

# Determination of Pollen Production and Quality of Some Local and Foreign Walnut Genotypes in Turkey

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**Abstract:** The pollen grains of 32 different walnut cultivars and types were tested in vitro to determine their viability, germination ability, and pollen production level. Viability of pollens was determined by TTC and FDA tests. The hanging drop method and agar in petri method were used for pollen germination. Pollen production of the cultigens was determined with the hemacytometric method. The rate of pollen viability of all the investigated cultigens was high (> 80%). The germination rate of the pollens ranged from 33.28% to 50.19% and the Van 4 cultivar had the highest pollen germination rate. Although similar results were obtained with the 2 germination tests, higher sucrose concentration (15%) resulted in better germination percentages. Pollen production of the walnut cultivars and types were higher than in previous reports. The range of total pollen grain production of the walnut trees was estimated to be 5-100 billion.

**Key Words:** Walnut, pollen, quality, production

## Bazı Yerli Ceviz Tipleri ve Yabancı Ceviz Çeşitlerinde Çiçek Tozu Kalitesi ve Üretim Miktarlarının Belirlenmesi Üzerine Bir Araştırma

**Özet:** Bu çalışmada 32 ceviz çeşit ve tipine ait çiçek tozlarının in vitro koşullarda canlılık ve çimlenme yetenekleri ile çiçek tozu üretim miktarları belirlenmiştir. Çiçek tozu canlılığını belirlemek amacıyla TTC ve FDA canlılık testleri uygulanmıştır. Çiçek tozu çimlendirme denemelerinde asılı damla ve petride agar metodu kullanılmıştır. Çeşitlerin çiçek tozu üretim miktarları hemisitometrik metotla saptanmıştır. Çiçek tozu canlılık testlerinde tüm çeşit ve tiplerde oldukça yüksek değerler elde edilmiştir (%80 nin üzeri). Çiçek tozu çimlenme oranı %33.28 ile %50.19 arasında değişmekte olup, Van 4 çeşidi en yüksek çimlenme oranına sahip olmuştur. Her iki çimlendirme testi de benzer sonuçları vermesine rağmen %15 lik sakaroz konsantrasyonundan daha yüksek çimlenme oranları belirlenmiştir. Ceviz tip ve çeşitlerin çiçek tozu üretim miktarlarının önceki çalışma sonuçlarına göre daha yüksek olduğu tesbit edilmiştir. Nitekim, bir ceviz ağacının toplam çiçek tozu üretiminin 5 ile 100 milyar kadar olabileceği hesaplanmıştır.

**Anahtar Sözcükler:** Ceviz, çiçek tozu, kalite, üretim

## Introduction

Walnuts are one of several fruit species indigenous to Anatolia, which has a long history of fruit cultivation (Şen, 1986). Turkey has 4.5 million walnut trees with an annual production of 126,000 metric tons, and is ranked fourth among the walnut-producing countries of the world (FAO, 2004). In Turkey, Kahramanmaraş province has become a prominent walnut production region with an increasing number of walnut trees (180,675 trees in 1993) (Şen, 1998).

Abundant and healthy flower development is necessary to ensure a sufficient fruit set, especially in

certain plants that produce edible seeds. Additionally, satisfactory pollination of these plants by a suitable pollinator and the occurrence of fertilization in the flower are crucial. To consider any cultivar (cv.) as a good pollenizer, it should produce well-developed pollen with both high vitality and germination rates. Furthermore, the level of morphological homogeneity and the quantity of pollen must be high, since all the pollen that is carried to the surface of the stigma may not reach the ovule.

The rate of vitality and germination of pollen, and its fertilization ability are important in selecting any cultivar as a pollenizer. There is usually a positive relationship

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between these parameters and the resulting fruit set in many fruit species (Visser, 1955; Dokuzoğuz, 1957; Ayfer, 1959; Griggs et al., 1971; Luza and Polito, 1985; Erdoğan and Ayfer, 1990; Eti, 1990). In vitro pollen viability and germination tests are widely used in determining pollination ability. The objectives of this study were to determine the quality and amount of pollen obtained from different walnut types and cvs.

## Materials and Methods

### Materials

Pollen of selected walnut types and cvs. from different parts of Turkey, and several American and European walnut cvs. were used in the experiment. The experiment began in 1996 by establishing an orchard at SEKAMER (Research and Adaptation Center of Nuts) consisting of 23 local and 9 foreign types or cvs. *Juglans regia* was used as rootstock. There were 5 trees planted per type or cv. Each tree was considered a replicate. The experiments were conducted in 2004, 2005, and 2006, and similar results were obtained each year. For this reason the average values of the investigated parameters are presented in this paper.

### Methods

Pollen viability and germination tests were performed according to Eti (1990).

#### Pollen viability tests

Triphenyl tetrazolium chloride (TTC) and fluorescein diacetate (FDA) tests were used to determine the pollen viability rate of walnut types. TTC and FDA solutions were prepared according to Norton (1966) and Heslop-Harrison (1970), respectively.

#### Pollen germination tests

Agar in petri dish and hanging drop tests were used (Stanley and Linskens, 1985). Initially, the 2 tests were conducted at different sucrose concentrations. The sucrose concentrations for the agar in petri dish test were 5%, 10%, and 15%, for the hanging drop test these values were 0%, 5%, 10%, 15%, and 20%.

### Pollen production rate

The following parameters were taken into consideration in determining the pollen production rate of the walnut types:

- number of staminate flowers per catkin (FC);
- number of anthers per flower (AF);
- number of pollen grains per flower (PF);
- number of pollen grains per anther (PA) = PF/AF;
- number of pollen grains per catkin (PC) = FC × PF;
- percentage of well-developed pollen (DP).

PF was determined using the hemacytometric method, according to Eti (1990). In order to obtain the average number of each component, 40 randomly selected catkins from each tree were collected. All the flowers on these catkins were counted and then the average number of the above components of each walnut type was determined.

Experimental data were analyzed using analysis of variance (ANOVA) and the means were statistically grouped by Tukey's (HSD) test. Data containing percentage values were angle transformed before the statistical analysis.

## Results and Discussion

### Pollen quality

#### Pollen viability

The results of TTC and FDA tests for averages in 2004, 2005, and 2006 are summarized in Table 1. There were slight differences in the pollen viability percentages; however, the pollen of Van 4 cv., Chandler cv., Ürgüp cv., Yalova 1 cv., and Maraş 18 cv. consistently demonstrated high pollen viability percentages.

In TTC tests, Van 4 cv. had the highest pollen viability (93.60%), followed by Chandler cv. (92.62%). Pollen viability determined by FDA test was 95.64% for Van 4 cv., 91.16% for Yalova 1 cv., 91.10% for Maraş 18 cv., 90.83% for Payne cv., 90.67% for Chandler cv., and 90.43% for Back cv. The pollen viability of the other types was over 80% in both tests. Therefore, regarding pollen viability, all the walnut cultivars can be considered good pollinators. TTC and FDA tests gave similar results. These findings are in accordance with previous studies (Eti, 1991; Mahanoğlu et al., 1993; Sütyemez, 1998).

Table 1. The viability percentage of pollen by TTC and FDA tests, and the germination percentages of pollen by agar in petri dish and hanging drop tests in walnut cultivars and types.

Varieties	TTC	FDA	Agar in petri dish	Hanging drop	
			1% agar + 10% Suc.	10% Suc.	15% Suc.
Van 6	89.34 a-f	89.70 b-f	46.28 a-d	40.70 a-d	44.74 bc
Şebin	88.62 a-f	89.57 b-f	44.71 a-e	37.87 cd	42.09 c-f
Yalova 3	83.67 fgh	85.58 c-i	38.95 g-l	36.67 cde	38.61 e-f
Bilecik	90.46 abc	88.36 b-h	36.98 klm	37.90 cd	38.84 e-h
Yalova 1	90.60 abc	91.16 ab	41.80 d-k	37.91 cd	44.11 bcd
Kaman 1	88.22 a-f	89.10 b-g	38.77 h-l	37.07 cd	42.75 c-f
Kaman 4	84.07 d-h	81.15 i	37.05 klm	35.41 de	36.63 gh
Franguette	83.98 e-h	85.48 d-i	34.38 lm	31.64 e	34.71 h
Serr	89.42 a-f	89.11 b-g	38.44 i-l	38.00 cd	44.28 bc
Chandler	92.62 ab	90.67 a-d	47.32 abc	37.51 cd	44.47 bc
Pedro	86.38 c-h	89.46 b-f	42.29 d-j	40.28 a-d	44.63 bc
Tokat 1	81.91 gh	80.82 i	40.16 e-k	36.30 cde	41.39 c-g
Kaman 3	84.43 d-h	83.29 hi	39.26 g-l	36.84 cde	38.03 fgh
Hartley	87.91 a-f	83.53 hi	42.40 c-i	39.92 a-d	44.46 bc
Yalova 4	84.67 c-h	85.12 f-i	39.67 f-k	38.87 bcd	42.84 c-f
Maraş 18	89.11 a-f	91.10 ab	44.63 a-e	37.20 cd	46.44 abc
Kaplan 86	89.96 a-d	89.16 b-g	43.57 b-h	39.68 a-d	45.27 abc
Kr 2	81.01 h	80.98 i	38.34 i-l	36.61 cde	35.37 h
Şen 1	85.38 c-h	83.43 hi	33.28 m	35.70 cde	36.12 h
R.de M. *	85.71 c-h	85.46 d-i	38.10 l-m	35.52 cde	38.32 e-h
Ürgüp	89.43 a-f	89.15 b-g	48.54 a	45.12 a	41.46 c-g
Back	88.48 a-f	90.43 a-e	47.57 ab	43.68 ab	48.82 ab
Payne	89.74 a-e	90.83 abc	44.35 a-f	39.08 bcd	43.44 cde
Van 4	93.60 a	95.64 a	48.27 ab	43.93 ab	50.19 a
77 H 1	85.01 c-h	83.95 ghi	41.40 d-k	36.71 cde	45.91 abc
Kr 1	86.23 c-h	85.65 c-i	37.38 j-m	35.70 cde	38.99 d-h
Şen 2	88.06 a-f	86.59 b-h	44.33 a-f	39.46 bcd	43.02 c-f
Maraş 10	87.57 b-g	83.35 hi	41.76 d-k	37.64 cd	43.27 c-f
Maraş 12	89.88 a-e	88.00 b-h	46.30 a-d	40.94 abc	46.27 abc
Maraş 19	85.81 c-h	85.25 e-i	41.81 d-k	39.82 a-d	45.24 abc
Sütyemez 1	85.35 c-h	83.15 hi	43.78 a-g	43.65 ab	45.02 abc
Sütyemez 2	89.38 a-f	89.34 b-f	41.89 d-k	39.06 bcd	42.66 c-f
LSD 1%	5.9306	5.2996	4.9453	3.1233	3.0051

R.de M. \*: Ronde de Montignac.

Suc.: Sucrose.

Data followed by the same letters are not significantly different (1%).

### Pollen germination

In the hanging drop test, the highest values were found at 10% and 15% sucrose concentrations, whereas high germination rates were only obtained at 10% sucrose concentration with the agar (1%) in petri dish test. As a result, only these concentrations are discussed in this paper.

The germination percentages of the pollen from each walnut type obtained with the 2 tests are summarized in Table 1.

In the agar in petri dish test (1% agar, 10% sucrose), the highest pollen germination rate was obtained from Ürgüp cv. (48.54%), which was followed by Van 4 cv. (48.27%), Back cv. (47.57%), and Chandler cv. (47.32%). Şen 1 cv. had the lowest germination rate (33.28%).

In the hanging drop test, sucrose concentration affected pollen germination. The higher sucrose concentration (15%), in general, improved the germination rates of the walnut pollens. The highest pollen germination rate was for Van 4 cv. (50.19%), followed by Back cv. (48.82%). The lowest value was obtained in Franquette cv. (34.71%).

Different germination media have been tested in studies on walnut pollen. The germination rate of walnut pollen belonging to 2 cultivars on media containing 1% agar, 15% sucrose, 0.02%  $H_3BO_3$ , and 0.05 Ca  $(NO_3)_2 \cdot 4H_2O$  was reported by Sağlam and Gülcan (1995). It seems that the addition of boric acid and Ca to germination media had a positive effect on germination. In another study, it was reported that the germination medium containing 0.75% agar and 15% sucrose positively affected walnut pollen (Cerovic et al., 1992).

The differences among the walnut types with regard to pollen germination in this study were statistically significant. In a previous study this difference was reported to be in the range of 43%-77% (Cerovic et al., 1992). The pollen germination rates of our walnut types appeared to be higher than the minimum required level (35%) for pollinators.

Taking into account these pollen viability and germination values, it may be concluded that these types can be used as pollinators for those that bloom in the same period. It should be stressed that the high germination rates found in vitro do not always indicate

good in vivo pollination rates for each cross combination, since overlap of the blooming period for monoecious walnut flowers has priority.

### Pollen production

The results of the components of pollen production of the walnut types and cultivars are summarized in Table 2.

In general, the results obtained from the pollen production study have potentially practical uses in fertilization biology. Ürgüp cv. had the highest FC ( $n = 148$ ), Kaplan 86 cv. had the highest AF ( $n = 22$ ), Kaplan 86 cv. had the highest PF ( $n = 134,800$ ), Tokat 1 cv. had the highest PA ( $n = 7666$ ), Kaplan 86 cv. had the highest PC ( $n = 19,546,087$ ), and Chandler Hartley cv., Van 4 cv., and Maraş 12 cv. had the highest DP (98%).

Eti (1991) reported that among the various entomophilous fruit species almond had the highest percentage of well-developed pollen grains; however, its overall pollen production rate was the lowest.

In addition to the quality of pollen, the components of pollen production studied above are important in determining the pollination ability of any cultivar or type. It can be seen from the amount of observed pollen production that most of the tested walnut types are above the satisfactory level for walnut.

There is controversy concerning the calculated number of pollen grains for walnut in the literature. According to Tormo Molina et al. (1997), the pollen grain production on a flower of a walnut tree could range from 30,000 to 100,000 and the pollen grain total of a walnut tree could be as much as 100-500 million. However, Şen (1986) reported that a mature walnut tree with 5000 staminate catkins might produce 1- 4 million pollen grains per catkin and estimated that a walnut tree could have 5-20 billion pollen grains. Our results agree more closely with Şen's finding, since we estimated 5-100 billion pollen grains per tree.

On the basis of pollen viability, germination percentage, and pollen production rate results, we conclude that all the cvs. and types tested in this study demonstrated characteristics of good pollinators. On the other hand, in establishing a walnut orchard with these cvs. and types, overlap of flowering, ecological conditions, and cultural practices should be appropriate in order to obtain high yield of high quality nuts.

Table 2. The values of pollen production components in walnut cultivars and types.

Varieties	FC*	AF*	PF*	PA*	PC*	DP (%)**
Van 6	113 mn	19 abc	116,790 e-j	6.146 ab	13,196,436 op	96 abc
Şebin	133 e-i	18 abc	118,507 e-i	6.603 ab	15,761,299 d-h	95 a-d
Yalova 3	122 j-m	17 bc	119,348 e-h	7.048 ab	14,558,472 h-m	96 abc
Bilecik	138 b-g	17 bc	127,555 bcd	7.502 a	14,602,521 bc	98 a
Yalova 1	110 n	18 abc	118,544 e-i	6.595 ab	13,039,840 p	95 a-d
Kaman 1	137 c-g	17 bc	121,522 d-g	7.148 ab	16,648,514 bcd	94 a-e
Kaman 4	135 d-h	19 abc	115,901 f-j	6.116 ab	15,646,648 d-i	91 de
Franguette	131 f-j	19 abc	116,595 e-j	6.136 ab	15,275,990 f-k	92 cde
Serr	135 d-h	18 abc	128,764 abc	7.153 ab	17,382,117 bc	95 a-d
Chandler	123 jkl	19 abc	130,594 ab	6.892 ab	16,063,172 d-g	98 a
Pedro	131 f-g	17 bc	115,228 g-j	6.801 ab	15,094,916 g-l	96 abc
Tokat 1	142 a-e	16 c	122,665 c-f	7.666 a	17,415,768 bc	95 a-d
Kaman 3	116 lmn	17 bc	117,218 e-j	6.894 ab	13,597,266 nop	97 ab
Hartley	125 i-l	18 abc	116,107 f-j	6.455 ab	14,513,375 i-n	98 a
Yalova 4	144 a-d	17 bc	122,509 c-f	7.206 ab	17,641,478 b	90 e
Maraş 18	140 a-f	19 abc	118,485 e-i	6.236 ab	16,587,970 b-e	97 ab
Kaplan 86	145 abc	22 a	134,800 a	6.126 ab	19,546,087 a	95 a-d
Kr 2	123 j-l	18 abc	116,335 f-j	6.463 ab	14,308,560 j-o	91 de
Şen 1	116 lmn	19 abc	132,476 ab	6.972 ab	15,366,434 e-j	93 b-e
R.de M. *	137 c-g	19 abc	119,673 e-h	6.315 ab	16,395,201 c-f	94 a-e
Ürgüp	148 a	17 bc	112,267 ijk	6.620 ab	16,615,516 bcd	96 abc
Back	131 f-j	17 bc	115,892 f-j	6.840 ab	15,181,943 f-k	94 a-e
Payne	130 g-j	18 abc	114,662 hij	6.390 ab	14,906,061 i-n	97 ab
Van 4	147 ab	18 abc	105,905 k	5.883 b	15,567,643 d-i	98 a
77 H 1	109 n	18 abc	106,745 k	5.929 b	11,633,655 q	95 a-d
Kr 1	142 a-e	18 abc	117,313 e-j	6.137 ab	16,658,539 bcd	93 b-e
Şen 2	124 i-l	17 bc	112,255 ijk	6.625 ab	13,918,704 l-p	90 e
Maraş 10	127 h-k	19 abc	111,435 jk	5.875 b	14,147,983 j-p	94 a-e
Maraş 12	122 j-m	18 abc	117,401 e-j	6.521 ab	14,322,922 j-o	98 a
Maraş 19	129 g-j	21 ab	123,395 cde	5.888 b	15,918,298 d-g	93 b-e
Sütyemez 1	118 k-n	18 abc	116,484 f-j	6.493 ab	13,745,836 m-p	92 cde
Sütyemez 2	124 i-l	17 bc	113,887 hij	6.698 ab	14,121,988 k-p	93 b-e
LSD 1%	5.3030	4.4785	6.8235	1.5684	1221.589	4.9351

R.de M.\*: Ronde de Monticnag

FC\*: Number of staminate flowers per catkin. AF\*: Number of anthers per flower.

PF\*: Number of pollen grains per flower. PA\*: Number of pollen grains per anther (PF/AF).

PC\*: Number of pollen grains per catkin (FC × PF).

DP\*\*: Percentage of well-developed pollen grains.

Data followed by the same letters are not significantly different (1%).

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