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Full Length Research Paper

In vitro pollen quantity, viability and germination tests in quince

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Quince (*Cydonia oblonga* Mill.) cvs. Ekmek, Eşme, Limon, Ege 2, Ege 22, Ege 25 and Quince-A rootstock pollens were collected in April from the unopened pink balloon-stage flowers on mature trees. The pollen amount was between 20063 pollen/flower ('Ege 25') and 11906 pollen/flower ('Limon') with hemacytometer. The lowest and highest pollen viability were determined from 79.8 (Quince A in day 14) to 97.8% ('Ege 2' in day 0) by IKI. The germination rates in different sucrose and boric acid concentrations in 1% agar changed from 0 ('Limon' in 0% sucrose) to 91.7% ('Ege 2' in 20% sucrose) and from 32.7 (Quince A in 0 mg/L boric acid) to 94.9% ('Ege 22' in 100 mg/L boric acid), respectively.

Key words: *Cydonia oblonga*, pollen, viability, germination.

INTRODUCTION

Quince (*Cydonia oblonga* Mill.) is a deciduous perennial angiosperm species that originated from North Iran, South Caucasia and Anatolia (Özçağıran et al., 2005). It is in the Pomoideae sub-family of Rosaceae family, relative of cultivated apple, pear and loquat. The diploid chromosome number of quince is 2n=2X=34. Turkey (96,282 t) is in the second place in world quince production, after China (105,000 t) (FAO, 2009). The quince fruit contains 83.8% water, 57 calorie, 0.4 g protein, 0.1 g fat, 15.3 g carbohydrate, 40 I.U. vitamin A and 197 mg K in 100 g edible portions (Westwood, 1993). Soft and juicy quince fruits are consumed raw. Jam and conservation in sugar are the other uses of the fruit. Quince is mainly produced by cuttings, mound layering and suckers (Hartmann et al., 1997). Although, quince is known as self-fertile, recent result shows self-incompatibility in different cross combinations (Nyéki et al., 2003).

Pollen studies have been conducted in pome, stone, nut and berry fruits. Eti (1991) studied pollen viability and germination rate in 10 cultivars of apple, pear, sweet cherry, sour cherry and plum, two cultivars in each species. The highest pollen germination ratio was obtained from 'Kırmızı Williams' pear (72%) in 20% sucrose in hanging drop method which was in agreement with TTC pollen viability test. Unfortunately, IKI test did not show any differentiation among different cultivars. Çetin (2006) studied pollen germination rate (0 to 15% sucrose in hanging drop) and pollen tube growth in pistil in 15 quince cultivars. Fruit set was compared among selfing and crossing combinations. Okusaka and Hiratsuka (2009) cultured pollen grains of Japanese pear (*Pyrus pyrifolia* Nakai) on a medium containing 10% sugar (sucrose, glucose or fructose), 1% agar and 0.01% boric acid (H_3BO_3) for pollen germination tests. The pollen was stained in acetocarmine solution for viability test. The pollen germination was completely inhibited at more than 3% fructose. It was observed that the Japanese pear pollen on fructose medium was similarly stained in acetocarmine as compared to that on sucrose. Albuquerque Junior et al. (2010) studied anther and pollen number in flowers and pollen germination in 12 apple cultivars in Brasil. The pollen germination rates were changed from 59.6 ('Suprema') to 73.2% ('Princesa'). Evrenosoğlu et al. (2010) found that the highest and the lowest pollen viability rate were 99.3 ('Williams') and 83.5% ('Limon') in IKI test in pear. The pollen germination rate ranged between 7.5 ('Ekşi') and 63.2% ('Ankara'). Sharafi (2010) searched for the best medium for pollen germination and pollen tube growth *in*

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vitro in five black hawthorn (*Crataegus douglasii*) and five red hawthorn (*Crataegus oxyacantha*) genotypes. The highest pollen germination was obtained from 10% sucrose + 0.005% boric acid medium (50.8%) solidified with 1.2% agar. Sharafi (2011) investigated pollen viability and pollen tube longevity in five different apple, pear and quince cultivars and genotypes in Iran. The highest pollen germination rate changed from 41.3 ('Ahar-almasi') to 94.1% ('Gizil-almasi') in apples, from 28.4 ('Shah-miveh' to 80.8% ('Sard-roud') in pear and from 37.2 (CO2) to 65.3% ('Gortoune-Sfahan') in quince. Yolaçtı (2006) analyzed the effect of different temperature regimes on the pollen germination and length of pollen tubes in quince. The highest pollen germination was obtained from control treatment (22 ± 1°C) with 91.47%. Increased and decreased pollen germination rates were observed at 5°C (1.67%), 10°C (7.72%), 15°C (22.65%), 20°C (22.20%), 25°C (74.99%), 30°C (3.29%) and 35°C (1.23%).

When literature was searched, information on the pollen number of quince was not found. The pollen viability and germination tests have not been performed in 'Ege 2' cultivar and a dwarfing rootstock, Quince A, yet. The aim of this study was to determine pollen amount of registered quince cultivars and a rootstock for the first time, and determine quantity, viability and germination rate of pollen in six certified quince cultivars and a rootstock.

MATERIALS AND METHODS

Quince flowers from 'Ekmek', 'Eşme' and 'Limon' cultivars, and Quince A rootstock were collected in April from trees located at Fruit Collection Orchard Kocakır-1 parcel, Department of Horticulture, Faculty of Agriculture, Adnan Menderes University, in Aydin planted in 1995. 'Ege 2', 'Ege 22' and 'Ege 25' flowers were collected from Aegean Agricultural Research Institute, Menemen, Izmir. The unopened flowers were collected in pink balloon stage in April. After petals and pistil were removed in the laboratory, anthers were spread over tissue paper under an incandescent lamp on a table overnight. In the next day, the pollen gains were collected in a small glass vial.

Pollen quantity

Twenty flowers were used in this study. The flowers were divided in two groups. Each group contained anthers from 10 flowers in small glass vials. The vials containing anthers were incubated in their lids open in front of a window receiving sunlight for the anthers to dehisce for several days (Eti, 1990; Günver-Dalkılıç and Dayı-Doğru, 2011). Then, 3 ml distilled water and little amount of diluted detergent was added into each vial. The anthers in a suspension were thoroughly crashed with a glass rod. A drop was placed on a two-counting area containing Thoma (hemacytometric) slide (0.1 mm in depth) to where a special cover slip was replaced. The pollens were placed on randomly chosen four large squares in each counted area with two replicates representing each group of flowers in vials. The average pollen grain amount per flower (n) was determined with the formula:

n = Pollen count × 3000 mm^3 / 0.1 mm / 10 flowers

Pollen viability test

IKI (0.5 g iodine (I) + 1 g potassium iodide (KI)) solution was prepared by dissolving I and KI in 100 ml distilled water. The pollen viability counts were made within few minutes in light microscope after pollens were placed on IKI solution. While the pollen grains stained with dark (brown in color) were counted as live, the pollen grains stained with light (pale yellow in color) or not stained at all were counted as dead. Two microscope slides were used. Four areas were counted in each slide (Eti, 1991; Günver-Dalkılıç and Dayı-Doğru, 2011). Pollen viability test were done in three different times. The first viability test was performed the same day (day 0) when the pollen grains were collected. The second and third viability tests were done in 8 and 14 days, respectively after pollen collection. Pollen was stored at 4°C until used.

Pollen germination tests

Concentrated agar (1%) in Petri dish containing different concentrations of sucrose (0, 5, 10, 15 and 20 mg/L) were used during the first pollen germination experiment. Then, in the second pollen germination test, 15% sucrose concentration was chosen. Pollens were dispersed on a medium containing 15% sucrose and different concentrations (0, 25, 50 and 100 mg/L) of boric acid solidified with 1% agar. Germinated pollen grains were count after 24 h (Günver-Dalkılıç and Dayı-Doğru, 2011).

Data analysis

The experiment was designed as completely randomized design (CRD) with four (pollen quantity, germination and boric acid tests) to eight (IKI test) replications. Statistical analyses were performed by using ANOVA procedure of TARİST computer package program. The differences among means were determined by Duncan's multiple range test ($P<0.05$). The percentage data were used by transferring to arc sinus. The original mean values are presented in the tables.

RESULTS AND DISCUSSION

Pollen quantity

After counting the pollen grains in hemacytometer, a significant difference ($P<0.05$) was found among quince cultivars studied (Figure 1). While 'Ege 25' (20063) had the highest pollen number, 'Eşme' (13219) and Limon (11906) had the lowest. This difference could be observed due to either genetic pattern and/or environmental growing conditions of the quince cultivars. This current experiment presented the first report for pollen number in the studied quince cultivars. Pırlak and Güleryüz (2005) found the pollen production of five cornelian cherry (*Cornus mas* L.) cultivars to be between 7082 and 13500 pollen/flower. Pollen production per flower was from 446448 to 992875 in pomegranate (*Punica granatum* L.) (Derin and Eti, 2001). The pollen number was from 50249.89 pollen/flower ('Red Chief') to 71675.44 pollen/flower ('Fuji') in apple (Öztürk, 2005). Albuquerque Junior et al. (2010) detected changing amount of pollen from 53658 pollen/flower ('Duquesa') to 103700 pollen/flower ('Imperatriz') in apple.

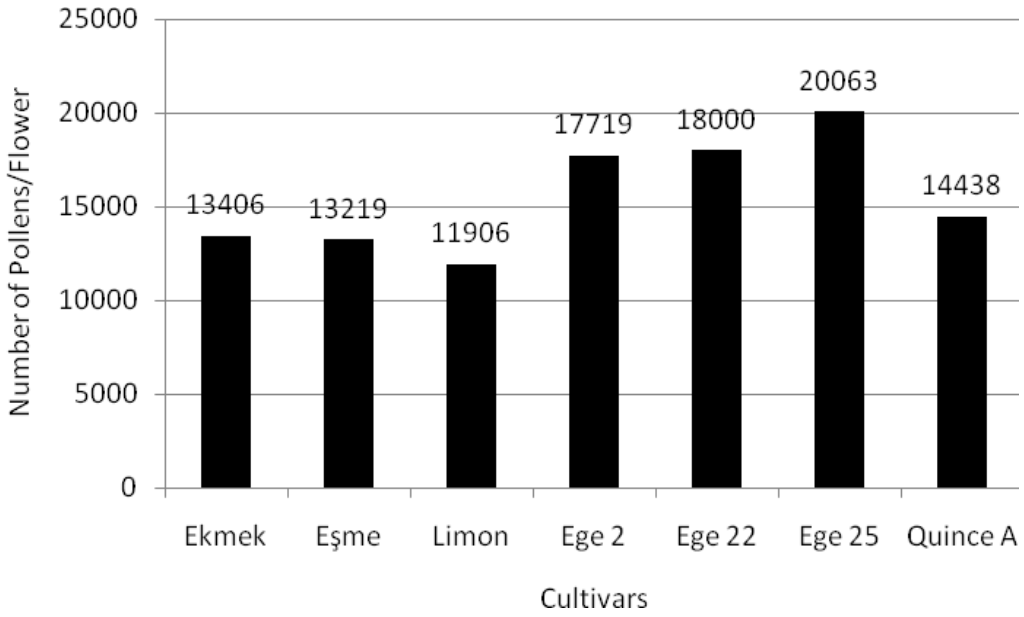

Figure 1. Number of pollens per flower in the quince cultivars.

Table 1. Viability of quince pollen in aging using 1% IKI (%).

Day	Cultivar							Mean
	Ekmek	Eşme	Limon	Ege 2	Ege 22	Ege 25	Quince A	
0	94.7	97.7	95.8[a]	97.8[a]	98.1	90.8[a]	92.1[a]	95.3
8	94.9	95.9	95.2[ab]	90.8[b]	95.8	82.9[b]	90.5[a]	92.3
14	97.1	97.1	91.3[b]	92.7[ab]	97.2	83.6[b]	79.8[b]	91.3
Mean	95.5	96.9	94.1	93.8	97.0	85.8	87.5	
P<0.05	n.s.	n.s.	*	*	n.s.	*	*	

*: The same letters show no difference among means in each column.

Pollen viability test

Using IKI, there were significant differences among the days of pollen stored at 4°C (Table 1). The pollen viability changed according to the cultivars. The pollen viability rate of 'Limon' (95.8%), 'Ege 25' (90.8%) and Quince A (92.1%) was high in the beginning of the experiment (day 0). The viability of the pollen grains was decreased to 91.3% in 'Limon', 83.6% in 'Ege 25' and 79.8% in Quince A in the day 14 of the experiment. The pollen viability of cornelian cherry genotypes changed from 73.02 to 86.79% in IKI test (Pırlak and Güleryüz, 2005). The pollen viability ratio changed from 83.5 ('Limon') to 99.3% ('Williams') for pear cultivars in IKI (Evrenosoğlu et al., 2010).

Pollen germination tests

The pollen germination rate in different sucrose concentrations changed, depending on different quince cultivars (Table 2). The highest pollen germination in the agar-containing medium containing 10% sucrose was obtained from 'Ekmek' (78.3%). The highest pollen germination ratio in 15% sucrose was obtained from 'Limon' (64.6%), 'Ege 22' (86.0%) and Quince A (53.9%). The 20% sucrose gave the highest germination ratios in 'Eşme' (75.5%), 'Ege 2' (91.7%) and 'Ege 25' (83.6%) cultivars. While 'Ege 22' could germinate as high as 67.8%, pollen grains of 'Limon' did not germinate in the medium without sucrose. Çetin (2006) reported that the highest pollen germination was obtained at 5% sucrose in 'Ekmek' (34.95%), 10% sucrose in 'Eşme 14' (83.60%) and 'Limon' (30.05%), 15% sucrose in 'Ege 22' (87.50%), 0% (control, H_2O) in 'Ege 25' (50.60%) quince cultivars. The results of the current study showed higher pollen germination rates in 'Ekmek', 'Limon' and 'Ege 25' at a higher sucrose concentration, and lower rates in 'Eşme' at a higher sucrose concentration. Similar results were obtained in 'Ege 22' at the same sucrose concentration.

Table 2. Germination rate of quince pollen in different sucrose concentrations (%) (1% agar).

Sucrose (%)	Cultivar							Mean
	Ekmek	Eşme	Limon	Ege 2	Ege 22	Ege 25	Quince A	
0	38.8^{c}	51.4^{b}	0.0^{d}	32.0^{d}	67.8^{b}	20.3^{c}	8.2^{c}	31.2
5	24.5^{d}	45.8^{b}	14.8^{c}	44.5^{c}	74.4^{ab}	36.8^{b}	27.1^{b}	38.2
10	78.3^{a}	66.7^{a}	42.3^{b}	55.7^{c}	83.0^{a}	69.0^{a}	45.1^{a}	62.9
15	63.8^{ab}	69.7^{a}	64.6^{a}	77.8^{b}	86.0^{a}	76.0^{a}	53.9^{a}	70.2
20	63.1^{b}	75.5^{a}	60.3^{a}	91.7^{a}	84.8^{a}	83.6^{a}	46.1^{a}	72.2
Mean	53.7	61.8	36.4	60.3	79.2	57.1	36.1	
P<0.05	*	*	*	*	*	*	*	

*: The same letters show no difference among means in each column.

Table 3. Effect of boric acid on germination rate of quince pollen (1% agar + 15% sucrose).

Boric acid (mg/L)	Cultivar							Mean
	Ekmek	Eşme	Limon	Ege 2	Ege 22	Ege 25	Quince A	
0	69.7	76.4	67.9^{a}	85.1^{b}	87.8^{b}	71.7^{b}	32.7^{c}	70.2
25	75.8	70.1	48.7^{b}	82.5^{b}	90.7^{ab}	82.9^{a}	75.7^{a}	75.2
50	82.0	71.8	75.5^{a}	94.8^{a}	88.5^{b}	84.0^{a}	43.1^{bc}	77.1
100	73.9	74.5	79.3^{a}	87.0^{ab}	94.9^{a}	82.2^{a}	52.0^{b}	77.7
Mean	75.4	73.2	67.9	87.3	90.5	80.2	50.9	
P<0.05	n.s.	n.s.	*	*	*	*	*	

*: The same letters show no difference among means in each column.

When pollens were stored at 22 ± 1°C, 91.47% of pollen germination could be obtained (Yolaçtı, 2006). The highest pollen germination rates were between 37.2% in CO_2 genotype and 65.3% in 'Gortoune-Sfahan' cultivar in quince (Sharafi, 2011). The germination rates changed from 7.02 (0% sucrose) to 57.54% (15% sucrose) in 'Red Chief' and 'Granny Smith', respectively in agar plate method (Öztürk, 2005). The highest pollen germination ratio was obtained from 'Kırmızı Williams' pear (72%) in 20% sucrose (Eti, 1991). The pollen germination ratio ranged from 7.5 ('Ekşi') to 63.2% ('Ankara') in pear cultivars (Evrenosoğlu et al., 2010). Albuquerque Junior et al. (2010) reported pollen germination rates between 59.6 ('Suprema') and 73.2% ('Princesa') in apple. The highest pollen germination rate in Japanese pear (36.0%) was recorded in 5% sucrose and 1% agar medium containing 0.01% boric acid (Okusaka and Hiratsuka, 2009). The highest pollen germination rate in cornelian cherry was obtained from 15 to 20% sucrose and 0.03% boric acid in hanging drop method (Pırlak and Güleryüz, 2005). Derin and Eti (2001) stated that the highest pollen germination was observed in 1% agar + 10% sucrose in agar plate method in pomegranate.

When different boric acid containing 1% agar + 15% sucrose media were tested on the germination rate of quince pollen grains, different quince cultivars showed different pollen germination rates (Table 3). 'Ekmek' and 'Eşme' did not statistically show any difference to boric acid concentrations. The highest germination rates were obtained from 25 mg/L boric acid in Quince A (75.7%), from 50 mg/L in 'Ege 2' (94.8%) and 'Ege 25' (84.0%), and from 100 mg/L in 'Limon' (79.3%) and 'Ege 22' (94.9%). Quince A pollen had the lowest germination rate (32.7%) in the control (0 mg/L) group. Sharafi (2010) obtained the highest pollen germination from 10% sucrose + 0.005% boric acid medium (50.8%) solidified with 1.2% agar in hawthorn.

In conclusion, the cultivars used in this study showed different pollen number, viability and germination rates in different *in vitro* media. The pollen amount, viability (IKI) and germination rates (sucrose and boric acid) ranged from 20063 pollen/flower ('Ege 25') to 11906 pollen/flower ('Limon'), from 79.8 (Quince A in day 14) to 97.8% ('Ege 2' in day 0), from 0 ('Limon' in 0% sucrose) to 91.7% ('Ege 2' in 20% sucrose), and from 32.7 (Quince A in 0 mg/L boric acid) to 94.9% ('Ege 22' in 100 mg/L boric acid), respectively. This study shows that although quince flowers contain comparable few pollen grains, their viability and germination can be high. These results are helpful for evaluating quince germplasm and their pollen characteristics to be used in cross breeding programs.

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