



Full Length Article

Determination of Pollen Production and Quality attributes of some Almond Cultivars (*Prunus dulcis*) and Selected Wild Almond (*Amygdalus orientalis*) Genotypes

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ABSTRACT

This study was carried out to establish pollen production, viability and germination ratios for five almond genotypes of *Amygdalus orientalis* Mill. (Orientalis 5, Orientalis 6, Orientalis 7, Orientalis 9 & Orientalis 10) and four cultivars of *Prunus dulcis* Mill., (Nonpareil, Texas, Ferragnes & Ferraduel). For determination of pollen viability of selected almond genotypes, 2,3,5 Triphenyltetrazolium chloride and Fluorescent diacetat tests were made. Pollen germination ratios *in vitro* conditions were determined with petri dishes methods. For *A. orientalis* genotypes, pollen viability ratios according to the TTC test were found to be 61.80% and 72.88% in 2006 and 2007, respectively whereas the same ratios according to the FDA test carried out in 2006 and 2007 were 63.44% and 57.50%, respectively. The number of anthers in one flower, the number of pollen in one anther and the number of pollen in one flower were found higher in cultivars, whereas these traits turned out to be lower in *A. orientalis* genotypes. 'Orientalis 6' was notable pollen viability, the number of anthers in one flower, the number of pollen in one anther and the number of pollen in one flower, while the highest pollen germination percentage was recovered from 'Orientalis 9' had in 2006 and from 'Orientalis 5' in 2007. Morphological homogeneity (%) was found to be high in all orientalis types both experimental years. The results obtained with *A. orientalis* genotypes proved that they could well be employed as pollinizer for hybrid improving work to be made with almond cultivars. © 2012 Friends Science Publishers

Key Words: Genetic resource; Almond species; Pollen; Viability; Germination; Production

INTRODUCTION

Turkey with its suitable ecology for horticultural plants' cultivation has very rich genetic resources due to the presence at the junction of migration road and long lasting civilization living in the Anatolia. As occurred for many species, Turkey is the genetic centers for almond species as well (Demir, 1990; Ağaoğlu *et al.*, 1995). In addition, *A. orientalis* (Mill), *A. turcomanica* (Lincz), *A. fenzliana* (Fritch) Lipsky, *A. trichamygdalus* (Hand-Mazz) Woronov, *A. arabica* (Olivier), and *A. webbii* (Spach) are naturally grown in Turkey (Browicz & Zielinski, 1984). Present knowledge indicates that plant genetic resources are the backbone of agriculture, which play a positive and unique role in the development of new cultivars including the restructuring of existing ones (Malik & Singh, 2006).

Unfavorable environmental and ecological conditions require breeding resistant lines or cultivars. For this purpose, *A. davidiana* (Carr.) and *A. mira* Koehne are used for improving disease resistance of peach and breeding new cultivars with higher yield capacity; *A. orientalis* for increasing cold resistance and breeding self pollinated

individual; *A. bucharica* Korschinsky for increasing cold resistance, breeding self pollinated individuals and canopy vigor control. Other utilizations of the wild almond includes *A. kuramica* Korschinsky for breeding self pollinated and disease resistance for almond cultivars; *A. webbii* (Spach) Vieh. for breeding self pollinated and individuals and controlling canopy growth; and *Prunus dulcis* (Mill.) D.A. Webb for breeding studies of peach to obtain resistant individuals to disease and pests (Denisov, 1988; Kester *et al.*, 1991; Kester & Gradziel, 1996; Gradziel *et al.*, 2001).

Because of these advantages, determination of pollen viability, pollen germination properties of *A. orientalis* genotypes selected from Niğde (Central Anatolia) are important for almond breeding studies. There are some self pollinated and infertile almond species as observed for apricot and cherry species. Pollination is very critical for fruit set in infertile species. Knowledge about the pollination properties of species to be used for hybridization studies is very important.

The aims of this study were: (i) to determine pollen viability and germination ratio and pollen production in *Amygdalus orientalis* Mill, genotypes and (ii) to compare

their properties to the standard almond cultivars.

MATERIALS AND METHODS

Plant material: The genotypes of *A. orientalis* were collected from natural grown almond populations of Ulukışla (Tabaklı village, Niğde city) district (Bayazit, 2007). Collected genotypes were the natural populations grown of the region and they were not given any cultural applications. The age of genotypes ranged between 10-21 years. Almond cultivars with 10 years age of cultivated on seed rootstock were obtained from Pozantı Agricultural Experimental Station, Çukurova University, were also used in the experiment (Table I). Pollen of selected almond species *A. orientalis* ('Orientalis 5', 'Orientalis 6', 'Orientalis 7', 'Orientalis 9', 'Orientalis 10') and *P. dulcis* ('Nonpareil', 'Texas', 'Ferragnes', 'Ferraduel') were used in the experiment. Balloon staged flowers of the almond accessions were collected and anthers were extracted and stored at room temperature (25°C) for one day. Pollen viability and germination percentages tests were then performed as described by Eti (1990).

In vitro pollen viability test: TTC and FDA tests were used to determine the pollen viability rate of wild almond species and cultivars. In TTC test, 0.2 g triphenyltetrazolium chloride (TTC) and 12 g sucrose were dissolved in 20 mL distilled water (Norton, 1966). One or two drops of TTC solution was put on a clean micro slide and pollen grains were sprinkled on these drops with a brush. Then, the drop was carefully covered by a cover glass without trapping air and kept for 2 h at ambient conditions. For this assay, two lamella for each genotypes and six regions of each lamella were investigated. Pollen grains were examined using a fluorescence microscopy (Euromex Microscopes, Holland). The viability of pollen was scored according to staining level: pollen with bold red color as viable, with light red color as semi-viable and with yellowish-green color or colorless as non-viable.

2 mg of fluorescent diacetat (FDA) were dissolved in 1 mL of absolute acetone then mixed with 9 mL of sucrose solution containing 1.71 g sucrose that was added drop by drop till the mixture become turbid (Heslop-Harrison & Heslop-Harrison, 1970). For the FDA test, as described for TTC test, two drops of FDA solution were put on a clean slide for each pollen sample. Five minutes after dusting out the pollen grains on these drops by means of a brush, the alive pollens were counted under fluorescence microscopy. The pollen with bright green color was alive, whereas pale green colored one was non-viable (Eti *et al.*, 1998).

In vitro pollen germination test: The pollen germination tests were conducted on petri dishes with 1% agar medium containing 0, 10, 15 or 20% of sucrose (Eti, 1990). After careful decantation of media into petri dishes, then pollen was sown at room temperature. For each genotype, two petri dishes and six regions in each petri were investigated using a fluoresane microscope (Euromex Microscopes, Holland),

and percentages of germination were determined.

Pollen production rate: Number of pollen grains per flower was determined using the hemacytometric method (Eti, 1990). The morphological homogeneity level of pollen was also investigated with the same method. To obtain the average number of each component, 30 randomly selected flowers from each tree were collected. All the flowers were counted and then the average number of the above components of each almond species and cultivars was determined.

The data were analyzed using SAS procedures (SAS, 2005). The variables expressed as percentages were normalized by a root square arcsine transformation. The means and standard deviations 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 Tukey test at 5% confidence level.

RESULT AND DISCUSSION

Pollen viability: Although the levels of pollen viability through the method of TTC for *A. orientalis* and *P. dulcis* genotypes are found insignificant ($p > 0.05$), there is a significant difference between the means through the method of FDA ($p < 0.05$) in the year 2006 (Table II).

While the highest pollen viability of 66.5% from 'Orientalis 9' genotype through the use of TTC test in 2006, 'Nonpareil' and 'Texas' cultivars produced very close pollen viability values close to the one for 'Orientalis 9'. For the year 2007, the highest pollen viability ratio was obtained from 'Orientalis 6' (86.1%). The FDA test yielded the highest values of pollen viability ratios for 'Texas' (76.2%) and 'Nonpareil' (74.9%) for the year 2006, while the lowest values for the pollen viability ratios were found for 'Ferraduel' and 'Orientalis 5' as 54.3% and 54.0%, respectively. However, for the year 2007, the highest pollen viability ratio was obtained for 'Ferraduel' cultivar with 84.4%. Eti *et al.* (1993) found that some of the almond cultivars produced TTC test-based pollen viability ratio as the highest ratio of 58.3% for 1988 and 82.2% for 1990 in Turkey. The FDA-based test of pollen viability ratio was between 37.9% and 71.6% for the year 1989 and 29.5% and 91.2% for the year 1990. Tosun *et al.* (2007) reported the pollen viability ratios for the selected almond genotypes varied between 71.8 and 85.9% in Isparta province, Turkey. The results of the current study on the pollen viability ratios for both of the cultivars and *A. orientalis* are higher than the ones from Eti *et al.* (1993) and similar to the ones from Tosun *et al.* (2007). In 2006, both FDA and TTC tests produced higher viability ratios for cultivars than those of *A. orientalis* genotypes. On the other hand, in 2007, the TTC test produced viability ratio of 72.9% for *A. orientalis* genotype, which was higher than the one for almond cultivar with 68.6%. The FDA tests in 2007 produced higher viability ratio for cultivars (72.2%) than for *A.*

Table I: Average age and location of almond genotypes

Accession	County/City	Average age (year)	Latitude	Longitude	Elevation (m)
Orientalis 5	Ulukışla/Niğde	21	37° 31' 28 N	34° 40' 54 E	1177
Orientalis 6	Ulukışla/Niğde	15	37° 31' 29 N	34° 40' 58 E	1166
Orientalis 7	Ulukışla/Niğde	20	37° 31' 29 N	34° 41' 00 E	1159
Orientalis 9	Ulukışla/Niğde	17	37° 40' 37 N	34° 40' 26 E	1408
Orientalis 10	Ulukışla/Niğde	10	37° 30' 32 N	34° 40' 33 E	1376
Nonpareil	POZMER	10	36° 28' 05 N	34° 53' 53 E	1050
Texas	POZMER	10	36° 28' 05 N	34° 53' 53 E	1050
Ferragnes	POZMER	10	36° 28' 05 N	34° 53' 53 E	1050
Ferraduel	POZMER	10	36° 28' 05 N	34° 53' 53 E	1050

Orientalis; *Amygdalus orientalis* Mill. POZMER; Pozantı Agricultural Research Center of Çukurova University

Table II: The viability percentages of TTC and FDA tests in selected *A. orientalis* Mill. genotypes and some *P. dulcis* Mill. cultivars for the years 2006 and 2007

Year	Accession	TTC			FDA	
		Viable	Semi-viable	Non-viable	Viable	Non-viable
2006	Orientalis 5	64.5	15.3 b	20.2 abc	54.0 b	46.0 a
	Orientalis 6	59.2	17.6 ab	23.2 ab	66.6 ab	33.4 ab
	Orientalis 7	57.2	17.8 ab	25.0 a	67.8 ab	32.3 ab
	Orientalis 9	66.5	20.7 ab	12.8 abc	65.3 ab	34.7 ab
	Orientalis 10	61.6	21.9 ab	16.5 abc	63.5 ab	36.5 ab
	Nonpareil	66.4	22.5 ab	11.1 abc	74.9 a	25.1 b
	Texas	66.5	22.8 ab	10.6 abc	76.2 a	23.8 b
	Ferragnes	65.1	25.1 a	9.85b c	64.9 ab	35.1 ab
	Ferraduel	62.7	25.1 a	12.2 abc	54.3 b	45.7 a
	HSD _{0.05}	ns	8.4	14.6	18.9	18.9
	<i>A. orientalis</i>	61.8	18.7	19.6	63.4	36.6
	<i>P. dulcis</i>	65.2	23.9	11.0	67.6	32.4
	Mean	63.3	21.0	15.7	65.3	34.7
	2007	Orientalis 5	61.4 de	11.1 a	27.5 a	57.9 bc
Orientalis 6		86.1 a	2.2 b	11.7cd	61.3 bc	38.7 ab
Orientalis 7		75.4 abc	11.0 a	13.6 cd	54.6 c	45.4 a
Orientalis 9		67.1 cde	9.4 a	23.5 ab	54.2 c	45.8 a
Orientalis 10		74.4 bc	11.6 a	14.1 bcd	59.4 bc	40.6 ab
Nonpareil		79.1 ab	12.7 a	8.3 d	69.8 ab	30.2 bc
Texas		72.8 bcd	13.2 a	14.0 bcd	65.9 bc	34.1 ab
Ferragnes		64.6 cde	15.4 a	20.0 abc	68.5 bc	31.5 ab
Ferraduel		57.8 e	15.8 a	26.4 a	84.4 a	15.6 c
HSD _{0.05}		11.5	7.0	9.6	15.0	14.9
<i>A. orientalis</i>		72.9	9.1	18.1	57.5	42.5
<i>P. dulcis</i>		68.6	14.27	17.15	72.2	27.9
Mean		71.0	11.38	17.65	64.0	36.0

orientalis genotypes (57.5%) as was the case in 2006. There were partial differentiations between the viability ratios for both of the tests in both years in the experiment.

Pollen germination: The levels of pollen germination at different sucrose doses for *A. orientalis* genotypes and *P. dulcis* cultivars in the experiment differentiated significantly ($p < 0.05$) (Table III). In both of the experimental years, ‘Ferraduel’ cultivar produced the highest pollen germination rate of all the sucrose doses while the ‘Orientalis 7’ genotype showed the lowest pollen germination rate in all sucrose doses in the experiment. In both of the years, pollen germination rates of cultivars were higher than those from *A. orientalis* genotypes grown in the 3 sucrose media. In addition, the germination rates varied in both of the years and genotypes used in the experiment, which is similar to the pollen viability test results. The rates of pollen viability and germination ratio increased in the year 2007 versus 2006.

The higher values in the second year of the experiment against the first year were observed for TTC test-based pollen viability of ‘Orientalis 6’ and pollen germination test-based results of ‘Orientalis 7’. The environmental conditions such as rain, high temperature, low relative humidity, salinity hazard and UV light beams, influenced pollen germination rates and viability levels (Asif *et al.*, 1983; Ortega *et al.*, 2007). Eti (1991) also reported that germination level is affected not only by the nutrient status of the nutrition media, but also by moisture content, pressure, temperature and pH of the media.

The Orientalis genotypes and cultivars showed higher germination rates for 10 and 15% sucrose doses than for 20% sucrose dose in both of the years (Table III). Eti (1991) reported that pollens of different fruit species and cultivars showed discrepancies among the germination rates at different sucrose doses. Eti *et al.* (1996) showed that the best germination medium is usually 15%-sucrose solution

Table III: The germination percentages at different sucrose doses (%) in *A. orientalis* genotypes and *P. dulcis* cultivars for the years 2006 and 2007

Year	Accession	Sucrose concentration (%)			
		0	10	15	20
2006	Orientalis 5	0.0	69.5 d	74.9 bc	55.8 cd
	Orientalis 6	0.0	75.1 dc	68.6 c	65.99 cd
	Orientalis 7	0.0	41.2 e	41.7 d	21.72 e
	Orientalis 9	0.0	80.1 c	77.5 bc	69.38 bc
	Orientalis 10	0.0	68.6 d	67.3 c	50.45 d
	Nonpareil	0.0	81.7 bc	75.8 bc	58.23 cd
	Texas	0.0	83.9 abc	80.3 abc	65.08 cd
	Ferragnes	0.0	91.3 ab	86.8 ab	84.12 ab
	Ferraduel	0.0	92.4 a	91.8 a	91.43 a
	HSD _{0.05}		10.1	13.2	17.10
	<i>A. orientalis</i>		66.9	66.0	52.67
	<i>P. dulcis</i>		87.4	83.7	74.72
	Mean		76.0	73.9	62.45
	2007	Orientalis 5	0.00	84.51 bc	90.29 abc
Orientalis 6		0.00	74.85 d	75.43 cd	37.27 d
Orientalis 7		0.00	77.26 cd	78.13 bcd	61.42 c
Orientalis 9		0.00	45.94 e	50.93 e	47.33 d
Orientalis 10		0.00	68.96 d	67.70 d	61.46 c
Nonpareil		0.00	88.92 ab	84.31 abc	72.79 bc
Texas		0.00	92.45 ab	93.25 ab	91.19 a
Ferragnes		0.00	94.39 a	91.90 ab	89.21 a
Ferraduel		0.00	96.89 a	95.37 a	93.67 a
HSD _{0.05}			9.47	16.08	13.22
<i>A. orientalis</i>			70.30	72.50	58.45
<i>P. dulcis</i>			93.16	91.21	86.72
Mean			80.46	80.81	71.01

for almond cultivars and genotypes, whereas some of genotypes prefer 10% and 20% sucrose solutions for the best germination rates. Our results are parallel to the ones of aforementioned studies. Some studies in the literature (Dicenta *et al.*, 2002) explained that pollen germination rates of self-fertile almond cultivars varied between 36% and 74%, whereas some of the almond genotypes grown in 15%-sucrose solution yielded germination rates between 35% and 82%. Our results of germination rates were higher than the results of the aforementioned studies.

Pollen production: The almond genotypes in the experiment showed significant differences ($p < 0.05$) for the number of anthers per flower for species and genotypes (Table IV). The almonds cultivars produced on average 28.7 anthers per flower in 2006, while the average number of anthers per flower recorded was 29.3 in 2007. *A. orientalis* genotypes produced 9.8 anthers per flower for 'Orientalis 7' in 2006 and 17.7 anthers per flower for 'Orientalis 6', averaging 13.5. For the year 2007, the number of anthers per flower ranged from 12.8 to 19.8, averaging 15.7, for 'Orientalis 9' and 'Orientalis 6', respectively. *A. orientalis* showed less number of anthers per flower than the one for almond cultivars for both of the years of the experiment. Godini (1981) reported that the number of anthers per flower varied from 20.4 to 37.2 for some of the almond cultivars. The current study resulted in similar values for the number of anthers as described by Godini (1981).

The number of pollens in anthers differentiated based on the almond genotypes and species. The number of

Table IV: The values of pollen production components in some *A. orientalis* genotypes and *P. dulcis* cultivars

Year	Accession	Anther number/flower	Pollen number/anther	Pollen number/flower	Morphological homogeneity (%)
2006	Orientalis 5	11.2 e	634.5 d	7131 e	100.0 a
	Orientalis 6	17.7 c	549.6 d	9747 e	82.0 c
	Orientalis 7	9.8 e	481.3 d	4720 e	100.0 a
	Orientalis 9	14.5 d	527.2 d	7637 e	100.0 a
	Orientalis 10	14.3 d	477.7 d	6835 e	94.4 abc
	Nonpareil	30.7 a	800.3 d	24533 d	92.6 abc
	Texas	22.0 b	1841.5 c	40482 c	84.5 bc
	Ferragnes	31.9 a	2913.1 a	92688 a	99.5 ab
	Ferraduel	30.4 a	2367.4 b	71929 b	93.8 abc
	HSD _{0.05}	1.6	513.8	12538	20.6
	<i>A. orientalis</i>	13.5	534.1	7214	95.3
	<i>P. dulcis</i>	28.7	1980.6	57408	92.6
	Mean	21.1	1257.3	32311	94.0
	2007	Orientalis 5	16.5 e	676.5 bc	11157 d
Orientalis 6		19.8 d	852.9 bc	16817 bcd	89.6 ab
Orientalis 7		13.9 f	1127.7 ab	15672 cd	100.0 a
Orientalis 9		12.8 f	955.3 bc	12152 d	95.6 a
Orientalis 10		14.9 fe	755.5 bc	11254 d	95.8 a
Nonpareil		29.2 b	866.8 bc	25172 b	86.5 ab
Texas		28.5 cb	363.4 c	10350 d	74.8 b
Ferragnes		26.2 c	1709.3 a	44810 a	98.0 a
Ferraduel		33.5 a	682.1 bc	22785 bc	93.3 ab
HSD _{0.05}		2.4	653.6	8611	21.7
<i>A. orientalis</i>		15.7	873.6	13410	96.2
<i>P. dulcis</i>		29.3	905.4	25779	88.2
Mean		22.4	889.49	19595	92.2

pollens in anthers during 2006 experiment was higher for cultivars, while both of the species produced similar number of pollens in anthers for the year 2007. The number of pollens in anthers of *A. orientalis* ranged from 477.7 to 634.5 for 'Orientalis 10' and 'Orientalis 5', respectively, in 2006, whereas it varied from 755.5 to 1127.7 for 'Orientalis 10' and 'Orientalis 7', respectively in 2007. The averages of number of pollens per anther in *A. orientalis* were 534.1 and 873.6 for 2006 and 2007, respectively. Godini (1981) reported that the number of pollens per anther for some cultivated almonds ranged from 1099 to 1787, yearly. The studies of stone fruits other than almond showed that the number of pollens per anther for apricot was between 1574-3757 (Mahanoğlu *et al.*, 1995), 1211 and 3042 for apricot (Asma, 2008), and 1782 and 3418 for cranberry (Pırlak & Gülerüz, 2005). The results of almond cultivars generally parallel to the results of the aforementioned works of the authors, whereas *A. orientalis* in our study showed lower number of pollens per anther in comparison to previous studies.

The number of pollens per flower significantly varied for almond species and genotypes in the current experiment (Table IV). The average value of number of pollens per flower was 57408 for 2006 and 25779 for 2007. *A. orientalis* genotypes produced pollens ranging from 4720 ('Orientalis 7') to 9747 ('Orientalis 6'), averaging 7214 for 2006, whereas these values ranged from 11157 ('Orientalis 5') through 16817 ('Orientalis 6'), averaging 13410, for 2007. Godini (1981) reported some of the almond cultivars produced the number of pollens per flower between 36960

and 56776 and Traynor (2001) explained these values to be between 42000 and 67000. Our number of pollens per flower for almond cultivars fell into the range of the above mentioned researchers' values. However, *A. orientalis* produced lower number of pollens per flower in our study in comparison to the studies mentioned above.

Table IV shows that the number of anthers per flower and the number of pollens per flower and per anther were produced in low numbers for *A. orientalis* genotypes, whereas *orientalis* genotypes and cultivars are similar to each other in terms of morphological homogeneity.

In order to be a high quality pollinator of a cultivar, it is important that the cultivar needs to be not only a plenty of pollen producer, but also to be of high ratio of pollen viability and germination (Stösser, 1984; Eti, 1990).

A. orientalis for increasing cold resistance and breeding self pollinated individual in almond breeding studies can be used (Denisov, 1988; Kester *et al.*, 1991; Kester & Gradziel, 1996; Gradziel *et al.*, 2001). The fact that the proximity of the values obtained from *A. orientalis* genotypes to cultivars in terms of not only pollen viability, pollen germination rate but also pollen production rate shows that these genotypes (*A. orientalis*), although not as high as the rate in cultivars, pollen production and pollen quality properties are higher.

CONCLUSION

The results showed that the pollens of *A. orientalis* genotypes can, particularly, be used in breeding studies on self-fertile and cold-resistant almonds. Before starting hybridizing these genotypes (*A. orientalis*), the properties of pollens need to be determined and then, based on these properties the breeding of almond cultivars should be performed. It is suggested, as a result of this study, that the pollens of genotypes of 'Orientalis 5', 'Orientalis 6', 'Orientalis 7', 'Orientalis 9' and 'Orientalis 10' can be used in breeding of hybridized self-fertile and cold-resistant almond cultivar studies in the future. As a result of determination of the pollen properties, the efficiency of breeding programs for the hybridization of almond species is increased.

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