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Analysis of pollen germination performance of pistachio hybrids and their male parents

Izzet Acar [1]*, Mehmet Uzun [1], H. Seyfettin Atli [1] and Sinan Eti [2]

*[1] Pistachio Research Institute,Universite Bulvari No: 136 Sahinbey, 27060 Gaziantep, Turkey. [2] Department of Horticulture, Faculty of Agriculture,University of Çukurova, 01330 Adana, Turkey. *e-mail: izzetacar@afae.gov.tr, izzetacar@yahoo.com*

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Abstract

This study was carried out at the hybrid pistachio plot of the Pistachio Research Institute, Turkey. Pollen of pistachio hybrids, by hybridizations of Kallahghochi, Siirt and Ohadi female cvs and Uygur, Atli and Kaska male cvs, was used in the study. The study compared the pollen germination percentage and pollen tube length of some pistachio male cvs with their offspring and showed that pollen germination of male parents was generally higher than that of their hybrids, except for Siirt × Uygur. The highest pollen germination percentage among the all hybrids and their male parents was obtained from Siirt × Uygur-Hybrid-2 (98.89%) and followed by Siirt × Kaska-Hybrid-2 (97.85%), which showed rather high pollen germination rates, while Siirt × Atli-Hybrid-2 had the lowest pollen germination (82.41%). The pollen tube length of the genotypes ranged from 680 µm (Ohadi × Kaska-Hybrid-3) to 1210 µm (Kallahghochi × Atli-Hybrid-1 and 2) and pollen tube development of Uygur was lower than of their offspring. In terms of pollen performance, Siirt × Kaska-Hybrid-2 was determined as the best F1 hybrid. The results of the study suggest that pollen germination and pollen tube growth may indicate pollinator performance of hybrid pistachio trees.

Key words: Pistachio, *Pistacia vera* L., pollen germination, pollen tube length, hybrid.

Introduction

The pistachio is a dioecious and wind pollinated fruit species. The pistillate and staminate flowers are produced in large inflorescences on different trees. Pollination is important for this species; the marketable part is the seed and, to obtain a good fruit set, adequate numbers of suitable male trees have to be interplanted in the orchards, located according to wind and rain conditions. To ensure adequate fruit setting, most of the pollen should remain in the environment for two or three days after the anthesis [3, 15, 28]. Commercial pistachio orchards usually contain one male tree for 8 to 11 females. However, there are significant pollinator problems at the pistachio orchards in Southeast Anatolian Region of Turkey. Suitable pollinators were determined [1, 3, 9] but their use has not become as widespread as was hoped. Pollinators have been found to be effective in fruit setting and they could be a factor in flower and fruit abscission in pistachio [4].

The pollen biology of the pistachio is well studied [2, 3, 5, 6, 9, 10, 14, 21, 22, 24, 28, 29] but there is no study of its offspring for pollen biology. According to some researchers, hybridization between two different taxa often produces offspring with reduced fitness, because the hybrids are infertile and inviable, or less fertile and less viable than the parent plants [12, 26]. However, hybrid individuals may have lower, equivalent or higher levels of fitness, compared to at least one of the parental species [7, 8], leading to an eventual fusion of the two parental taxa [17, 18]. Schwarzbach *et al*. [27], using morphological and physiological parameters, characterized hybrids according to features, which may be similar, intermediate or transgressive (exceeding more or less), in relation to parental traits.

Direct observations of pollen tube growth may provide some information on pollen performance, but will often not be able to account for the overall fitness of a certain genotype of pollen [16]. There is a long tradition of using pollen viability as an indicator of hybrid performance [13].

A study had been started in Pistachio Research Institute to obtain new male and female pistachio cultivars by hybridization of domestic and foreign pistachios in 1996, and 3500 male and female hybrids were obtained in the study [31]. The male hybrids used in the present experiment as pollen source were selected among these hybrids. The aim of the present research was to characterize the pollen germination and pollen tube growth of different F1 hybrids and their paternal generations, to determine the hybrid performance of pistachio.

Materials and Methods

Plant material: The present study was carried out at the hybrid pistachio plot of the Pistachio Research Institute in the Gaziantep province of Turkey. Pollen of pistachio hybrids and their male parents was used in the experiment. F1 hybrid combinations used in the experiment were: 'Kallahghochi × Uygur', 'Kallahghochi × Atli', 'Kallahghochi × Kaska'; 'Siirt × Uygur', 'Siirt × Atli', 'Siirt × Kaska'; 'Ohadi × Uygur', 'Ohadi × Atli' and 'Ohadi × Kaska'. Nine hybrid combinations were used in the study and three male trees were randomly marked for each hybrid combination. In this way, 27 hybrid male trees and their 3 male parents were used in the research.

Pollen collection and growth medium: Pollen from the hybrids was obtained at the beginning of blooming from inflorescences collected randomly from the trees. Inflorescences that had some flowers with dehiscent anthers were removed from male hybrid trees and their male parents, then brought into the laboratory and spread over tissue paper. Pollen that was shed overnight was sieved and collected under laboratory conditions.

In vitro germination was assessed with the hanging drop method. Pollen germination and pollen tube growth were determined by placing a small drop of germinating media on a cover glass; pollen grains were sown on the drops with a clean brush, and the cover glass was then inverted and rested on the cavity slide. Pollen was incubated in 20% (w/v) sucrose medium for 24 h at 25°C in dark conditions [3]. The male parents and the hybrids were then examined to identify any distinct differences in pollen germination and pollen tube growth.

Pollen germination and pollen tube measurements: Pollen germination was determined by direct microscopic observation (EUROMEX Bio-medical, Arnhem, Holland).

For each genotype, germination was recorded in three drops by counting three fields. A pollen grain was considered germinated when pollen tube length was at least equal to or greater than the grain diameter [20]. Germination percentage was determined by dividing the number of germinated pollen grains per field of view by the total number of pollen grains per field of view and was expressed as percentage.

Measurements of pollen tube length were recorded directly by an ocular micrometer fitted to the eyepiece of the microscope. Mean pollen tube length was calculated as the average length of 9 pollen tubes measured from each drop after 24 h.

Statistical analysis: The replicated values on pollen germination and tube length were analyzed using one-way ANOVA (MSTAT, Michigan State University, Lansing). All percentage data were arcsine transformed. Duncan's Multiple Range Test was used for mean separation. Significant differences were determined at $p \leq 0.05$.

Results

Pollen germination: The effects of male parents on the *in vitro* pollen germination of nine hybrids of Kallahghochi, Siirt and Ohadi cultivars were evaluated and expressed as the percentage of germinated pollen grains (Table 1).

Hybrid differences were observed for germination percentage (Table 1 and Fig.1). Pollen germination percentage ranged from 82.41% (Siirt × Atli, Hybrid-2) to 92.85% (Atli, Control), with a mean of 89.43% for Atli male parents; from 86.36% (Kallahghochi × Uygur, Hybrid-1) to 98.89% (Siirt × Uygur, Hybrid-3), with a mean of 92.41% for Uygur male parent; and from 87.22% (Siirt × Kaska, Hybrid-3) to 97.85% (Siirt × Kaska, Hybrid-2), with a mean of 93.56% for Kaska male parent.

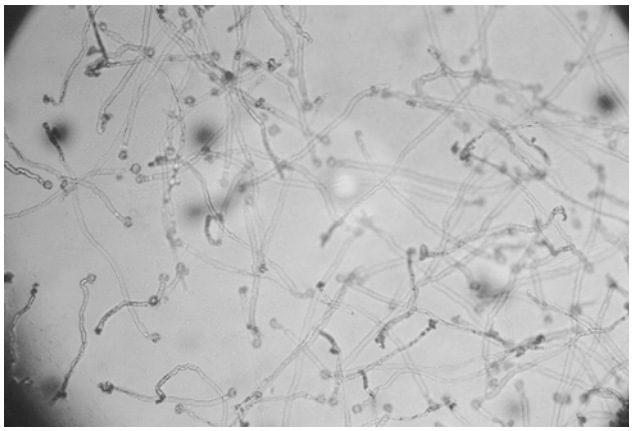

Figure 1. Light microscopy micrograph of Ohadi × Uygur hybrid pollen germinated in media containing 20% (w/v) sucrose after 24 h with the hanging drop pollen germination method (x 100).

Pollen tube growth: Hybrids differed significantly in pollen tube length (PTL) in respect of their male parents (Table 2). Pollen tubes were measured 24 h after germination in the *in vitro* medium. Pollen tube length for Atli male parent ranged from 780 µm for Siirt × Atli-Hybrid-2 to 1210 µm for Kallahghochi × Atli-Hybrid-1 and 2, with a mean of 1101 µm. The PTLs of the Kallahghochi hybrids were higher than the hybrids of Siirt, Ohadi and male parents (control). The lowest PTL was observed for Siirt hybrids. The PTL of Uygur male parent and their hybrids ranged from 859 µm for Uygur-Control to 1043 µm for Ohadi × Uygur-Hybrid-2, with an average of 962 µm. The PTLs of all hybrids of Uygur were higher than their male parent (control). Pollen tube length for Kaska male parent ranged from 680 µm for Ohadi × Kaska-Hybrid-

Table 1. Pollen germination rates of Atli, Uygur and Kaska male pistachio cvs and their hybrids (%).

Female parents	Hybrids	Pollen germination (%, ±SD)		
		Male parents		
		Atli	Uygur	Kaska
	Control	92.85±0.26 a*	92.80±2.26 bcd	94.57±1.86 b
	Hybrid–1	88.29±3.05 b	86.36±2.55 d	95.80±1.78 ab
Kallahghochi	Hybrid–2	89.82±2.41 ab	93.54±3.44 bc	90.60±2.37 bc
	Hybrid–3	88.33±1.28 b	92.69±1.31 bcd	95.31±2.14 ab
	Hybrid–1	87.25±4.59 b	94.41±4.44 b	94.28±2.25 b
Siirt	Hybrid–2	82.41±9.12 c	89.26±3.55 bcd	97.85±1.87 a
	Hybrid–3	90.62±0.29 ab	98.89±1.92 a	87.22±0.48 c
	Hybrid–1	90.54±2.81 ab	93.69±2.61 bc	92.08±2.54 bc
Ohadi	Hybrid–2	92.84±3.28 a	88.45±0.38 cd	94.50±2.36 b
	Hybrid–3	91.36±2.14 ab	94.00±2.58 bc	93.39±2.67 b
MEAN		89.43±3.10	92.41±3.56	93.56±2.98
LSD ($p \leq 0.05$)		3.38	5.64	5.37

*The letters following the numbers indicate different groups determined by Duncan's test ($p \leq 0.05$).

Table 2. Pollen tube growth of Atli, Uygur and Kaska male pistachio cvs and their hybrids (µm).

Female parents	Hybrids	Pollen tube length (µm, ±SD)		
		Male parents		
		Atli	Uygur	Kaska
	Control	1193±58.6 ab*	859±30.9 d	1110±70 a
	Hybrid–1	1210±10.0 a	911±38.4 cd	1097±35.1 ab
Kallahghochi	Hybrid–2	1210±45.8 a	997±15.3 ab	943±28.9 d
	Hybrid–3	1189±44.9 ab	980±52.9 abc	1023±20.8 bc
	Hybrid–1	1127±68.1 b	917±47.3 bcd	1100±79.4 ab
Siirt	Hybrid–2	780±36.1 d	1010±72.1 a	1133±63.5 a
	Hybrid–3	987±40.4 c	890±26.5 d	977±20.8 cd
	Hybrid–1	1143±49.3 ab	973±50.3 abc	1087±15.3 ab
Ohadi	Hybrid–2	1153±37.9 ab	1043±11.5 a	1090±50.0 ab
	Hybrid–3	1017±37.9 c	1037±40.4 a	680±30.0 e
MEAN		1101±136.7	962±63.8	1024±135.7
LSD ($p \leq 0.05$)		77.15	75.70	76.09

*The letters following the numbers indicate different groups determined by Duncan's test ($p \leq 0.05$).

3 to 1133 µm for Siirt × Kaska-Hybrid-2, with a mean of 1024 µm.

The analysis of variance revealed a highly significant effect of the main factors (parents and hybrid performances) and a significant effect of the male parent/female parent interaction. Male-female parent interaction was significant for pollen germination and PTL indicating that the effect of hybrid performance was parent-dependent (Fig. 2).

Mean pollen germination rates and PTL of 3 hybrids each of Kallahghochi, Siirt and Ohadi were calculated and compared with their male parent (Fig. 2). Average pollen germination percentages of Atli and Kaska were higher than of their hybrids; pollen germination of Uygur was higher than that of Kallahghochi × Uygur and Ohadi × Uygur hybrids and lower than that of Siirt × Uygur hybrids. Pollen tube growth in Kallahghochi × Atli was greater than in Atli and the PTL of Uygur hybrids were higher than Uygur. Pollen tube growth of the remaining hybrids was lower than that of their male parents. Both pollen germination and PTL were lowest in Siirt × Atli hybrids.

Discussion

The test most commonly used to assess pollen performance is *in vitro* germination. While an artificial medium cannot fully simulate the complex pollen-stigma interaction *in vivo*, pollen tube development *in vitro* appeared to be normal under the appropriate conditions (Fig. 1).

The study compared the pollen performance of some pistachio male cvs with their offspring and showed that pollen germination of male parents was generally higher than that of their hybrids, except for Siirt × Uygur (Fig. 2). The highest pollen germination percentage among all the hybrids and their male parents was obtained from Siirt × Uygur-Hybrid-2 (98.89%), followed by Siirt × Kaska-Hybrid-2 (97.85%), which showed rather high pollen germination rates, while Siirt × Atli-Hybrid-2 had the lowest value, with 82.41% (Table 1). Pollen germination in the 20% sucrose medium was substantially increased with the hanging drop pollen germination method (Fig. 1). Pollination and pollen performance are the main factors effective in pistachio productivity, because the marketable product is the seed. Pollen germination percentages of the male genotypes and their offspring evaluated in our study were high when compared with the results of other researchers [2, 3, 5, 6, 9, 10, 21, 24, 30]. The PTL of the genotypes were similar to germination, but the pollen tube development of Uygur was lower than of their offspring (Fig. 2). Among all the genotypes, the lowest PTL (680 µm) was observed for Ohadi × Kaska-Hybrid-3, while Kallahghochi × Atli-Hybrid-1 and 2 (1210 µm) had the highest value (Table 2). When all genotypes were compared with each other, the best pollen performance was observed in the Siirt × Kaska-Hybrid-2, in respect of both pollen germination percentage and PTL (Tables 1 and 2).

Grant [19] reported that under natural conditions, however, different environmental pressures can affect hybrids and parental species in different ways. Hybrids may not be formed under natural conditions, due to other factors besides pre- or postzygotic mechanisms. In some cases, breeding systems may confer barriers against hybrid formation. According to Niklas [23], postzygotic barriers are those processes that cause hybrid inviability, sterility or breakdown. It is already known that there is no self or inter-incompatibility between *Pistacia* species [11]. That means these species may naturally or artificially pollinate and fertilize each other. For this reason, there are many natural *Pistacia* hybrids in the natural growing areas.

Hybrids may be morphologically intermediate to their parents, but may also constitute a mosaic of them [25]. Pistachio male cvs and their hybrids had high pollen germination percentages, which may increase nut production, as well as seed formation. The pollen germination percentages of male parents used in the present study were similar, but the comparison of pollen tube growth between paternal genotypes indicated that Uygur did not perform as well as Atli and Kaska. Acar [3] reported that the pollen tubes of different male types reached the ovules of Kirmizi, Siirt and Ohadi pistachio cvs between 24 and 48 h; that is, actual fertilization occurs between 24 and 48 h once pollen is released, depending on the speed of growth of the pollen tube.

Finally, under *in vitro* conditions, pollen germination showed that Atli, Uygur and Kaska male pistachio cvs were generally better than their F1 hybrids, except for Siirt × Uygur. Pollen tube length indicated that Uygur showed the lowest development of all hybrids, while Kallahghochi × Atli had the highest value. The hybrids of Kaska and Atli had lower PTL than their parent, except for Kallahghochi × Atli. The results of the study suggest that pollen germination and pollen tube growth may indicate pollinator performance of hybrid pistachio trees.

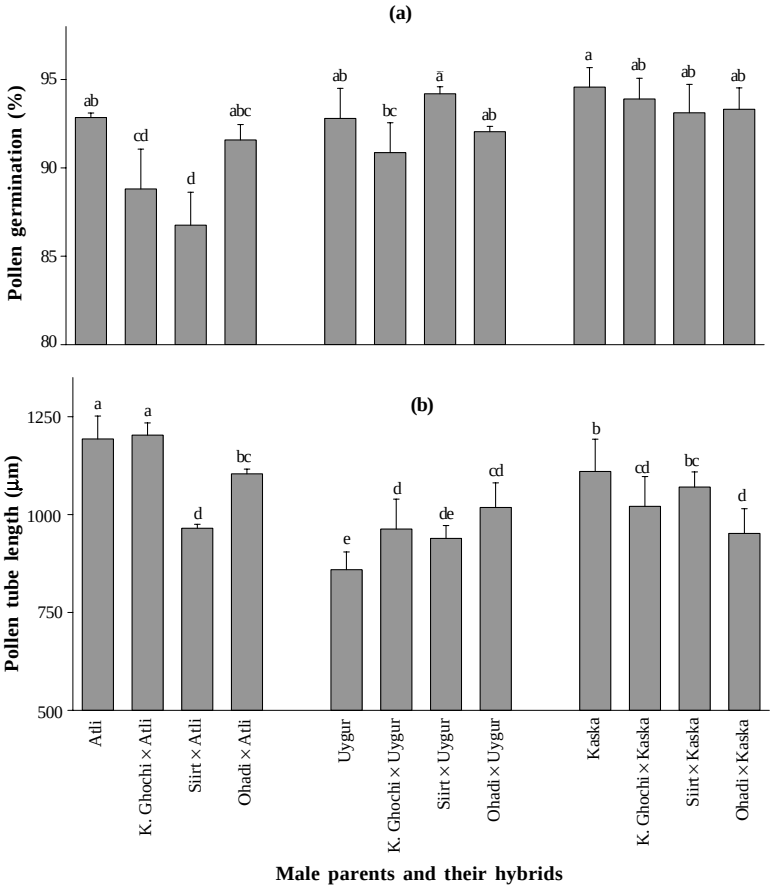

Figure 2. Mean and standard deviations of pollen germination percentage (a) and pollen tube length (b) of Atli, Uygur and Kaska male pistachio cvs and their hybrids.
The value of each hybrid was calculated as the mean of 3 hybrids. Two-factor analysis of variance and multiple mean comparisons Duncan test were carried out. Differences in letters above the bars indicate lineages that differ significantly at $p \leq 0.05$.

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