., grape, kiwifruit, sour cherry, chestnut), cornelian cherry cultivars have three germination regions (Abreu et al., 2006; Ahmedullah, 1983; Jiang et al., 2004; Mert and Soylu, 2007; Miaja et al., 2000).

The surface of cornelian cherry pollen was covered with thread-like pollenkitt as shown in Figure 2B. Pollenkitt is the most striking pollen surface coat of many insect- as well as wind-pollinated species (Weber, 1996). It mainly comprises neutral lipids, including carotenoid pigments (Pacini and Hesse, 2005).

The pollen grains of the cultivars studied exhibited significant differences between the cultivars with their length (polar) and width (equatorial) diameters (Table 1), which ranged from 23.63 to 25.13  $\mu\text{m}$  and from 24.25 to 27.13  $\mu\text{m}$ , respectively. Erdtman (1966) found that the length of pollen grains

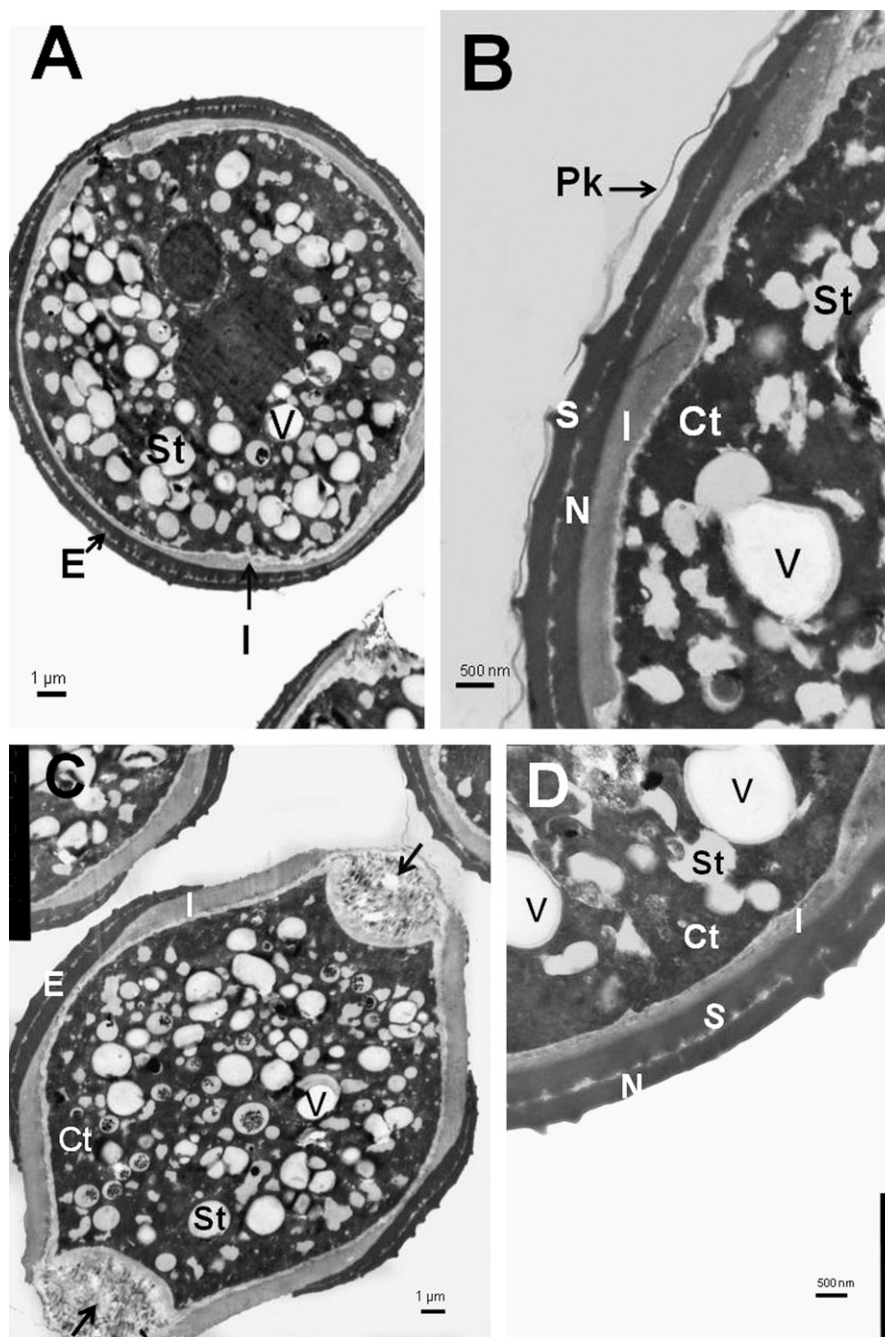


Fig. 2. Transmission electron micrographs (TEM) of pollen grain and pollen wall in cornelian cherry cultivars Iri Bardak (A–B) and Uzun Memeli (C–D). Transverse section ( $\times 3,000$ ) through pollen grain showing cytoplasm, vacuole, starch granules, and apertures (arrows) (A, C) (bar = 1  $\mu\text{m}$ ). Transverse section ( $\times 8,000$  and  $\times 10,000$ ) of pollen wall showing sexine, nexine, and intine (B, D) (bar = 500 nm). A = aperture; Ct = cytoplasm; E = exine; I = intine; N = nexine; Pk = pollenkitt; S = sexine; St = starch; V = vacuole.

of *Cornaceae* varied between 15 and 67  $\mu\text{m}$ . Our results are in accordance with these values. However, our findings were within a much narrower range than the values reported by Erdtman (1966). Cornelian cherry cultivars examined in the present study have smaller pollen grains than peach, European plums, apple, and Japanese plums (*Prunus salicina* Lindl.) (Fogle, 1977a, 1977b). The equatorial length of the pollen grains was generally much greater than the polar length, except Bugur cultivar. Two types of pollen

shape index were determined among the cornelian cherry cultivars studied (Table 1). The cultivars Degirmendere, Erkenci Degirmendere, Iri Bardak, Yuvarlak Bardak, and Uzun Memeli have oblate spheroidal-type pollen grains with a shape index of 1.0 or greater. 'Bugur' has prolate spheroidal pollen grains with a shape index of 1.02 or less (Table 1). Erdtman (1966) stated that the *Cornaceae* family has oblate spheroidal-prolate pollen grains. Our results support this finding.

**Anatomical structure of pollen grains.** The microspore comprises a large nucleus, a large vacuole, and the distribution of various cytoplasmic organelles is relatively sparse (Fig. 2A, C). The dense, evenly distributed cytoplasm in a mature pollen grain, as observed with light microscopy, was confirmed by TEM (Fig. 2A, C). Starch granules, protein bodies, and the pollen wall are shown in Figure 1D. The cornelian cherry pollen wall consisted of two layers: the outer exine layer and the inner intine layer (Fig. 2). The pollen walls of all the cornelian cherry cultivars were of similar appearance in cross-section. The exine is subdivided into two components, sexine and nexine, and is of the tectate type (Fig. 2B, D) as shown by Esau (1977). The tectum is continuous and has small spines. Murray (1997) also found that *Cornus* pollen grains have a tectate sexine with many spinules, which is in accordance with the current findings.

The thickness of these layers varied significantly among the cultivars, as shown in Table 2. For each pollen grain, the thickness of the intine layer was variable. Around the aperture region, the intine layer was found to be thicker, whereas the exine layer was thinner (Fig. 2).

**Abnormalities.** Alongside normal pollen grains, each cultivar examined also displayed a proportion of abnormally formed pollen grains. Percentages of abnormal pollen grains among the cultivars ranged from 3.9% to 27.6% (see Table 1). Some were almost completely filled with aborted pollen grains with remnants of the degenerated tapetum remaining between them (Fig. 3B). The following types of abnormal pollen grains were observed in the cultivars examined: 1) shrunken pollen grains of abnormal appearance with little cytoplasm (Fig. 3B–D); 2) pollen grains of normal appearance with little or no cytoplasm (Fig. 3D); and 3) more or less shrunken, abnormal pollen grains of elliptical shape with a colpate-type aperture (Fig. 3A). Similar abnormalities have been observed in other plant species. Abnormal pollens that have no germinative pores (acolporated form) in *Vitis vinifera* cultivars were observed (Abreu et al., 2006; Ahmedullah, 1983). Li et al. (2005) observed that *Tritileymus* exhibited amorphous pollen grains with abnormal shapes. These microspores show a large number of germination pores. Radice and Galati (2006) observed a large proportion of nonuniform pollen grains within anthers of the ‘Forastero’ peach (*Prunus persica* Batsch). Moreover, pollen grains with empty cytoplasm were also reported in some plant species, e.g., four interspecific hybrids of the genus *Actinidia* (Jiang et al., 2004), in 23 sterile lines of peach (Laishui et al., 1999), in original *ms1* and *ms2* mutations of *Cucumis melo* (McCreight, 1984), in wheat (*Triticum aestivum*) (De Vries and Ie, 1970), and in (Mert and Soyulu, 2007) chestnut. In the present study, the maximum proportion of abnormal pollen grains among the cornelian cherry cultivars examined was 27.6% in the Bugur cultivar. Values for the

Table 1. Dimensions of pollen grains and abnormal pollen grain ratios together with length:width ratios and shapes in cornelian cherry (*Cornus mas* L.) cultivars.

Cultivars	Abnormal pollen (%)	Pollen length [mean ± SE (µm)]	Pollen width [mean ± SE (µm)]	Length:width ratio	Shape
Degirmendere	4.7	24.50 ± 1.54 ab <sup>2</sup>	26.51 ± 1.71 ab	0.9242	Oblate spheroidal
Erkenci Degirmendere	7.0	25.13 ± 0.99 a	26.75 ± 1.18 ab	0.9394	Oblate spheroidal
İri Bardak	3.9	23.63 ± 1.28 c	26.00 ± 1.26 b	0.9088	Oblate spheroidal
Yuvarlak Bardak	6.3	24.25 ± 1.18 bc	26.50 ± 1.26 ab	0.9151	Oblate spheroidal
Uzun Memeli	6.5	24.88 ± 0.56 ab	27.13 ± 0.92 a	0.9171	Oblate spheroidal
Bugur	27.6	24.88 ± 1.28 ab	24.25 ± 2.00 c	1.0259	Prolate spheroidal

<sup>2</sup>Mean values followed by different lower case letters are different significantly by Duncan’s multiple range test at  $P \leq 0.05$ .

Table 2. Intine, exine, and total wall (exine + intine) thickness of pollen grains of cornelian cherry cultivars.

Cultivars	Intine thickness [mean ± SE (nm)]	Exine thickness [mean ± SE (nm)]	Intine + exine thickness [mean ± SE (nm)]
Degirmendere	233.1 ± 79.7 c <sup>2</sup>	842.7 ± 161.6 a	1,175.8 ± 149.1 a
Erkenci Degirmendere	367.5 ± 139.2 ab	510.2 ± 57.7 c	877.7 ± 141.0 b
İri Bardak	344.2 ± 145.2 ab	714.0 ± 173.4 b	1,058.2 ± 193.1 a
Yuvarlak Bardak	277.5 ± 63.2 bc	573.8 ± 89.8 c	851.4 ± 51.2 b
Uzun Memeli	406.4 ± 124.5 a	748.4 ± 207.3 ab	1,154.8 ± 302.6 a
Bugur	405.4 ± 91.6 a	695.6 ± 115.0 b	1,101.0 ± 126.4 a

<sup>2</sup>Mean values followed by different lower case letters are different significantly by Duncan’s multiple range test at  $P \leq 0.05$ .

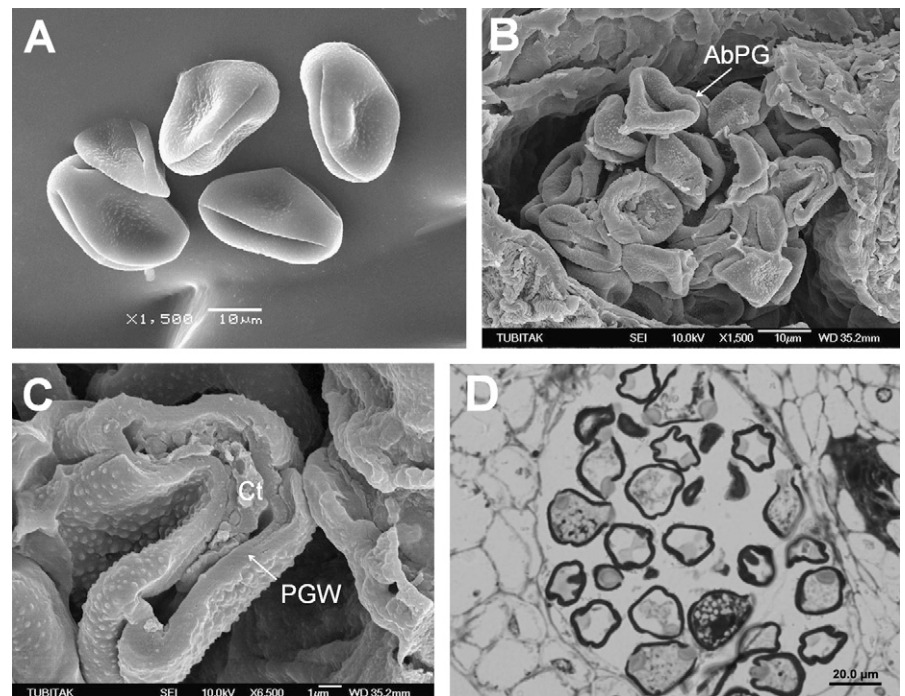


Fig. 3. Scanning electron micrograph (SEM) images of pollen grains of cornelian cherry cultivars. View of normal and shrunken pollen grains with elliptically colpate type of aperture in Bugur cultivar (A). View of abnormal and shrunken pollen grains in Uzun Memeli cultivar (B–C). Transverse section of anther locule with abnormal and normal pollen grains of cornelian cherry ‘Erkenci Degirmendere’ (D). Transverse sections stained with toluidine blue and photographed using light microscopy. AbPG = abnormal pollen grain; Ct = cytoplasm; PGW = pollen grain wall.

other cultivars ranged from 3.9% to 7.0% in this respect. These values showed that there are no significant problems of pollen abnormality among the cultivars examined with the exception of ‘Bugur’.

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