ISSN 1819-1894

Asian Journal of Agricultural Research

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OPEN ACCESS Asian Journal of Agricultural Research

ISSN 1819-1894
DOI: 10.3923/ajar.2017.93.97

Research Article
Determination of Pollen Quality and Production in New Walnut Cultivars

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Abstract

Background and Objective: Walnut (*Juglans regia* L.) is a monoecious plant and wind-pollinated. In addition, there is no self and cross-incompatibility in this fruit. The germination rate and viability of pollens and its fertilization ability must be known to select a cultivar as a pollinator. Therefore, this study aimed to determine pollen production, pollen viability and pollination of some walnut cultivars *in vitro*. **Materials and Methods:** This study was conducted on four different walnut cultivars, i.e., "Diriliş, 15 Temmuz, Maraş 12 and Bayrak". In the study, pollen viability rate, pollen germination rate, amount of pollen production and normal developed pollen rates were determined. The pollen viability studies with TTC and FDA tests and pollen germination experiments with agar in the petri and Hanging drop methods. Experimental data were analyzed using analysis of variance (one-way ANOVA), by Tukey's (HSD) test. **Results:** In this study, generally the germination rate of pollens reached a high level in both germination tests. However, the highest germination rate was obtained from sucrose concentration by 15% in hanging drop test. The pollen viability studies with both tests and pollen germination experiments both methods, the viability values ranged from 87.53% (Maraş 12) and 92.70% (15 Temmuz), germination levels were between 5.18% and 50.14%. **Conclusion:** As a result of this research, all walnut cultivars were adequate in terms of pollinator potential while Bayrak and 15 Temmuz was highest.

Key words: Fertilization biology, pollinator, germination, walnut, pollen grains

Citation: Akide Özcan, Şakir Burak Bükücü and Mehmet Sütyemez, 2017. Determination of pollen quality and production in new walnut cultivars. Asian J. Agric. Res., 11: 93-97.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Walnut (*Juglans regia* L.) is the major walnut species cultivated for nut production and is one of the most widespread tree nut species in the world. Widely used in various fields around the world, walnut (*Juglans regia* L.) has been so far studied from many perspectives. Variety of studies have always focused on the improvement of a more productive and high quality. Walnut (*Juglans regia* L.) is a monoecious plant and wind-pollinated. In addition, there is no self and cross-incompatibility in this fruit.

In spite of its self-compatible nature, breeding and research programs encounter difficulties in terms of obtaining sufficient pollen quantities at the time of pistillate flowers receptivity because of the dichogamous nature of the species, which has protandrous or protogynous mating types[1]. Since the life span of walnut pollens are short under natural conditions, it must be handled with care when it expose to high temperature and relative humidity at maturity which cause viability loses[1].

The germination rate and viability of pollens and its fertilization ability must be known to select a cultivar as a pollinator. Despite some exceptions, there is usually a positive correlation between these parameters and the resulting fruit set in many fruit species[1-13]. *In vitro* pollen viability and germination tests are widely used in determining pollination ability.

Pollen viability and germination tests are conducted in laboratories under *in vitro* conditions in order to determine whether pollinators and pollens are functional.

In terms of fertilization biology, a high pollen quality (pollen viability, pollen germination and well-developed pollen rates) and a high number of pollen production is important for a satisfying yield.

This study aims to determine pollen production, pollen viability and pollination of some new walnut cultivars *in vitro*.

MATERIALS AND METHODS

Materials: In this study 4 different walnut cultivars were used as material that conducted in 2017. Among cultivars used in the study were "Diriliş and 15 Temmuz", which were improved as hybrid cultivars and "Maraş 12 and Bayrak", which were selection cultivars.

Methods

Pollen viability tests: In this study, two tests, i.e., fluorescein diacetate (FDA) according to Heslop-Harrison and Heslop-Harrison[14] and triphenyl tetrazolium chloride (TTC) according to Norton[15], were used to determine the pollen viability rate of walnut cultivars.

Pollen germination tests: "Agar in petri" and "Hanging drop" methods were used for pollen germination tests under *in vitro* conditions[12]. While sucrose concentrations of 5, 10 and 15% added to an agar dilution of 1% were used in agar in petri method, sucrose solutions of 0, 5, 10 and 15% were used as an environment for pollen germination in Hanging drop method[16].

Pollen production: The pollen production quantities of the cultivars under experiment were determined using "Hemocytometic Method"[7].

The following parameters were taken into consideration in the determination of the pollen production rate of the walnut cultivars: 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).

The percentage of well-developed pollen was also tested by the same method. In order to obtain the average number of each component, 50 randomly selected catkins were collected from each tree. All the flowers on these catkins were counted and the average number of the above-mentioned components of each walnut cultivar was determined[16,17].

Statistics analysis: Experimental data were analyzed using analysis of variance (one-way ANOVA) and the means were statistically grouped by Tukey's (HSD) test. Data were analyzed using a SAS based program and means were grouped by using Tukey's test ($p<0.05$, $p<0.01$).

RESULTS AND DISCUSSION

Pollen viability: The pollen viability rates of walnut cultivars are given in Table 1. It can be observed that the pollen viability rates of these cultivars are generally high (over 85%). While "15 Temmuz" cultivar yielded the highest viability rate (92.70%) in TTC test, "Bayrak" cultivar yielded the highest viability rate (92.09%) in FDA test. Sutyemez[18] reported that the pollen viability rates of different walnut cultivars varied between 81.01 and 93.60% in TTC test while in FDA it was between 80.98 and 95.64%. In this study, the results of TTC and FDA tests were similar to previous studies[1,9-11,13,18].

These pollen viability levels suggest that the walnut cultivars in question can be easily selected as pollinators.

Table 1: Viability percentage of pollen by TTC and FDA tests in walnut cultivars

	TTC		FDA	
Cultivars	Non-viable (%)	Viable (%)	Non-viable (%)	Viable (%)
Diriliş	9.52	90.48	11.26	88.74
15 Temmuz	7.30	92.70	8.84	91.16
Bayrak	8.66	91.34	7.91	92.09
Maraş 12	12.47	87.53	11.78	88.22
LSD 1%	ns	ns	ns	ns

ns: Non significant, LSD: Least significant differences test, data followed by the same letters are not significantly different (1%)

Table 2: Germination rates of pollen by agar in petri and Hanging drop tests in walnut cultivars

	Agar in petri (1% agar+sucrose)			Hanging drop				
Cultivars	5% Suc.	10% Suc.	15% Suc.	0% Suc.	5% Suc.	10% Suc.	15% Suc.	20% Suc.
Diriliş	33.17	45.51^{ab}	28.40^{ab}	5.93^{bc}	24.95	39.17^{a}	46.70	21.32^{ab}
15 Temmuz	31.92	47.35^{a}	27.46^{b}	5.18^{c}	26.15	36.44^{b}	48.59	23.13^{a}
Bayrak	32.39	44.10^{b}	29.67^{a}	6.53^{ab}	26.41	39.08^{ab}	50.14	20.40^{b}
Maraş 12	29.86	45.22^{ab}	29.74^{a}	7.19^{a}	25.78	37.11^{ab}	47.65	20.94^{b}
LSD 1%				0.594				
LSD 5%	ns	2.080	1.340		ns	1.802	ns	1.425

Suc: Sucrose, ns: Non Significant, LSD: Least Significant Differences Test, Data followed by the same letters are not significantly different (1 and 5%)

Table 3: Pollen production components in walnut cultivars

Cultivars	FC*	AF*	PF*	PA*	PC*	DP*
Diriliş	128^{ab}	18	116873^{ab}	6492	14955944^{b}	97
15 Temmuz	117^{b}	18	113628^{b}	6332	13294476^{c}	94
Bayrak	140^{a}	17	123517^{a}	7366	17292563^{a}	97
Maraş 12	125^{ab}	18	116452^{ab}	6469	14555894^{bc}	98
LSD 1%	12181	ns	6322	ns	1167368	ns

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, ns: Non Significant, LSD: Least significant differences test, data followed by the same letters are not significantly different (1%)

Pollen germination: The results of pollen germination tests are given in Table 2. In the germination test performed using agar in petri method (agar of 1%+sucrose concentrations of 5, 10 and 15%), the highest pollen germination rate was obtained from 15 Temmuz with an agar dilution of 1%+a sucrose concentration of 10% by 47.35%. On the other hand, the highest pollen germination rate in walnut cultivars germinated using Hanging drop method belongs to Bayrak cultivar with an agar dilution of 1%+a sucrose concentration of 10% by 50.14%.

It can be stated that walnut cultivars yielded a high germination rate in both environments (Table 2). It was also reported in various studies on other fruit cultivars that a correlation is likely to be observed between pollen germination and viability tests when a suitable germination environment is selected[2,3,8-11,13].

In their study on pollens of 21 walnut cultivars, Luza and Polito[1] obtained the highest germination rate from an agar dilution of 0.65%+10 mM $CaCl_2$+0.16 mM boric acid+two sucrose solutions of 20 and 15% and reported that the germination values in the same solutions varied between 43 and 77%. Mert[10] obtained generally the highest pollen germination rates from a sucrose concentration of 15 and 20% in Hanging drop method on walnut cultivars.

It was also reported that the highest germination rate in a germination test on two walnut cultivars was obtained from an agar dilution of 1+15% sucrose+0.02% H_3BO_3+0.05% $Ca(NO_3)_2 \cdot 4H_2O$ by 61% and that this rate decreased in the course of the time depending on the pollen storage conditions[7,16].

Sutyemez[18] attempted to determine the germination rates of walnut cultivars. This study reported that the highest pollen germination rate in the germination tests belonged to a sucrose concentration of 15% by 50.19% in a Hanging drop test and 51 agar+10% sucrose concentration by 48.54% in a petri test.

The pollen viability and germination rates obtained in this study suggest that these cultivars can be easily used as pollinators for other genotypes depending on inflorescence conditions in the same period.

Pollen production: The pollen production of walnut cultivars in this study is given in Table 3. While the number of anthers per flower in these walnut cultivars was determined as 17-18,

the number of staminate flowers per catkin varied between 117 (15 Temmuz) and 140 (Bayrak). The number of pollen grains per flower varied between 113628 (15 Temmuz) and 123517 (Bayrak). On the other hand, the number of pollen grains per anther varied between 6312 (15 Temmuz) and 7265 (Bayrak). The number of pollen grains per catkin was calculated between 13294476 (15 Temmuz) and 17292380 (Bayrak). The percentage of well-developed pollen for the walnut cultivars in question were calculated as remarkably high (94-97%).

The calculated number of pollen grains for walnut is quite controversial in the literature. According to Sutyemez[18], the pollen grain production on a flower of a walnut tree could vary between 30000 and 100000 and the pollen grain total of a walnut tree could even reach 100-500 millions. However, Sen[19] reported that a mature walnut tree with 5000 staminate catkins might produce 1-4 million(s) of pollen grains per catkin and estimated that a walnut tree could yield 5-20 billions of pollen grains. The results of the present study overlap Sen's[19] finding since the number of pollen grains per tree was estimated as 5-100 billions.

In a study on the determination of pollens in walnut cultivars, it was reported that the number of anthers per flower varied between 16 and 22 and the number of staminate flowers per catkin varied between 109 and 149. In the same study, the number of pollen grains per flower varied between 105.905 and 134800 while the number of pollen grains per anther varied between 5883 and 7502. The number of pollen grains per catkin, on the other hand, were calculated as between 11633655 and 19546087[18].

The percentage of well-developed pollens, a high pollen viability and germination rate, the total number of pollen production and a high level of morphological homogeneity for pollens bear utmost importance in order to select a cultivar as an efficient pollinator[3,7-9,11,16].

In terms of fertilization biology, this study demonstrated that a high pollen quality (pollen viability, germination, morphological homogeneity) and a high level of pollen production is vital for a high level of pollination ability.

In this study, which is based on determining the pollinating properties of new walnut varieties, it has been determined that the quality of pollen and the production quantity of values of the varieties are very high. These varieties have been found to have sufficient pollinating ability in terms of fertilization biology.

CONCLUSION

In this study, there were slight differences in the pollen viability percentages, however, Bayrak cultivar and 15 Temmuz cultivars consistently demonstrated high pollen viability percentages. In TTC tests, 15 Temmuz cultivar has the highest pollen viability (92.70%). Pollen viability determined by FDA test was 95.64% for Bayrak cultivar. In the germination test performed using Hanging drop test method, Bayrak cultivar has the highest pollen germination rate (50.14%) which was obtained 15% sucrose concentration. In the Hanging drop test, sucrose concentration affected pollen germination. In the agar in petri test (1% agar+10% sucrose), the highest pollen germination rate was obtained from 15 Temmuz cultivar (47.35%). In general, the results obtained from this study have potentially practical using in fertilization biology in the walnut cultivation.

SIGNIFICANCE STATEMENT

In this study, the cases of fertilization biology of the new walnut varieties obtained by cross-breeding are presented. Thus, in the gardens to be established with these varieties, the conditions that will negatively affect the fruit set will be removed.

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