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Full Length Research Paper

Determination of pollen viability, germination ratios and morphology of eight apricot genotypes

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Turkey is a leading apricot producing country, with the majority of dried apricot production centered in Malatya. Currently, there are breeding programs to develop superior apricot cultivars for several different utilizations. Determining the components of reproduction biology is critical for optimizing yields from apricot orchards and is therefore important for breeding programs. In this study, the pollen viability and germination ratios were determined for eight apricot cultivars. The genotypes tested included a local cultivar (Kabaaşı), foreign-origin cultivars (Roksana and Canino), and selections from the İnönü University program (Levent, Özal, Akyürek, 44-2005-01, 44 K 07). The results indicated that viable, semi-viable and dead pollen rates differed among cultivars, where Roksana had the least amount of viable pollen (41.5%). The genotypes had their highest germination rates at 20°C, whereas Roksana and Levent had the lowest germination rates (46.8 and 48.5%). The germination rates were also affected by sucrose concentrations, and media containing a 15% sucrose concentration had the highest germination rates, while Roksana again had the lowest germination rate (36.4%). While the differences in anther number/flower were not significantly different among genotypes, there were significant differences in pollen number for both anther and flower bases. 44-2005-01 and Canino had the highest pollen numbers. Pollen morphology was also evaluated using a Scanning Electron Microscope (SEM). Although some size and index differences were measured, the pollen of the genotypes was generally similar in morphology. The findings provide important insight into improving our understanding of apricot reproduction biology.

Key words: Flower biology, malatya, *Prunus armeniaca*, scanning electron microscope.

INTRODUCTION

Apricot (*Prunus armeniaca* L.) is a temperate zone fruit species that can be grown in many regions of the world under climatically diverse conditions. These regions include Siberia, Central Asia, China and Japan (Asma, 2007). Apricot belongs to the Roseceae family and has perfect flowers. Flowers have 5 sepals, 5 petals, 25-30 stamens and 1 pistil. Each bud has a single flower that emerges before the leaves. The petals are red-pink in color during the early stages; however, they become white upon full flowering. Sepals are dark red in color and cover the flower. The flowering dates may deviate 8-10 days among cultivars, while even more deviation (15-20 days) can be observed for a single cultivar across different years. Flowering periods may differ based on cultivar and climate conditions, but usually last 5-8 days. This period may be extended up to 15 days during unusually cool spring weather. Flowering starts from the lower parts of trees and continues towards the upper regions (Asma, 2000).

Flowering and fertilization are critical for fruit set in stone fruits. There are several barriers for fertilization. Poor or malfunctioning pollen production is among these barriers in apricot genotypes. It is known that internal and external factors limit pollen production, viability and germination rates (Özbek, 1993). For example, low temperature during flowering reduces pollen germination rate and inhibits pollen tube growth, thereby resulting in poor fertilization and yield (Vitagliano and Viti, 1989).

Because of its importance in fertilization and therefore production, several studies have investigated pollen viability and germination for apricot cultivars. For example, Bolat and Güleryüz (1994) studied the effect of several different types of media on pollen viability and germination rates for Hasanbey, Karacabey, Şalak, Şekerpare

and Tokaloğlu. They found that the cultivars tested had significant differences in pollen viability and germination rates. Hasanbey and Karacabey had the highest viability and germination rates. Paydaş et al. (2006) studied the pollen quality and production of 62 apricot cultivars. Pollen viability differed among cultivars; 31 K 03 had the highest viability (89%) while Tokaloğlu had the lowest. Pollen germination rates ranged from 34 to 79%.

Determining pollen morphology and comparing the differences among species and cultivars improves our understanding of reproductive biology. SEM is an excellent tool for this purpose. For example, using SEM Günen et al. (2007) determined the pollen morphology and revealed critical differences among apple, pear, quince, apricot, peach, plum, almond, walnut, chestnut, pomegranate and persimmon.

Apricot is particularly prone to erratic fruit set. Several reasons, such as adverse weather conditions at flowering or pollination failure, can explain this behavior in some circumstances. However, poor fruit set with no apparent causes is frequently observed. The term “flower quality” is used to express something inherent to the flower that is reflected in the subsequent fruit set (Rodrigo et al., 2006).

Knowledge of reproduction biology, particularly pollen production and quality, is critical for the newly-developed cultivars. The objective of this study was to compare apricot genotypes from distinct backgrounds for their pollen viability, germination rates and morphology by SEM.

MATERIALS AND METHODS

Plant materials

The study was conducted using eight apricot cultivars grown at the Apricot Research Center at İnönü University. The trees were eight years old, grafted onto seedling-grown apricot rootstocks and planted in 7 x 7 m plots. Five of the genotypes studied were from the apricot breeding programs of İnonü University (Levent, Özal, Akyürek, 44-2005-01, 44 K 07). Kabaaşı is a commonly grown local apricot cultivar with good quality parameters. The genotypes tested also included two foreign-origin cultivars, Roksana and Canino, which perform well under Malatya conditions. Roksana has especially excellent adaptation to Malatya conditions. It has large, high quality fruits that are very suitable for table consumption. However, information on its pollen production and quality is lacking, although some information regarding the cultivar’s performance in Malatya can be found in Asma (2000), Asma and Birhanlı (2004) and Asma and Öztürk (2005). For the viability and germination test, approximately 300 flowers were randomly collected from each genotype. The anthers from the flowers sampled at the popcorn stage were separated from other parts and kept at 22°C for 24 h. The pollen was stored at 4°C prior to experiments.

In vitro pollen viability test

The viability of the pollen was determined on 1% 2,3,5-triphenyl tetrazolium chloride (TTC). This assay is referred to as TTC and is commonly applied in apricot research (Eti, 1990). For this assay, two lamella for each genotype and three regions of each lamella were investigated; viable, semi-viable and dead pollen numbers and their percentages were determined. Viable pollen was dyed in red, semi-viable pollen dyed in light red-pink and dead pollen not dyed at all. Examples of viable, semi-viable and dead pollen are presented in Figure 1.

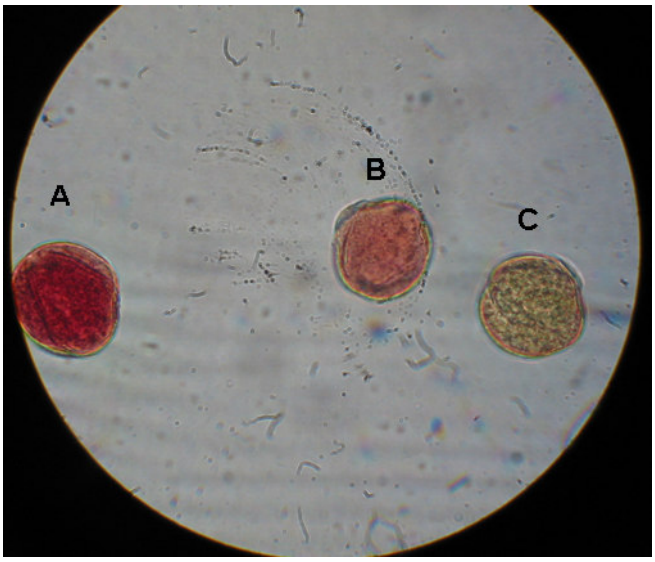

Figure 1. Views of viable (A), semi-viable (B) and dead pollen (C).

In vitro pollen germination test

The germination tests were conducted on Petri dishes with 1% agar medium containing either 10, 15 or 20% sucrose (Eti, 1990). The Petri dishes were kept at 20°C. To determine the effect of temperature, one of the treatments (1% agar + 15% sucrose) was kept at 10, 20 or 30°C. For each genotype, two Petri dishes and three regions in each Petri were investigated, and percentages of germination were determined.

Determination of pollen production

Pollen production was assayed by the Hemocytometric Lamella Method (Eti, 1990). The pollen was obtained as described above. Pollen from each flower was put into a jar and 2 ml of water was added to the jar. The suspension was dropped into counting compartments on hemocytometric lamella. The numbers of pollen were determined using the relevant rate calculation.

Determination of pollen morphology by SEM

Pollen morphology was determined by using SEM. Pollen was covered under pressure on a vacuum evaporator. To avoid dispersion of the pollen under pressure, clear bands were placed on the structure of the microscope and pollen were allowed to stick to them. The pollen was viewed under these conditions using SEM, and the samples were photographed. The pollen dimensions were measured for 10 pollen of each cultivar.

Statistical analysis

The data were analyzed using SAS procedures (SAS, 1990). The variables expressed as percentages were normalized by a root square arcsin transformation. The means and standard deviations

Table 1. Pollen viability rates of apricot (*Prunus armeniaca*) genotypes.

Genotype	Rate (%)		
	Viable	Semi-viable	Dead
Levent	52.7 bc*	22.7 a	24.6 b
Özal	72.5 ab	19.4 a	8.1 c
Akyürek	59.8 b	14.9 b	25.3 b
44–2005–01	66.3 ab	17.8 a	15.9 bc
44 K 07	62.6 b	20.3 a	17.1 bc
Roksana	41.5 c	19.6 a	38.9 a
Canino	77.2 a	7.4 bc	15.4 bc
Kabaaşı	63.9 b	14.1 b	22.0 b

*Different letters indicate significant differences based on LSD conducted at 5%.

Table 2. Germination rates of apricot (*Prunus armeniaca*) genotypes at various temperature treatments.

Genotype	Germination rate (%)		
	10 °C	20 °C	30 °C
Levent	41.6 c*	48.5 c	35.3 bc
Özal	59.1 b	76.7 a	44.9 b
Akyürek	52.7 bc	61.6 b	43.2 b
44–2005–01	59.8 b	71.5 ab	55.3 a
44 K 07	61.3 b	69.4 ab	57.6 a
Roksana	29.3 d	46.8 c	26.1 c
Canino	71.1 a	79.8 a	39.4 bc
Kabaaşı	52.6 bc	68.6 ab	48.9 ab
Mean	53.4 B**	65.4 A	43.8 C

*Different letters in small caps indicate significant differences among genotypes based on LSD conducted at 5%.
**Different letters in caps indicate significant differences among treatments based on LSD conducted at 5%.

were calculated using the TABULATE procedure. The GLM procedure was used to calculate analysis of variance tables, where significant differences between means were determined by the least significant difference (LSD) method at 5%.

RESULTS AND DISCUSSION

Pollen viability rates significantly differed among the genotypes tested (Table 1). The highest viable pollen rate was recovered from Canino (77.2%), while the lowest from Roksana (41.5%). The semi-viable pollen ratio had less variability when compared to the viable and dead pollen rates. Kabaaşı had the lowest (14.1%) and Levent had the highest rates (22.7%). The highest dead pollen rate was found in Roksana (38.9%) while the lowest was from Özal (8.1%). Genotypic differences among apricot cultivars for pollen viability have been previously reported. For example, Yaegaki et al. (2002) studied pollen quantity in 59 fruiting and 23 flowering cultivars of apricot and found that approximately 60% of the fruiting cultivars showed high pollen quantity, while approximately 70% of the flowering cultivars showed little or no pollen. They also revealed significant correlation coefficients among pollen quantity, rate of stained pollen and rate of germinated pollen. Variation of pollen quality among Turkish apricot cultivars has also been reported by Paydaş et al. (2006).

The temperature significantly affected germination rates for the apricot genotypes tested (Table 2). All genotypes had their highest rates at 20°C (65.4%) followed by 10°C (53.4%). The lowest germination rates were recovered from 30°C (43.8%). At 20°C, the germination rates ranged from 48.5% (Levent) to 79.8% (Canino). The same genotypes had the highest and lowest means (41.6 and 71.1%) at 10°C. However, different patterns were obtained at 30°C. The highest germination rates were recovered from the selections 44-2005-01 and 44 K 07 (55.3 and 57.6%). Roksana had the lowest rate at 30°C (26.1%). Pırlak and Bolat (2001) studied how different temperature treatments (5, 10, 15 and 20°C) affected pollen germination of apricot and sweet cherry cultivars. The treatments significantly differed and the highest germination rates were recovered from 15 and 20°C. Using 'Hasanbey', 'Mahmudun Erigi', 'Karacabey', 'Salak' and 'Sekerpare', Pırlak (2002) studied the germianation rates at the same temperature treatments. The highest germination rates were obtained from 15 and 20°C as well. Similar results were also reported in Pırlak and Bolat (1999). Other stone fruits have also produced similar responses to the temperature treatments (Hedhly et al., 2003; Hedhly et al., 2005).

Similar to temperature treatments, sucrose treatments also resulted in significant differences in mean numbers (Table 3). The highest means were obtained from 15% sucrose treatment (64.5%). 20% sucrose had a higher germination rate (54.4%) when compared to 10% (47.3%). Regardless of the sucrose concentrations, Roksana had the lowest germination rates (17.1, 36.4 and 22.5% for 10, 15 and 20%, respectively). The fact that this experiment was conducted at 20°C and Roksana was in the mean group with the lowest germination rate at 20°C may be related to these results. At 20% sucrose, Özal (68.2%) and Canino (66.3) had the highest germination rates while 44-2005-01 and Kabaaşı had the highest means at 10% sucrose treatment. Mahanoglu et al. (1995) studied pollen production, viability and germination on Precoce de Colomer, Beliana, Priana, Feriana and Canino. They found that the highest germinations rates were recovered from 15 and 10% sucrose concentrations. Polat and Pırlak (1999) found that a 15% sucrose concentration gave the highest germination rates for Şekerpare apricot. Indeed, from their previous studies Polat and Pırlak (1999) and Pırlak (2002) considered a 15% sucrose concentration as the most suitable sucrose concentration for pollen germination, and conducted their temperature treatments on this concentration. Therefore,

Table 3. Germination rates of apricot (*Prunus armeniaca*) genotypes at various sucrose treatments.

Genotype	Germination rate (%)		
	1% Agar, 10% sucrose	1 % Agar, 15% sucrose	1% Agar, 20% sucrose
Levent	45.1 b*	53.3 c	50.9 b
Özal	43.9 b	78.4 a	68.2 a
Akyürek	41.6 b	58.8 bc	52.7 b
44–2005–01	61.3 a	69.0 b	58.7 b
44 K 07	54.9 b	70.2 b	62.3 ab
Roksana	17.1 d	36.4 d	22.5 c
Canino	55.4 ab	81.9 a	66.3 a
Kabaaşı	59.3 a	68.2 b	53.5 b
Mean	47.3 C	64.5 A	54.4 B

Table 4. Pollen number for apricot (*Prunus armeniaca*) genotypes.

Genotype	Anther number / flower	Pollen number / anther	Pollen number / flower
Levent	28.55 ^{ns}	2.215 bc	63.238 bc
Özal	29.10	2.114 bc	61.517 bc
Akyürek	28.90	1.211 cde	34.980 e
44–2005–01	31.05	3.347 a	103.925 a
44 K 07	28.60	1.525 cd	43.615 de
Roksana	33.20	1.916 cd	63.611 bc
Canino	32.95	3.042 a	100.234 a
Kabaaşı	30.20	2.517 ab	76.013 b

our results are in general agreement with these studies.

Anther numbers were not significantly different among the genotypes (Table 4). However, pollen numbers expressed as both “per anther” and “per flower” were different for genotypes. Pollen number/anther was highest for 44-2005-01 (3.347) and Canino (3.042), while Akyürek had the lowest mean (1.211). The separations of the genotypes to the mean groups slightly changed for pollen number/flower; however, the same genotypes had the highest and lowest means. Genotypic differences for pollen production for apricot cultivars have previously been reported by Alburquerque et al. (2004), Davarynejad et al. (1995) and Mahanoğlu et al. (1995).

Pollen morphology was examined by SEM. Sample views from several genotypes are presented in Figure 2. Flower morphology results are presented in Table 5. Overall, the pollen morphology was similar among the genotypes. All genotypes were classified as having monad pollen that were iso-polar symmetric. The shapes of the pollen were sub-oblate and the pollen edges were circular. Pollen aperture was classified as tritem, while the pore shapes were colpat. The pollen from all genotypes was classified as large and the actual dimensions ranged from 50.18 to 60.83 µ (width and length, respectively). The indexes were around 2, where Levent (1.86) and 44-2005-01 (2.20) had the lowest and highest values, respectively.

In this study, we determined the pollen viability and pollen germination rates in various conditions for eight apricot cultivars with diverse genetic background. These genotypes represent the breeding program at Inönü University. The results presented here are important for improving our understanding of apricot reproduction biology, not only at Inönü University but throughout the world.

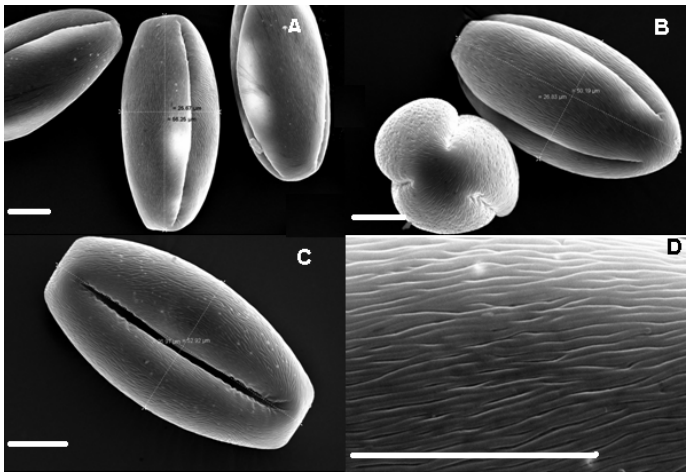

Figure 2. View of pollen from different cultivars (A = Roksana, B = Levent, C = Özal, D = Canino). Bars indicate 10 µ.

Table 5. Several pollen characteristics of selected apricot (Prunus armeniaca) genotypes.

Characteristics	Levent	Özal	Akyürek	44-2005-01	44 K 07	Roksana	Canino	Kabaaşı
Pollen release	Monad	Monad	Monad	Monad	Monad	Monad	Monad	Monad
Symmetry	iso-polar symmetric	iso-polar symmetric	iso-polar symmetric	iso-polar symmetric	iso-polar symmetric	iso-polar symmetric	iso-polar symmetric	iso-polar symmetric
Size (µ)	Large	Large	Large	Large	Large	Large	Large	Large
	50.68-57.02	52.09-55.40	50.83-56.88	54.11-60.83	52.36-59.55	53.11-56.84	54.14-58.82	50.18-55.24
Index	1.86	1.96	1.92	2.20	2.19	2.07	2.15	1.98
Shape	Sub-oblate	Sub-oblate	Sub-oblate	Sub-oblate	Sub-oblate	Sub-oblate	Sub-oblate	Sub-oblate
Pollen edge	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Pore aperture	Tritrem	Tritrem	Tritrem	Tritrem	Tritrem	Tritrem	Tritrem	Tritrem
Pore shape	Colpat	Colpat	Colpat	Colpat	Colpat	Colpat	Colpat	Colpat

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