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Araştırma Makalesi/*Research Article (Original Paper)*

Determination of Pollen Germination Rates and Pollen Quantities of Some Hybrid Walnut Genotypes

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Abstract: The aim of this research is to determine the rate of pollen germination and pollen production of 29 hybrid walnut genotypes. For this aim, the germination percentage of the pollens was examined by using the agar in the petri and hanging drop methods. Pollen production amount of the genotypes was determined with the hemacytometric method. The highest germination ratio of genotype 30 was obtained by the agar in the petri method in 1% agar+10% sucrose (54.65%). In 15% sucrose concentration in the hanging drop method, which is the best medium for all 29 hybrid walnut genotypes, pollen germination rate was 51.84% (genotype 9). The amount of pollen in a flower and an anther is very high in all genotypes. The consequence indicates that different treatments had a significant effect on the germination percentage. As a result of the findings obtained from this study, genotypes can be used as a pollinator.

Keywords: Germination, Hybrid walnut genotype, Pollen, Production

Bazı Melez Ceviz Genotiplerinin Çiçek Tozu Çimlenme Oranlarının ve Üretim Miktarlarının Belirlenmesi

Öz: Bu çalışma, 29 melez ceviz genotipinin çiçek tozu çimlenme ve üretim miktarının belirlenmesi amacıyla yapılmıştır. Çiçek tozlarının çimlenme oranlarının belirlenmesinde petride agar ve asılı damla yöntemleri, çiçek tozu üretim miktarlarının belirlenmesinde ise hemasitometrik yöntem kullanılmıştır. En yüksek çiçek tozu çimlenme değeri (%54.65) genotip 30'da petride agar yönteminde %1 agar+%10 sakkaroz konsantrasyonundan, asılı damlada ise %51.84 (genotip 9) ile %15 sakkaroz konsantrasyonunda tespit edilmiştir. Tüm genotiplerde çiçek tozu üretim miktarları genelde yüksek bulunmuştur. Bu çalışmada elde edilen bulgulara göre tüm genotiplerin tozlayıcılık yeteneklerinin genelde yüksek olduğu belirlenmiştir.

Anahtar kelimeler: Çimlenme, Melez ceviz genotipleri, Çiçek tozu, Üretim

Introduction

Juglans regia L. (Common walnut) is a monoecious and anemophilous plant with its flowers being able to be fertilized by its own pollen. Despite being able to be fertilized by its own pollen, the walnut bears dichogamous flowers that are either protandrous or protogynous, which may raise some complications for breeding researches to obtain sufficient pollen at the time female flowers receptivity (Luza and Polito 1985). Walnut pollen loses its viability rapidly under natural conditions, thus, extra care such as temperature, humidity, lightness or maturity, is required to prolong pollen viability if stored or kept artificially (Luza and Polito 1985).

Since it is one of the main criteria in the selection of pollinator varieties, it is very important to know the viability, germination and pollination abilities of pollen. There is usually a linear connection between the viability and germination abilities of pollen in many fruit species and fruit attitudes, apart from in certain special circumstance (Kobel 1944; Dokuzoğuz 1957; Ayfer 1959; Griggs et al. 1971; Bilgin and Mısırlı 2017; Bükücü et al. 2018).

Despite some exceptions, pollen germination ratio, viability, and its pollination ability have direct relationships with fruit set. *In vitro* pollen viability and germination tests are extensively used in determining pollination capability. One of the most important issues affecting yield in fruit cultivation is the performance of the selected cultivars in terms of fertilization biology. For this reason, it is necessary to know the pollination features of the selected cultivars and to pollinate each other and to realize the pollination of the selected cultivars (Luza and Polito 1985; Sulusoglu 2014).

It is very important for a cultivar to be described as a good pollinator in terms of the healthy development of pollen, viability and germination abilities, as well as the total amount of pollen produced in flowers and the levels of morphological homogeneity of pollen, are high (Stösser and Anvari 1981; Stösser 1984; Eti 1990).

The objective of the present paper is to provide detect pollen germination, pollen production and pollination ability of some new hybrid walnut genotypes *in vitro*.

Material and Methods

In this search conducted in 2017, 29 different walnut cultivars were used as material. Pollen germination tests conducted under *in vitro* conditions were compared with "agar in petri" and "hanging drop" methods at a constant temperature of 20°C (Stanley and Linskens 1985). 5%, 10%, and 15% sucrose concentrations were used in agar in petri method, while distilled water, 5%, 10%, 15%, and 20% sucrose concentrations were used as pollen germination medium in hanging drop method. Pollen germination tests were performed through 5 replications for each genotype and for each application. The experiment was carried out through eight replications in randomly chosen four fields on each microscope slide. The amount of pollen production of genotypes studied in the scope of the present research was determined using hemocytometer method (Eti 1990). On hemocytometer lam, there are two counting chambers, each divided into several squares and having a certain depth. For the purpose of counting, the anthers of 20 flowers that have not bloomed yet were determined in two replications and placed in a bottle. 2 ml of water was added to each bottle after the anther in the bottles which were left without their caps were dried. After this suspension was shaken well, a drop was applied to the counting chamber on hemocytometer lam, and special lamella was put on them to close. The volume between lam pit and lamella was determined. The amount of pollen in this volume was determined by counting in 4 random areas for each replication, and the amount of pollen in 10 flowers in the total volume of suspension was determined by proportion. From here, the amount of pollen in a flower and an anther can be determined. Thanks to this test, the percentage of normal-looking pollen can be determined from a morphological perspective, and the ratio of normal-developed pollen can be determined as well.

Data were analyzed using a SAS based package program. Variance analysis (One-way ANOVA) was used for statistical analysis and the Tukey (HSD) test was used to reveal the difference between groups ($p<0.05$).

Results and Discussion

The results obtained from the germination test were obtained in Table 1. In the germination test conduct using agar in petri method, the highest germination percentage (38.82%) was obtained from pollen belonging to genotype 1, followed by genotype 30 (37.95%) and genotype 3 (37.69%) agar in petri, 1% agar+5% in sucrose concentration. The lowest germination percentage (25.84%) was obtained from the pollen of genotype 21 (Table 1). The study also demonstrated that germination rates obtained from 1% agar+10% sucrose concentration was generally higher compared to other sucrose concentrations. In this medium (1% agar+10% sucrose), the highest germination values were acquired from genotype 30 (54.65%), genotype 6 (54.60%), genotype 1 (52.39%) and genotype 29 (51.33%). In the same germination medium, the lowest germination percentage (34.01%) was found to be in the pollen genotype 24. In all three media, values belonging to other genotypes were included in the intermediate group (Table 1).

As a result of the "hanging drop" method, which is another germination test, it was found that germination rates were between 3.65% (genotype 25) and 11.76% (genotype 7) in distilled water medium, between 22.89% (genotype 26) and 33.92% (genotype 2) in 5% sucrose concentration, between 29.65% (genotype 5) and 44.23% (genotype 19) in 10% sucrose concentration, and between 32.98% (genotype 13) and 51.84% (genotype 9) in 15% sucrose concentrations. It was also observed that the difference was between 15.47% (genotype 23) and 28.75% (genotype 24) in 20% sucrose concentration (Table 2).

In a study conducted by Mert (2009), pollen obtained from Yalova 3, Kaplan 86, Şebin, Franquette, Hartley and Pedro walnut cultivars were used to determine the effects of different temperature and sucrose concentrations (10%, 15% or 20%). The pollen germination rates of these walnut cultivars increased significantly in proportion to temperature. The highest germination rates were obtained at a temperature of 27±1°C in both years (26.94-73.98%; 22.78-70.86%). All cultivars of pollen viability rates (>75%) were found to be high. In addition, the highest percentage of pollen germination was obtained from 15% and 20% sucrose concentrations in both years.

Özcan et al. (2017) the pollen germination ratio of 15 Temmuz, Diriliş, Bayrak and Maraş 12 walnut cultivars were determined using "agar in petri" and "hanging drop" methods. In this study, pollen germination rates were high for both tests. In general, maximum germination ratio was determined in 15% concentration in hanging drop method. In this medium, pollen germination rates were reported to vary between 5.18% and 50.14%.

Table 1. Germination ratio of pollen by agar in petri test in walnut genotypes

Genotypes	1% agar + 5% sucrose*	1% agar + 10% sucrose*	1% agar + 15% sucrose*
Genotype 1	38.82 a	52.39 abc	24.63 ijk
Genotype 2	27.12 i	46.23 e..i	28.46 d..j
Genotype 3	37.69 ab	48.67 c..f	25.23 h..k
Genotype 4	26.95 i	40.88 j..m	27.63 e..k
Genotype 5	37.11 abc	47.67 d..g	28.97 d..j
Genotype 6	36.14 a..d	54.60 a	26.02 g..k
Genotype 7	28.98 g..i	42.95 h..k	32.00 a..f
Genotype 8	27.97 hi	44.10 g..j	24.67 ijk
Genotype 9	34.63 b..e	42.06 i..l	29.85 c..i
Genotype 10	36.79 abc	42.17 i..l	32.45 a..f
Genotype 11	27.12 d..g	42.32 i..l	28.69 d..j
Genotype 12	33.19 c..f	47.62 d..g	32.74 a..e
Genotype 13	26.13 i	43.95 g..j	33.00 a..e
Genotype 14	28.95 g..i	39.20 klm	27.22 f..k
Genotype 15	37.25 abc	42.97 h..k	29.96 c..i
Genotype 16	29.92 f..i	48.89 ab	35.62 ab
Genotype 17	27.86 hi	45.37 f..i	28.36 d..j
Genotype 18	31.67 e..h	37.63 mn	26.83 f..k
Genotype 19	31.45 e..h	34.20 n	29.63 d..i
Genotype 20	33.95 b..f	47.52 d..g	33.65 a..d
Genotype 21	25.84 i	37.12 mn	22.15 k
Genotype 22	25.88 i	37.02 mn	23.87 jk
Genotype 23	32.27 d..g	46.96 e..h	32.09 a..g
Genotype 24	26.46 i	34.01 n	26.00 g..k
Genotype 25	31.29 e..h	50.33 b..e	35.32 abc
Genotype 26	26.36 i	38.74 lm	29.24 d..j
Genotype 28	32.62 d..g	36.91 mn	28.63 d..j
Genotype 29	33.54 c..f	51.33 a..d	35.69 b..h
Genotype 30	37.95 ab	54.65 a	36.45 a
HSD: 5%	3.5786	3.6846	4.7377

***:** Statistical analysis was performed according to angle transformation. Data followed by the same letters are not significantly different (5%) by Tukey's HSD.

In this research, normal-developed pollen ratio and pollen production ratio of the walnut cultivars were determined. For this purpose, the number of staminate flowers per catkin (FC), the number of anthers per flower (AF), the number of pollen grains per flower (PF), the number of pollen grains per anther (PA) = PF/AF, the number of pollen grains per catkin (PC) = FC×PF, the percentage of well-developed pollen (DP).

Among the walnut genotypes, genotype 2 had also the highest number of male flowers in a catkin (FC) (160.12 grain), while genotype 9 had the lowest number (98.67 grain).

Similar results were obtained from the walnut genotypes in terms of average number of anther in a flower (AF). In this respect, it was found out that the highest value was 23.54 grain and genotype 15 and 19, and the lowest value (15.63 grain) was in genotype 22. The mean anther numbers of other genotypes vary between these values (Table 3). The on the average number of pollen in a flower of (PF) genotypes ranged from 96 300 grain (genotype 3) to 147 372 grain (genotype 29).

In terms of average number of pollen (PA) in anther, which is found through the mean number of pollen in a male flower divided by the mean number of anther in male flowers, the highest number among genotypes was 8 973.5 grain and 8 692.6 grain with genotype 22 and genotype 25, respectively, while the lowest number was 5 022.7 grain with genotype 19.

Table 2. Germination ratio of pollen by hanging drop test in walnut genotypes

Genotypes	Distilled water	5% Sucrose	10% Sucrose	15% Sucrose	20% Sucrose
Genotype 1	10.17 a..d	33.76 ab	37.69 f..i	50.23 a	16.78 lmn
Genotype 2	9.75 a..e	33.92 a	43.21 ab	49.28 abc	22.69 fgh
Genotype 3	9.96 a..e	29.85 f	35.98 g..k	47.63 bcd	18.62 klm
Genotype 4	9.52 a..e	29.64 f	34.22 ijk	38.33 ij	23.87 d..h
Genotype 5	8.95 a..f	27.88 gh	29.65 l	35.20 kl	26.25 a..d
Genotype 6	8.12 a..g	33.01 abc	37.52 f..i	46.00 d	19.90 ijk
Genotype 7	11.76 a	32.41 cd	33.64 jk	37.62 ijk	24.16 d..h
Genotype 8	7.21 d..h	26.74 ij	32.62 kl	37.85 ij	22.97 fgh
Genotype 9	7.79 b..g	29.45 f	38.75 d..h	51.84 a	25.62 b..e
Genotype 10	11.63 ab	32.65 bc	39.63 b..g	50.78 a	19.54 ijk
Genotype 11	10.58 a..d	29.52 f	34.23 ijk	42.52 fg	19.26 jk
Genotype 12	9.36 a..f	24.86 k	33.67 jk	47.36 cd	24.63 c..g
Genotype 13	10.12 a..e	31.97 cde	42.65 abc	32.98 l	24.62 c..g
Genotype 14	8.99 a..f	32.98 abc	42.87 a	39.63 hi	28.12 ab
Genotype 15	5.59 fgh	28.03 gh	39.96 f..j	36.52 jk	22.00 ghi
Genotype 16	6.89 d..h	27.95 gh	33.63 jk	40.96 gh	24.88 c..f
Genotype 17	9.21 a..f	31.12 e	37.62 f..i	38.52 hij	26.98 abc
Genotype 18	7.21 d..h	26.70 ij	37.46 f..i	46.32 d	21.69 hij
Genotype 19	6.27 e..h	33.74 ab	44.23 a	47.26 cd	23.45 e..h
Genotype 20	9.74 a..e	28.95 fg	33.62 jk	36.97 jk	27.21 abc
Genotype 21	11.25 abc	31.21 e	42.86 abc	42.26 fg	26.95 abc
Genotype 22	8.54 a..g	33.65 ab	38.24 e..h	46.37 d	16.48 mn
Genotype 23	8.97 a..f	32.64 bc	40.25 b..f	49.89 ab	15.47 n
Genotype 24	7.15 c..g	31.28 de	39.56 c..g	37.52 ijk	28.75 a
Genotype 25	3.65 h	25.67 jk	42.27 a..d	43.67 ef	18.96 kl
Genotype 26	8.19 a..g	22.89 l	36.20 h..k	49.33 abc	22.77 fgh
Genotype 28	4.88 g..h	32.47 cd	44.21 a	50.71 a	24.12 d..h
Genotype 29	10.63 a..d	29.78 f	41.75 a..e	49.87 ab	26.94 abc
Genotype 30	9.36 a..f	27.65 hi	33.62 jk	45.23 de	22.68 fgh
HSD: 5%	3.2600	1.0430	3.1570	2.2170	2.2813

***:** Statistical analysis was performed according to angle transformation. Data followed by the same letters are not significantly different (5%) by Tukey's HSD.

The total number of pollen in one catkin (PC) is obtained through the average number of flowers in one catkin divided by the average number of pollen in one catkin. In this category, the highest and lowest values among genotypes (18 788 801 and 11 011 247) were determined to belong to walnut genotypes 2 and 11, respectively. On the other hand, the values of other genotypes have formed intermediate groups.

The highest values of pollen in walnut genotypes (DP) were 98.78% with genotype 17. The lowest value for this category (94.65%) was obtained from genotype 6 (Table 3).

In order to define a cultivar used in fruit cultivation as a good pollinator, it must possess healthy development of pollen, viability and germination abilities as well as the total amount of pollen produced in flowers and the normal levels of pollen because all of the pollinated pollen cannot reach seed drafts by developing (Oberle and Goertzen 1952; Anvari 1977; Hansen 1981; Stösser and Anvari 1981; Seilheimer and Stösser 1982; Stösser 1984; Eti 1990).

Our study indicated that the values we found in terms of the number of male flowers in a catkin were similar to the values reported by Sütyemez (1998; 2007). Şen (2011) reported that the total number of catkin in a walnut tree was 5.000, while the number of male flowers in a catkin varied between 10 to 100 grains. In our study, the number of male flowers in a catkin was determined as 155 grain, and it was higher than the values reported by Şen (2011).

Table 3. Pollen production constituent in walnut genotypes

Genotypes	FC*	AF*	PF*	PA*	PC*	DP* (%) **
Genotype 1	108.54 k	16.21 mn	111 369 k	6 870.3 de	12.087 991 jk	95.72 e..h
Genotype 2	160.12 a	15.75 o	117 342 ij	7 450.2 cd	18.788 801 a	96.25d..g
Genotype 3	119.65 fg	15.89 no	96 300 l	6 060.4 hij	11.522 295 kl	97.85 abc
Genotype 4	113.23 i	18.62 h	140 321 b	7 536.0 cd	15.888 546 e	94.91 gh
Genotype 5	110.27 j	18.74 h	122 952 fg	6 560.9 fg	13.557 917 i	98.22 ab
Genotype 6	99.89 n	16.21 mn	118 324 ij	7 299.4 cde	11.819 384 jk	94.65 h
Genotype 7	126.64 d	16.63 kl	142 269 b	8 554.9 ab	18.016 946 ab	95.14 fgh
Genotype 8	110.52 j	21.20 d	139 647 b	6 587.1 fg	15.433 786 ef	98.67 a
Genotype 9	98.67 n	19.64 fg	117 254 ij	5 970.1 ijk	11.569 452 kl	98.41 a
Genotype 10	103.16 m	16.99 jk	115 321 j	6 787.5 fg	11.896 514 gk	95.63 e..h
Genotype 11	99.57 n	18.67 h	110 588 k	5 923.2 jk	11. 011 247 l	96.72 cde
Genotype 12	110.62 j	20.33 e	112 321 k	5 524.8 klm	12.424 949 j	98.22 ab
Genotype 13	107.24 k	16.49 lm	126 238 e	7 655.4 cd	13.537 763 i	97.69 abc
Genotype 14	113.65 hi	21.36 d	121 325 gh	5 680.0 jkl	13.788 586 hi	95.24 fgh
Genotype 15	99.54 n	23.54 a	119 698 hi	5 084.8 mn	11.914 738 jk	97.83 abc
Genotype 16	129.67 c	17.21 j	142 637 b	8 288.0 b	18.495 739 a	96.54 c..f
Genotype 17	129.50 c	19.32 g	130 227 d	6 740.5 fg	16.864 396 cd	98.78 a
Genotype 18	114.69 h	19.78 f	126 214 e	6 380.8 ghi	14.475 483 gh	97.65 a..d
Genotype 19	124.52 e	23.54 a	118 235 ij	5 022.7 n	14.722 622 fg	97.58 a..d
Genotype 20	112.51 i	19.32 g	110 250 k	5 706.5 jkl	12.404 227 j	97.42 a..d
Genotype 21	126.78 d	21.80 c	142 366 b	6 530.5 fg	18.049 161 ab	96.87 b..e
Genotype 22	110.10 j	15.63 o	140 257 b	8 973.5 a	15.442 295 ef	98.58 a
Genotype 23	123.62 e	16.20 mn	125 241 ef	7 730.9 c	15.482 292 ef	97.56 a..d
Genotype 24	124.57 e	17.64 i	133 652 c	7 576.6 cd	16.649 029 d	95.18 fgh
Genotype 25	136.21 b	15.67 o	136 214 c	8 692.6 ab	18.553 708 a	96.78 cde
Genotype 26	113.20 i	22.55 b	122 625 fg	5 437.9 lmn	13.881 150 hi	98.52 a
Genotype 28	130.21 c	19.57 fg	134 752 b	6 885.6 ef	17.546 057 bc	95.23 fgh
Genotype 29	119.32 g	22.78 b	147 372 a	6 469.3 fgh	17.584 427 bc	96.47 c..f
Genotype 30	120.63 f	19.67 fg	127 620 de	6 488.0 fgh	15.394 800 ef	98.22 ab
HSD: 5%	1.2179	0.3645	2.7552	4.2061	7.4273	1.2062

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.
HSD test, data followed by the same letters are not significantly different (5%).
****:** Statistical analysis was performed according to angle transformation.

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