Variations and abnormalities of stamen morphologies in cornelian cherry (*Cornus mas* L.) cultivars

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ABSTRACT: Stamen morphologies of six cornelian cherry cultivars (Degirmendere, Erkenci Degirmendere, İri Bardak, Yuvarlak Bardak, Uzun Memeli, and Bugur) (*Cornus mas* L.) were evaluated using scanning electron, light and stereo microscopes. Flowers of cornelian cherry cultivars generally had four stamens, but a few of them had three or five stamens. Abnormal and normal stamens were found together in the cultivars examined. Percentages of abnormal stamens among the cultivars ranged from 12% to 22%. Various types of stamen abnormalities were identified in the cultivars such as variations in filament length, abnormalities in anther structure, fusion of stamen to other floral organs. Pollen grain numbers per anther varied between 1,380 and 4,240 among the cultivars. Abnormal anther surface morphology in the cultivars consisted of uneven and swollen epidermal cells. Anther dimensions varied between 624.00 and 1,001.25 µm in length and 460.50 and 745.50 µm in width. Anthers of all the cultivars had subprolate and prolate type of shapes.

Keywords: anther; *Cornus mas* L.; morphology

Cornus (commonly known as dogwoods) contains about 45 species of trees, shrubs, and herbs with woody rhizomes. The genus is distributed throughout the northern hemisphere, with centers of diversity in eastern Asia, eastern North America, the Pacific Northwest, and the mountains of Central America (Murrell 1993). Cornelian cherry (*Cornus mas* L.) is a widely distributed species endemic in Europe. It grows at altitudes of up to 1,400 m. Bloom time begins early in the spring and an ample amount of pollen is released by the anthers. Fruits are very valuable for fresh consumption and for processing to produce syrups, juices, jams and other traditional products. This species has significant potential for cultivation in monoculture.

The species of *Cornus* are remarkably uniform in floral structures and foliar morphology. All members of the genus possess tetramerous flowers, a nectariferous disc surrounding the style, a one-seeded, usually bilocular drupe (Murrell 1993). Some researches were conducted in *Cornaceae* and in some *Cornus* species concerning flower anatomy, botanical and morphological characteristics (Wilkinson 1944; Eyde 1987; Murrell 1993; Xiang et al. 2003; Xiang, Boufford 2005).

Structural characteristics of anthers and various kinds of stamen abnormalities of many fruit and plant species were described by the researchers (Westwood, Challice 1978; Kaul 1988; Sawhney, Bhadula 1988; Chaudhury 1993; Sessions 1997; Lillecrapp et al. 1999; Sanders et al. 1999; Farbos et al. 2001; Aihua et al. 2004; Mert, Soylu 2006). Abnormality and/or sterility level of the male and female organs have great importance in fruit set and yield in many fruit species. The information about the structure of the floral organs of cultivated species should be needed due to this importance. However, we were not able to find any information on stamen morphology and anatomy of cornelian cherry cultivars, which is one of the economically valuable species in Turkey. The aim of this study was to determine the stamen morphology, dimensions and anatomical structure of the anthers and pollen production capabilities of some of the cornelian cherry cultivars grown in Turkey.

MATERIAL AND METHODS

Studies were carried out on flowers of six cultivars of cornelian cherry: Degirmendere, Erkenci Degirmendere, İri Bardak, Yuvarlak Bardak, Uzun Memeli, and Bugur. Flower and stamen morphology were observed by examining 300 flowers of each cultivar. Structures of the anthers were also examined under a stereomicroscope (SZ6045TR, Olympus Optical Co. Ltd., Tokyo, Japan). Abnormal anthers and stamens were determined and classified.

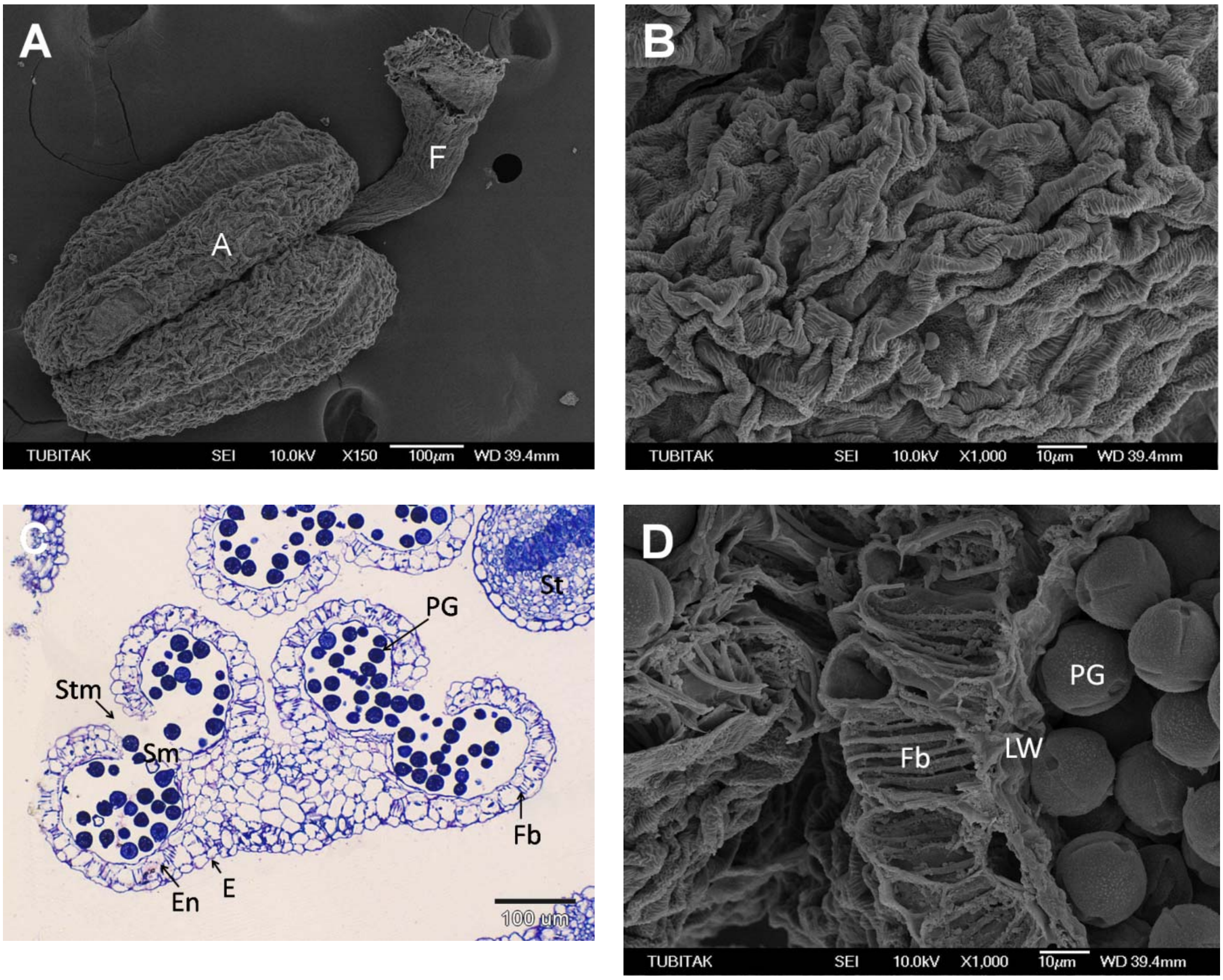


Fig. 1. Scanning electron micrographs of external anther morphology of Yuvarlak Bardak whole anther (bar = 100 µm) (A), anther surface structure (bar = 10 µm) (B), anther wall (bar = 10 µm) (D). Transverse section of four locules in an Erkenci Degirmendere anther stained with toluidine blue and photographed using light microscope (bar = 100 µm) (C)

A – anther, E – epidermis, En – endothecium, F – filament, Fb – fibrous band, LW – inner locule wall, PG – pollen grain, Sm – septum, St – style, Stm – stomium

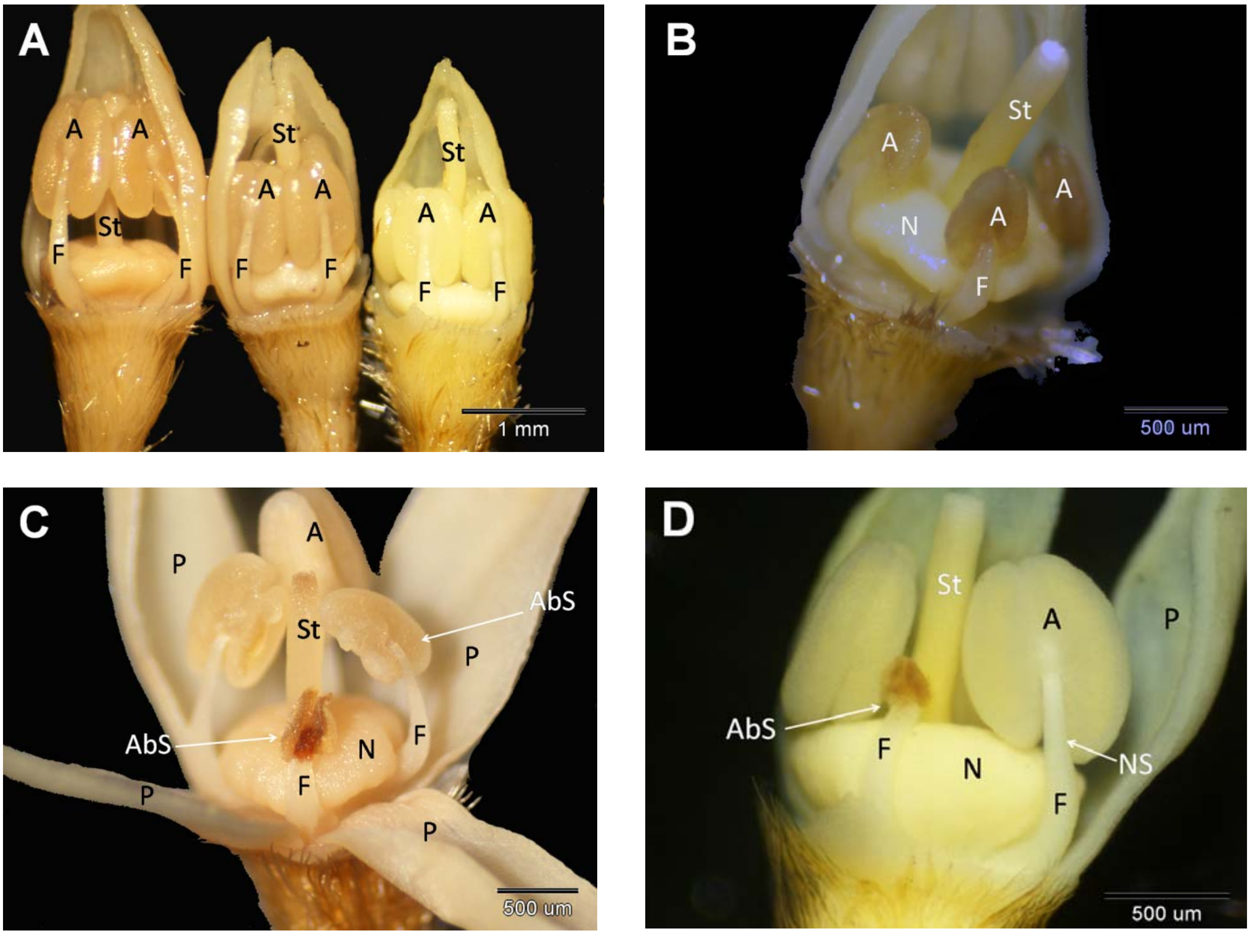
All photographs were taken using an Olympus DP 20 camera.

Dimensions of the anthers. The anther samples were placed on microscope slides, a drop of glycerin was poured and they were covered with a cover slip. An ocular micrometer was used to measure the dimensions of 50 anthers. In addition, length:width (L:W) ratios were calculated, and shape indexes were determined according to Erdtman (1966).

Determination of the pollen production per anther. The amount of pollen production per anther was determined using the hemacytometric method.

Scanning electron microscope (SEM). Anther samples were fixed in FAA solution (10 parts formalin:5 parts glacial acetic acid:50 parts ethanol:35 parts water, by volume) (Sass 1958) and were dehydrated for 10 min each in an ethanol series (50%, 70%, 95%, and twice in 100%), to the critical dry point. Anthers were mounted on SEM stubs and coated with gold-palladium (Polaron SC7620; VG Microtech, Uckfield, UK), and examined with a JSM-5600LV SEM (Jeol).

Light microscope (LM). Flower cluster samples fixed in the FAA solution were washed three times in phosphate buffer (pH 7.2) for 20 min. Then the anthers were fixed in 1% osmium tetroxide for 2 h, washed four times in a phosphate buffer, and then dehydrated for 10 min each in an ethanol series (50%, 70%, 95%, and twice with a 100%). The samples were gradually infiltrated and embedded in Spurr epoxy resin, and sectioned (1 µm) using an



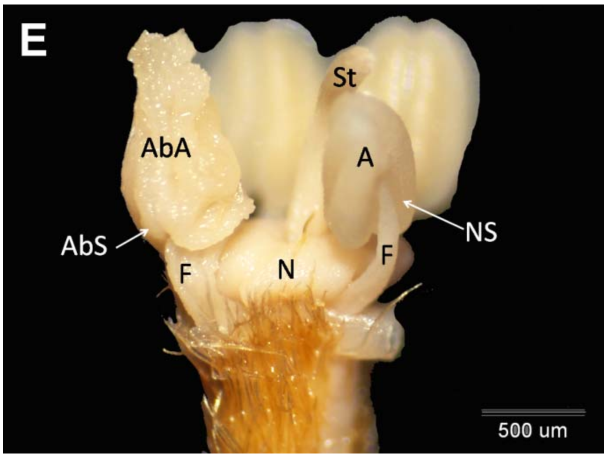


Fig. 2. Cornelian cherry flowers photographed with a stereo microscope. (A) View of flowers with stamens which have short, medium and long filaments in cultivar Erkenci Degirmendere (bar = 1 mm); (B) View of stamens which have short filaments and small anthers in cultivar İri Bardak (bar = 500 µm); (C, D) View of the flowers with abnormal stamens in cultivar Uzun Memeli (bar = 500 µm); (E) View of abortive and normal stamens in cultivar Degirmendere (bar = 500 µm)
A – anther, AbA – abnormal anther, AbS – abnormal stamen, F – filament, N – nectary tissue, NS – normal stamen, P – petal, St – style

ultramicrotome (Reichert Supernova, Leica, Wien, Austria) with a glass knife. Anther sections were stained with 1% toluidine blue and examined under an LM (BH-2, Olympus Optical Co. Ltd., Tokyo, Japan). All photographs were taken using an Olympus DP 20 camera.

Statistical analysis. The data were analyzed using the MSTAT-C statistical software, and means were compared using the Duncan's multiple range test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Variations in stamen morphology and pollen production. Flowers of cornelian cherry generally had four stamens. However some of the flowers were found to have 3 or 5 stamens. This result is similar to previous findings (Watson, Dallwitz 1992; Xiang, Boufford 2005). The stamen consisted of two morphologically distinct parts: the anther and the filament (Fig. 1A). Anthers had two lobes and

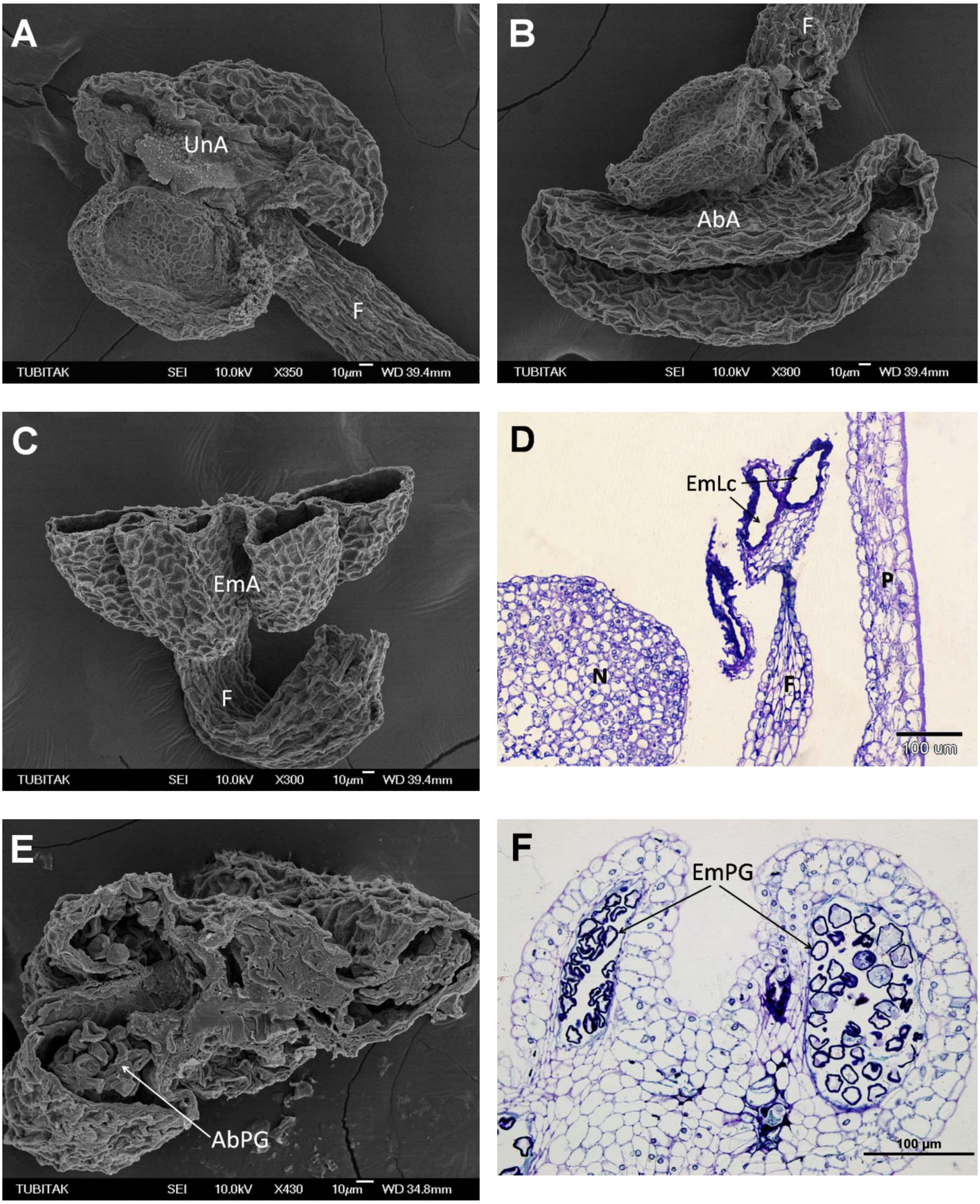


Fig. 3. Scanning electron micrographs of abnormal anthers (A, B, C, E; bar = 10 μm) and transverse sections of empty anthers and defective-pollen grains in anthers stained with toluidine blue and photographed using light microscope (D and F; bar = 100 μm). Undeveloped anther and abnormal anther of cultivar Degirmendere (A and B). Transverse section of empty (pollenless) anthers of cultivar Erkenci Degirmendere (C and D). Transverse sections of anther locules with defective pollen grains of cultivars İri Bardak (E) and Erkenci Degirmendere (F)

AbA – abnormal anther, AbPG – abnormal pollen grain, EmA – empty anther, EmLc – empty locule, EmPG – empty pollen grains, F – filament, N – nectary tissue, P – petal, UnA – undeveloped anther

Table 1. Dimensions of anthers of cornelian cherry (*Cornus mas* L.) cultivars together with length/width ratios, shapes, abnormal anther ratios and number of pollen grain per anther

Cultivars	Number of pollen grains per anther	Anther length (L, μm) (mean ± SE)	Anther width (W, μm) (mean ± SE)	L/W ratio	Shape	Abnormal anthers (%)
Degirmendere	4240a*	927.00 ± 59.94b	733.00 ± 45.02a	1.26	subprolate	16
Erkenci Degirmen-dere	2880ab	914.50 ± 74.93b	745.50 ± 67.33a	1.23	subprolate	22
İri Bardak	2800abc	999.50 ± 90.35a	696.50 ± 52.92b	1.44	prolate	19
Yuvarlak Bardak	2600bc	824.00 ± 58.24c	691.50 ± 58.38b	1.19	subprolate	13
Uzun Memeli	3280ab	1,001.25 ± 37.59a	742.50 ± 38.98a	1.35	prolate	14
Bugur	1380 c	624.00 ± 49.98d	460.50 ± 41.37c	1.36	prolate	12

*Mean values followed by different lower-case letters differ significantly by Duncan's multiple range test at $P \leq 0.05$, SE – standard error

each lobe contained two pollen sacs (Figs. 1A and C). Anthers split along the stomium to release the mature pollen grains. Pollen grain number per anther is given in the Table 1. This number varied significantly among the cultivars and the value changed between 1,380 and 4,240. According to this result pollen production capability of the cultivars is significantly different and Bugur is the less productive cultivar in this respect. This result showed us that pollinator quality of the cultivars is variable.

The surrounding tissues of the anthers consist of a surface epidermis, endothecium, middle layers, and tapetum (Figs. 1C and D) as featured by Esau (1977).

Some abnormal stamens were observed along with the normal stamens in the same flower samples examined (Figs. 2C, D and E). The percentage of abnormal stamens varied between 12% and 22% among 100 stamens and some were found in all of the cultivars (Table 1). These abnormalities were few and probably do not significantly affect the pollination and fertilization process. Some variations in stamen morphology were observed, such as the stamens with short, medium or normal filaments (Figs. 2A and B), abnormal anther development, or fusion of the stamen to other floral organs. These abnormalities were observed in all cultivars. Stamens with short filaments had small or abortive anthers (Figs. 2C and D, 3A and B). These anthers cannot produce pollen grains. Abortive small anthers which produce no pollen have also been reported in other plant species (Duvick 1965; Kaul 1988). Sanders et al. (1999) found some mutants of *Arabidopsis thaliana* (L.) Heynh. without pollen grains which could be attributed to meiotic aberrations during meiosis or abnormalities of the locule cells. Similarly, in some male-sterile apricot (*Prunus armeniaca* L.) cultivars shrunken anthers were observed with few or no pollen grains (Lillecrapp et al. 1999). In maize, the gibberellin (GA)-deficient *dwarf* mutants *d2, d3,* and *d5* and the anther-ear mutants *an1* and *an2*, had smaller anthers than normal, and produced no pollen (Duvick 1965; Kaul 1988). A tomato mutant, stamenless-2, was described whose stamens were shorter and paler in colour than wild-type stamens (Sawhney, Bhadula 1988). Alloplasmic male-sterile tobacco flowers contain 4 or 5 shortened filaments with shrivelled anthers (Farbos et al. 2001).

Stamens with medium-length filaments have different types of abortive anthers. These stamens showed the following variations:

(1) anthers with normal appearance but no pollen grains (Figs. 3C and D),

(2) anthers with normal appearance with abnormal pollen grains (Figs. 3E and F),

(3) anthers with normal appearance but consisted of undifferentiated masses of cells (Fig. 4A),

(4) anthers which have normal and abnormal locules together (Figs. 4B, C and D). In these abnormal anthers only one lobe was normally developed, and the other was undeveloped (Figs. 4B). Normally developed lobes can generally produce pollen grains (Figs. 4C and D). However some of them had no pollen grains.

(5) Abnormally developed anthers with abnormal filaments (Fig. 2E). These abnormalities have also been observed in other plant species. Malformed anthers were reported in a dominant mutant, *ms4,* in cotton (*Gossypium* L.) (Allison, Fisher in Chaudhury 1993). Aihua et al. (2004) observed that pear cultivar Xinli No. 7 had shrivelled anthers

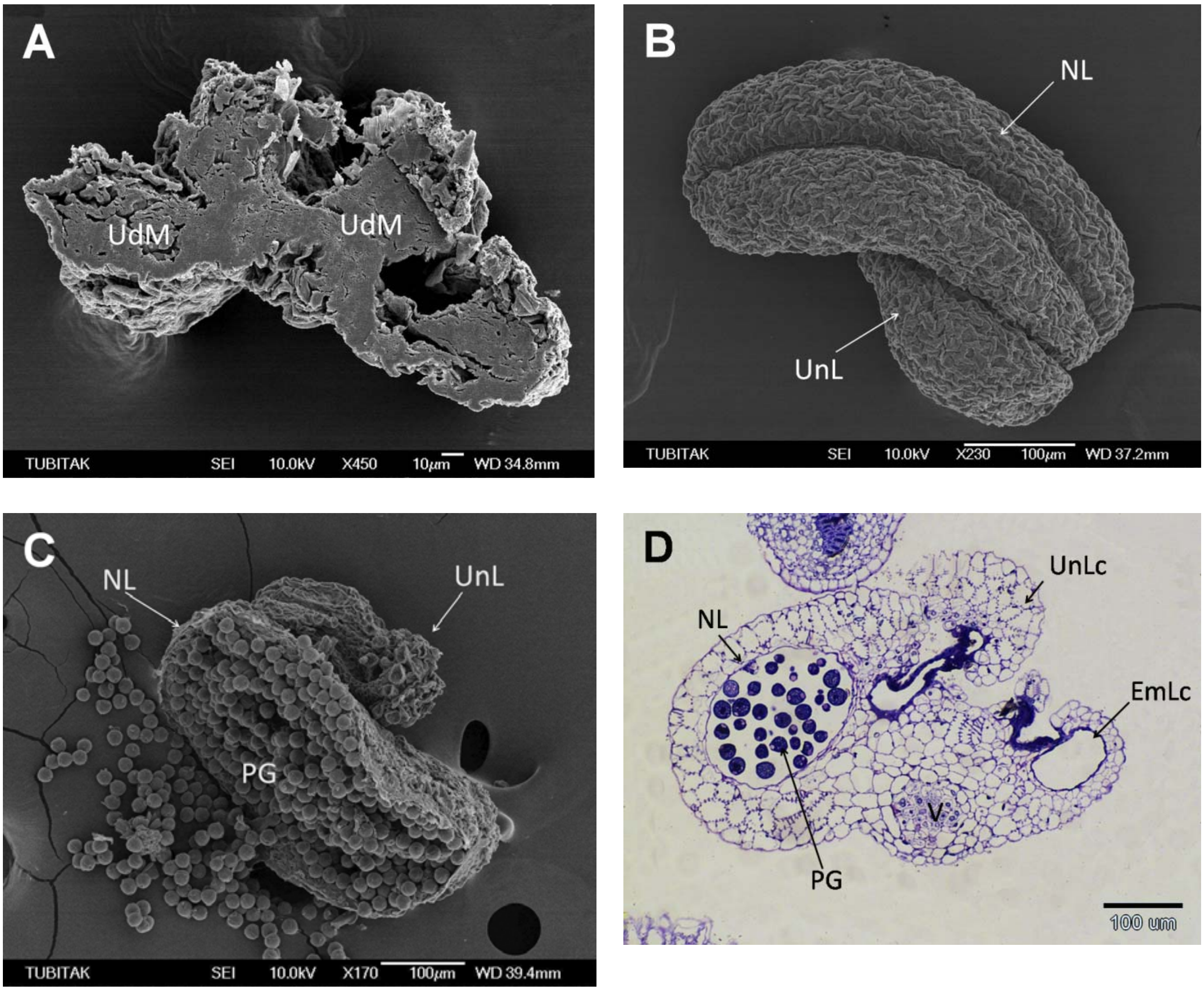


Fig. 4. Scanning electron micrographs of abnormal anthers (A, B and C) and transverse section of abnormal stamen stained with toluidine blue and photographed using light microscope (D) (all bars = 100 μm). Anthers with pollen production in normal lobes and with abnormal lobes with no pollen

EmLc – empty locule, NL – normal lobe, PG – pollen grain, UnL – undeveloped-lobe, UnLc – undeveloped-locule, UdM – undifferentiated mass of cells

with few pollen grains or empty anthers. Mert and Soylu (2006) reported that male-sterile chestnut cultivar Vakit Kestanesi has small anthers with short filaments. These anthers consisted of undifferentiated masses of cells. On the other hand, some of the anthers of male-sterile chestnut cultivar Osmanoglu had both normal and abnormal lobes.

Some of the stamens examined in this study had fused to corolla or pistil parts of the flowers (Figs. 5A, B, C and D). Anthers developed abnormally in all these variations. However the ratios of all these abnormalities were low. Similar abnormalities have been observed in other plant species. For example, some of the stamens in the flowers of *Cuscuta campestris* developed abnormally fused to petals (Ing-Liao et al. 2005), and in alloplasmic male-sterile tobacco flowers of some the stamens fused with the carpel wall (Farbos et al. 2001).

Anther surface morphology and dimensions. Surface structure of the anthers was different among the cultivars. As shown in Fig. 1B, the surface of anthers in the cultivar Yuvarlak Bardak had swollen-uneven epidermal cells. All cornelian cherry cultivars had small anthers and the dimensions were significantly different from each other (Table 1). Erkenci Degirmendere (745.50 μm) had the widest and Uzun Memeli (1,001.25 μm) had the longest anthers. Both values were significantly lower in Bugur (460.50 μm, 624.00 μm) compared with the other cultivars (Table 1). Anthers of all the cultivars are

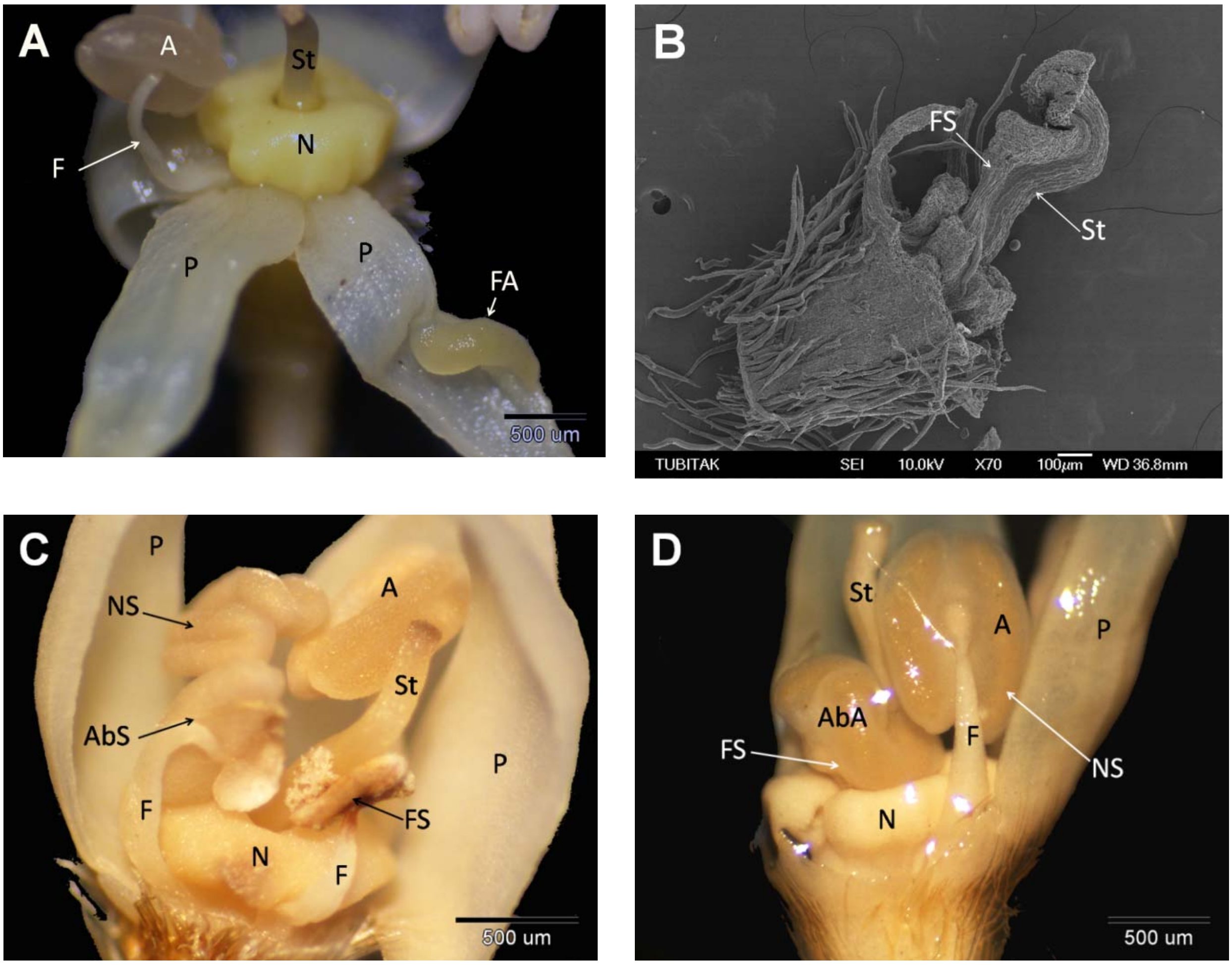


Fig. 5. Cornelian cherry flowers photographed with a stereo microscope (A, C and D) and a scanning electron microscope (B). View of a stamen fused to a corolla in an anther of cultivar Degirmendere (A). Stamen fused to the wall of the style in cultivar İri Bardak (B). View of a stamen partly fused to the style in cultivar Erkenci Degirmendere, (C) view of an abnormal stamen that originated from nectary tissue in the same cultivar (D)
A – anther, AbA – abnormal anther, AbS – abnormal stamen, F – filament, FA – fused anther, FS – fused stamen, N – nectary tissue, NS – normal stamen, P – petal, St – style

of subprolate and prolate type in shape. Xiang and Boufford (2005) stated that *Cornus* L. has ellipsoid to narrowly ellipsoid or oblong shaped anthers.

In conclusion we have found various kinds of abnormalities in stamen morphology and anatomy of cornelian cherry cultivars. However, these abnormalities were rather few (not more than 22%). It is unlikely that this would meaningfully affect the fruit set since cornelian cherry cultivars can produce so many flowers on their shoots, and one anther can produce 1,380–4,240 pollen grains (Table 1). We did not find a complete or effective level of sterility in any of the cultivars examined. These findings are the first detailed report on stamen structure and pollen production capabilities in cornelian cherry cultivars.

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Změny a abnormality morfologie tyčinek u kultivarů dřínu jarního (*Cornus mas* L.)

ABSTRAKT: Morfologie tyčinek šesti odrůd (Degirmendere, Erkenci Degirmendere, İri Bardak, Yuvarlak Bardak, Uzun Memeli a Bugur) dřínu jarního (*Cornus mas* L.) byly hodnoceny za použití tří typů snímacích mikroskopů (elektronového, světelného a stereo). Květy hodnocených odrůd dřínu jarního měly nejčastěji čtyři tyčinky, ale některé z nich měly tři nebo pět tyčinek. U hodnocených odrůd se vyskytovaly jak abnormální, tak i normální tyčinky. Podíl abnormálních tyčinek kolísal u odrůd mezi 12 % a 22 %. Navíc byly u sledovaných kultivarů identifikovány různé typy abnormalit tyčinek jako změny v délce nitek, anomálie ve struktuře prašníků, fúze tyčinek do jiných květních orgánů. Počty pylových zrn na jednom prašníku se u odrůd pohybovaly v rozmezí od 1 380 do 4 240. Abnormální morfologie povrchu prašníku u zkoumaných kultivarů spočívala v nestejných a nabobtnalých epidermálních buňkách. Rozměry prašníku se pohybovaly mezi 624.00 a 1 001.25 μm délky a 460.50 a 745.50 μm šířky. Prašníky všech kultivarů měly poloprotáhý nebo protáhlý tvar.

Klíčová slova: prašník; *Cornus mas* L.; morfologie

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