Anther and Pollen Morphology and Anatomy in Walnut (Juglans regia L.)

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Abstract. The morphology and ultrastructure of anthers and pollen grains were described for eight walnut (Juglans regia L.) cultivars (Şebin, Bilecik, Kaman I, Kaplan 86, Yalova 3, Pedro, Hartley, and Franquette) using light microscopy and scanning and transmission electron microscopy. Anther and pollen dimensions and pollen production capabilities were also determined. The mean number of stamens per staminate flower (14.90 to 20.03) and mean anther dimensions (1412.5 to 2553.5 μm in length and 849.0 to 1145.5 μm in width) differed significantly among the cultivars. The number of pollen grains per anther also varied significantly and ranged from 4720 to 9840 among the cultivars. The pollen grains of walnut cultivars are spherical in shape (nearly circular) and pantocoporate (with many colporate apertures on the exine). The pollen surface is microechinulate with spiniferous projections. The germination pores are circular and nonbordered. Pollen grain length varied from 33.35 to 37.50 μm, pollen width from 24.43 to 29.32 μm, exine: 793.1 to 1161.1 nm; and total wall: 954.4 to 1327.8 nm.

Juglans regia L. is typical of Juglandaceae in that it is monoeious, wind-pollinated, and self-compatible. Despite its self-compatibility, breeding and research programs have encountered difficulties acquiring sufficient quantities of pollen when the pistillate flowers are receptive because of the dichogamous nature of the species, which has both protandrous and protogynous mating types (Luza and Polito, 1985). The leaves and blossoms of the walnut tree normally appear in the spring. Staminate flowers on walnut plants are spherical in shape (nearly circular) and pantocoporate (with many colporate apertures on the exine). The pollen surface is microechinulate with spiniferous projections. The germination pores are circular and nonbordered. Pollen grain length varied from 33.35 to 37.50 μm, pollen width from 24.43 to 29.32 μm, exine: 793.1 to 1161.1 nm; and total wall: 954.4 to 1327.8 nm.

Results and Discussion

Anther morphology and dimensions. Walnut is a monoeious fruit species, and the catkins form a spike-like structure (Abbe, 1974). The number of the stamens per staminate flower has been described for several walnut cultivars. The number of stamens per flower varied from 17 to 22. Similarly Molina et al. (1996) found that the number of stamens per staminate flower ranged from 12.9 to 15.5. Although our results were somewhat different from the previously published reports, they are generally in the same range as the earlier measurements. Anther dimensions were also significantly different among the cultivars (Table 1). 'Bilecik' (1145.5 μm) has the widest anthers, and 'Kaman 1' (2553.5 μm) has the longest anthers. Both values were significantly lower in 'Franquette' (849.0 μm).
1871.5 μm) compared with the other cultivars (Table 1). Anthers of all the cultivars are of the perprolate and prolate types in shape.

The stamen consists of two morphologically distinct parts: the anther and the filament. Anthers of walnut cultivars have four microsporangia arranged in pairs of two lobes; each pair is separated by parenchymatous and vascular tissue at the connective site of the anther (Fig. 1A, C) as described by Luza and Polito (1988). The tissues surrounding the anther consist of a surface epidermis, endothecium, middle layers, and a tapetum (Fig. 1B). The mature walnut pollen sac contains pollen grains and a new completely collapsed tapetum (Fig. 1A, C, D).

Significant differences were observed in walnut cultivars with respect to the number of pollen grains in each anther. The pollen number per anther varied from 4720 to 9840 among the cultivars (Table 1). Su¨tyemez (2007) found that the pollen number per anther ranged from 6126 to 7502. Our results support this finding.

Pollen surface morphology and dimensions. The pollen grains in all of the examined walnut cultivars were spherical (nearly circular) and pantocolporate (Fig. 2A–B). The pollen is circular in polar view (Fig. 2A) and suboblate in equatorial view (Fig. 2B). The pollen surface is microechinate with spiniferous projections (Fig. 2C). At anthesis, the pollen grains, which are not fully hydrated, are collapsed at the polar ends (Fig. 2D). The germination pores are circular and nonbordered (Fig. 2C). Evrenosoglu and Misirli (2009) obtained similar results and stated that the pollen grains of walnut cultivars were pantocolporate and nearly circular (spherical).

The pollen grains from the cultivars used in this study have been shown to vary significantly with respect to diameter when measured along their length (polar) and width (equatorial) and ranged from 33.35 to 37.50 μm and from 39.30 to 43.15 μm, respectively (Table 2). This is consistent with the findings of previous researchers (Evrenosoglu and Misirli, 2009; Molina et al., 1996). However, walnut pollen grains are also larger than those measured in other nut species such as chestnut (*Castanea sativa* Mill.), 9 to 21 μm (length × width) (Beyhan and Serdar, 2009; Bounous et al., 1992; Mert and Soylu, 2007), and pistachio (*Pistacia atlantica* Desf., Anacardiaceae), 33.7 to 37.2 μm (length × width) (Belhadj et al., 2007). Pollen grains from the examined walnut cultivars have a suboblate shape.

### Table 1. The mean stamen number of the staminate flower, number of pollen grain per anther, dimensions of anther, and length/width ratios in walnut (*Juglans regia* L.) cultivars.

| Cultivar | Stamen (no./flower) | Pollen grain (no./anther) | Anther length (L) [mean ± se (μm)] | Anther width (W) [mean ± se (μm)] | Length:width ratio | Anther shape |
|----------|---------------------|--------------------------|-----------------------------------|-----------------------------------|--------------------|--------------|
| Sehil    | 18.75 ± 1.99 bc     | 7920 ab                  | 2463.5 ± 156.4 b                  | 980.5 ± 121.4 c                  | 2:51               | Perprolate   |
| Kaplan   | 16.16 ± 1.20 d      | 7280 abc                 | 2082.0 ± 171.3 d                  | 1032.5 ± 76.1 b                  | 2:02               | Perprolate   |
| Yalova   | 20.00 ± 1.36 a      | 9840 a                   | 2553.5 ± 213.6 a                  | 978.5 ± 101.1 c                  | 2:61               | Perprolate   |
| Bilecik  | 18.90 ± 1.29 e      | 4720 c                   | 1412.5 ± 121.0 f                  | 872.5 ± 74.4 c                   | 1:62               | Prolate      |
| Kaman    | 14.90 ± 1.39 g      | 6800 bc                  | 2161.5 ± 128.4 c                  | 938.5 ± 55.1 d                   | 2:30               | Perprolate   |
| Pedro    | 18.06 ± 0.58 c      | 4800 c                   | 1871.5 ± 175.3 e                  | 849.0 ± 100.1 e                  | 2:20               | Perprolate   |
| Hartley  | 20.03 ± 1.18 a      | 4920 c                   | 1855.0 ± 142.2 d                  | 945.5 ± 96.8 c                   | 2:61               | Perprolate   |
| Franquette| 19.33 ± 2.26 ab     | 7280 abc                 | 2082.0 ± 171.3 d                  | 1032.5 ± 76.1 b                  | 2:02               | Perprolate   |

Mean values followed by different lower-case letters different significantly by Duncan’s multiple range test at P ≤ 0.05.
Table 2. Dimensions of pollen grains together with length:width ratios and shapes in walnut (Juglans regia L.) cultivars.

| Cultivar        | Pollen length [mean ± se (μm)] | Pollen width [mean ± se (μm)] | Length:width ratio | Shape          |
|-----------------|---------------------------------|--------------------------------|--------------------|----------------|
| Sebun           | 34.00 ± 1.39 a                  | 42.45 ± 1.75 a                 | 0.81               | Suboblate      |
| Kaplan 86       | 37.50 ± 1.82 a                  | 43.05 ± 2.16 a                 | 0.87               | Suboblate      |
| Yalova 3        | 34.25 ± 2.03 bc                 | 40.65 ± 2.24 bc                | 0.84               | Suboblate      |
| Bilecik         | 35.00 ± 2.08 b                  | 41.10 ± 2.32 c                 | 0.85               | Suboblate      |
| Hartley         | 33.35 ± 1.64 d                  | 40.20 ± 2.25 c                 | 0.83               | Suboblate      |
| Franquette      | 33.95 ± 1.58 cd                 | 39.30 ± 2.20 d                 | 0.86               | Suboblate      |

Mean values followed by different lower-case letters are different significantly by Duncan’s multiple range test at $P \leq 0.05$.

Fig. 3. Transmission electron micrographs (TEM) of pollen grain and pollen wall in walnut cultivars Pedro (A–B) and Kaman 1 (C–D). Transverse section (1,500×) through pollen grain showing cytoplasm, vacuole, starch granules and oncus (A, C) (bar = 1 μm). Transverse section (10,000× and 6,000×) of pollen wall showing tectum, bacula, nexine, intine, and spines (B, D) (bar =1 μm); b = bacula; e = exine; i = intine; n = nexine; nc = nucleus; o = oncus; s = spines; t = tectum.

Table 3. Intine, exine, and total wall (exine + intine) thickness of pollen grains in walnut (Juglans regia L.) cultivars.

| Cultivar        | Intine thickness [mean ± se (nm)] | Exine thickness [mean ± se (nm)] | Intine + exine thickness [mean ± se (nm)] |
|-----------------|-----------------------------------|---------------------------------|------------------------------------------|
| Sebun           | 155.5 ± 98.8 ab                   | 1053.6 ± 129.7 a                | 1211.1 ± 189.8 a                         |
| Kaplan 86       | 200.0 ± 120.6 a                   | 1111.1 ± 111.3 a                | 1311.1 ± 213.4 a                         |
| Yalova 3        | 166.6 ± 107.3 ab                  | 1161.1 ± 146.2 a                | 1327.8 ± 192.2 a                         |
| Bilecik         | 161.8 ± 50.8 ab                   | 793.1 ± 66.5 b                  | 954.4 ± 101.4 b                          |
| Kaman 1         | 125.0 ± 35.1 be                   | 844.4 ± 121.7 b                 | 969.4 ± 121.8 b                          |
| Pedro           | 161.1 ± 44.5 ab                   | 816.7 ± 166.1 b                 | 977.8 ± 166.6 b                          |
| Hartley         | 82.1 ± 35.3 c                     | 895.6 ± 198.5 b                 | 977.6 ± 209.3 b                          |
| Franquette      | 132.8 ± 36.4 abc                  | 853.3 ± 123.4 b                 | 992.2 ± 125.0 b                          |

Mean values followed by different lower-case letters are different significantly by Duncan’s multiple range test at $P \leq 0.05$.

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