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Full Length Research Paper

Pollen quality and pollen production in some almond culvitars under Kaharamanmaras (Turkey) ecological conditions

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Pollen quality and pollen production of 21 almond cultivars under Kaharamanmaras ecological conditions were analyzed to see the effect on fruit yield and quality. Pollen viability tests with TTC and FDA methods and pollen germination tests were performed by hanging drop method in sucrose (10, 15 and 20%, respectively) containing media and on agar media containing sucrose (0, 5, 10 15 and 20%, respectively) in petri dish. The amount of pollen production and pollen morphological homogeneity levels were also determined. In pollen viability tests, Masbovera, False Baresa, Tuano and Nonpareil varieties; in pollen germinating test Cristomorto, Yaltinski and Gloretia varieties; in the determination of pollen production Bertina, Felisia, Super Nova and Masbovera varieties had the highest values. Morphological homogeneities were found to be high in all the cultivars.

Key words: Pollen, almond cultivars, morphological homogeneity, Kaharamanmaras.

INTRODUCTION

Almond is known as a drought tolerant species compared to many other fruit species. Hot summer days and nights with no precipitation, Mediterranean climate, is ideal for almond culture. Although almond is widely cultured around the world, the ecological conditions limit the yield and the quality. Almond has a short chilling requirement, in turns, blooms very early in the spring. The low temperature and high precipitation in spring have negative effects on almond pollination.

Almond culture has not reached the desired level in Turkey, the yield has almost not changed for years. This is partially related to unfavorable eccological conditions, as well as self and cross incompatibility of almond cultivars. Despite the fact that Turkey is one of the primary genetic centers for almond, almond culture is concentrated mostly in Datca peninsula of the Aegean region (Dokuzoguz and Gülcan, 1979). However, in the South-East and Mediterranean regions, commerical almond orchards have been established for the last couple of decades.

Low temperatures during flowering impede pollens to germinate in flower stigma or pollen tube formation (Moffett and Rodney, 1973; Drescher and Engel, 1976; Robinson, 1979; Stephen, 1981). Low temperatures along with high precipitation also obstruct bees' activity essential for almond pollination.

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

MATERIALS AND METHODS

The experiment was conducted at Kahramanmaras Sutcu Imam University (KSÜ), Prof. Dr. Nurettin Kaska Nuts Research Center in K.Maras, Turkey in 2010. Nine years old almond trees were used as the plant material. Ferragnes, Ferraduel, Nonpareil, Cristomorto, Tuano, Texas, Garrigues, Drake, Yaltinski, Picantili, D. M. Largueta, Lauranne, Ayles, Felisia, Masbovera, Bertina, Glorieta, Super Nova, Guara, False Barese and Ne Plus Ultra were almod varieties employed in the study.

The preparation of the pollen for the viability and germination tests was done according to Eti, (1990).

Pollen production

For each cultivar, randomly sampled 40 flowers were used. Number of pollen grains per flower (PF) was determined according to the hemacytometric method (Eti, 1990).

Table 1. Pollen production paremerts and pollen homogeneity.

Cultivar	AF*	PF*	PA*	DP*
Ferragnes	36.00$^{b..f}$	109.380^{de}	3.038$^{a..d}$	98.90
Ferraduel	38.40$^{a..d}$	106.670^{de}	2.855^{cd}	99.20
Nonpareil	38.25$^{a..d}$	112.715^{cd}	2.946^{bcd}	96.90
Cristomorto	36.80$^{b..f}$	110.465^{cd}	3.001$^{a..d}$	99.40
Tuano	39.30^{ab}	128.510^{ab}	3.269^{abc}	93.10
Texas	35.45$^{c..f}$	125.305^{b}	3.534^{a}	98.90
Garrigues	35.10^{def}	93.870^{f}	2.674^{d}	98.00
Drake	36.20$^{b..f}$	111.790^{cd}	3.088$^{a..d}$	99.70
Yaltinski	37.70$^{a..e}$	110.145^{cd}	2.921^{bcd}	95.80
Picantili	34.50^{ef}	91.400^{f}	2.649^{d}	96.20
D.M. Largueta	33.50^{f}	94.055^{f}	2.807^{cd}	97.80
Lauranne	36.60$^{b..f}$	110.120^{cd}	3.008$^{a..d}$	97.00
Ayles	38.65^{abc}	92.210^{f}	2.385^{d}	98.50
Felisia	39.45^{ab}	124.615^{b}	3.158$^{a..d}$	97.80
Masbovera	35.40$^{c..f}$	116.270^{c}	3.284^{abc}	99.50
Bertina	40.80^{a}	133.830^{a}	3.280^{abc}	97.40
Glorieta	38.40$^{a..d}$	107.045^{de}	2.787^{cd}	96.50
Super Nova	38.60^{abc}	134.420^{a}	3.482^{ab}	96.30
Guara	39.00^{ab}	103.205^{e}	2.646^{d}	96.40
False Barese	36.25$^{b..f}$	109.910^{cd}	3.032$^{a..d}$	99.00
Ne Plus Ultra	37.20$^{b..e}$	110.730^{cd}	2.976$^{a..d}$	98.20

Means represented with the same letter in a column are not significantly different at P = 0.001; AF*: number of anthers per flower; PF*: number of pollen grains per flower; PA*: number of pollen grains per anther = PF/AF; DP*: percentage of well-developed pollen; Means represented with the same letter in a column are not significantly different.

The amount of pollen per anther was determined using the following Formula:

PA = PF/AF;

Where PA is the number of pollen grain per anther, PF is the number of pollen grains per flower, AF is number of anthers per flower and DP is percentage of well-developed pollen.

Pollen viability tests

Triphenyl tetrazolium chloride (TTC) and Fluorescein diacetate (FDA) tests were used to determine the pollen viability rates of almond cultivars. TTC solution was prepared according to Norton (1966) and FDA solution was prepared according to Heslop-Harriison (1970).

Pollen germination tests

"Agar in petri dish" and "hanging drop" tests were used (Stanley and Linskens, 1985). Initially, the two tests were conducted with a range of sucrose concentrations. The sucrose concentrations were 10, 15 and 20% for "agar in petri dish" method and 0, 5, 10, 15 and 20% for "hanging drop" method.

For statistical analysis, percentage data were subjected to angular transformation. Data were analyzed using analysis of variance (ANOVA). Differences between means were determined by Tukey's LSD test.

RESULTS AND DISCUSSION

Pollen production

For a cultivar to be used as a pollinizer, in addition to high pollen viability and pollen germination rates, it is important that its anthers produce high amounts of pollens. Because not all pollens germinated on stigma reach the carpels, thus for succesfull fertilization, pollinizer cultivars producing high amount of pollens are desired (Stösser, 1981; Stösser, 1984). The pollen production tests showed that male flowers of eight cultivars produced sufficient amount of pollen (Table 1).

The results obtained from the present study have potentially practical uses in fertilization biology. 'Bertina and Felisia' had the highest AF (40.80 and 39.45), Super Nova and Bertina' had the highest PF (134.420 and 133.830), 'Texas, Masbovera and Bertina ' had the highest PA (3534, 3284 and 3280) and 'Drake'and Masbovera' had the highest DP (99.70% and %99.50) as shown in Table 1. Similar results were obtained from a study conducted by Eti et al. (1996).

No statistical differences were found among almond cultivars in terms of morphological homogeneity level or normally developed pollen percentage. However, considering all data, we may conclude that the cultivars

Table 2. Pollen viability (%) of almond cultivars determined by TTC test.

Cultivar	TTC Test		
	Viable pollen (%)	Semi–viable pollen (%)	Dead pollen (%)
Ferragnes	72.30^{f}	14.20^{bcd}	13.50^{b}
Ferraduel	70.40^{h}	15.40$^{a..d}$	14.20^{c}
Nonpareil	74.10^{de}	16.20^{abc}	19.70^{a}
Cristomorto	78.50^{a}	14.80^{bcd}	7.10^{fg}
Tuano	68.20^{i}	16.20^{abc}	15.60^{b}
Texas	74.10^{de}	16.55^{abc}	9.35^{ef}
Garrigues	72.70^{ef}	14.30^{bcd}	13.00^{bcd}
Drake	76.45^{b}	13.80^{cd}	9.75^{def}
Yaltinski	79.20^{a}	17.25^{abc}	3.55^{h}
Picantili	70.30^{h}	17.60^{ab}	12.10^{cde}
D. M. Largueta	75.40^{bcd}	16.50^{abc}	8.10^{fg}
Lauranne	74.80^{cd}	12.10^{d}	13.10^{bcd}
Ayles	74.10^{de}	14.05^{cd}	11.85^{cde}
Felisia	70.15^{h}	16.90^{abc}	12.95^{bcd}
Masbovera	78.40^{a}	15.20$^{a..d}$	6.40^{fgh}
Bertina	76.50^{b}	14.30^{bcd}	9.20^{ef}
Glorieta	76.20^{bc}	18.50^{a}	5.30^{gh}
Super Nova	76.80^{b}	14.40^{bcd}	8.80^{ef}
Guara	70.65^{gh}	16.20^{abc}	13.15^{bcd}
False Barese	78.50^{a}	15.10$^{a..d}$	6.40^{fgh}
Ne Plus Ultra	71.85^{fg}	14.70^{bcd}	13.45^{bc}

had over 90% homogeneity level (Table 1).

Eti (1991) reported that among the various entomophyl fruit species, almond had the highest percentage of well-developed pollen grain; however, its overall pollen production rate was lowest.

Pollen viability

The results of TTC and FDA tests are summarized in Tables 2 and 3. Viable pollen numbers were recorded higher in FDA test than in TTC test (Tables 2 and 3). However, TTC test covers partially-viable pollens, while FDA test covers only full-viable pollens. Thus both tests may give similar results when the partially-viable pollens are omitted.

In TTC test, the percentage pollen viability varied between 75.80% (Masbovera, False Barese cultivars) to 68.20% (Tuano cultivar). In FDA test, percentage pollen viability changed between 81.55% (Nonpareil cultivar) to 60.15% (Tuano 1 cultivar).

The pollen viabilities of remaining cultivars were over 70% in both tests. Therefore, in terms of pollen viability, all the almond cultivars can be considered as good pollinators.

Eti et al. (1996) reported that some of the almond varieties had a viable pollen rate of 35 to 59% when TTC test was used, and of 29 to 83% when FDA test was used.

To find out pollen viability in different fruit species, TTC and FDA were used in several studies and the results were generally similar to each other (Seilheimer and Stösser, 1982; Eti, 1991; Mahanoğlu et al., 1995).

In this study, TTC and FDA tests gave similar results. This finding is in accordance with the previous studies (Seilheimer and Stösser, 1982; Eti, 1991; Mahanoğlu et al., 1995; Sütyemez and Eti, 1999, 2006).

Pollen germination

To determine pollen germination amount, “agar in petri dish” method and “hanging drop” methods were used. In “hanging drop” method of sucrose solutions 0, 5, 10, 15, 20% used, 15% sucrose solution provided the highest numbers of germination pollen (Table 4). When hanging drop method was used, Cristomorto and Yaltinski had the highest pollen germination rate 72.80 and 70.60%, respectively. In the pollen germination test using the “agar in petri dish” method with 1% agar, 10, 15 and 20% sucrose, the highest germination rate (82%) was found with Glorieata on 15% sucrose solution and the lowest 38.50% in Drake cultivar on 10% sucrose solution (Table 5).

The pollen germination percentages of almond

Table 3. Pollen viability (%) of almond cultivars determined by FDA tests.

Cultivar	FDA Test	
	Viable	Dead
Ferragnes	79.15^{f}	20.85^{ghi}
Ferraduel	75.20^{k}	24.80^{cd}
Nonpareil	81.55^{a}	18.45^{k}
Cristomorto	80.30^{de}	19.70^{ijk}
Tuano	60.15^{n}	39.85^{a}
Texas	78.40^{g}	21.60^{fgh}
Garrigues	76.70^{j}	23.30^{de}
Drake	75.00^{kl}	25.00^{c}
Yaltinski	81.15^{ab}	18.25^{k}
Picantili	74.80^{kl}	25.20^{c}
D. M. Largueta	77.50^{hi}	22.50^{ef}
Lauranne	78.10^{gh}	21.90^{efg}
Ayles	74.40^{l}	25.60^{c}
Felisia	62.20^{m}	37.80^{b}
Masbovera	80.65^{bcd}	19.35^{ijk}
Bertina	79.80^{ef}	20.20^{hij}
Glorieta	80.20^{de}	19.80^{ijk}
Super Nova	76.90^{ij}	23.10^{ef}
Guara	80.40^{cde}	19.60^{ijk}
False Barese	81.10^{abc}	18.90^{jk}
Ne Plus Ultra	77.45^{hi}	22.55^{ef}

Means represented with the same letter in a column are not significantly different at P = 0.001.

Table 4. Pollen germination (%) of almond cultivars determined by hanging drop method.

Cultivar	Sucrose concentration				
	0%	5%	10%	15%	20%
Ferragnes	20.35^{l}	38.40	51.20$^{d..g}$	65.40^{d}	64.65^{e}
Ferraduel	27.80^{h}	40.15^{fg}	53.05$^{a..g}$	63.25^{e}	66.90^{c}
Nonpareil	32.10^{c}	39.70^{g}	48.60^{g}	60.30^{h}	62.70^{f}
Cristomorto	30.75^{e}	44.90^{a}	56.25$^{a..g}$	72.80^{a}	68.40^{b}
Tuano	29.80^{f}	40.25^{f}	59.25^{abc}	57.85^{j}	55.30^{l}
Texas	29.30^{fg}	43.10^{c}	53.10$^{a..g}$	55.70^{l}	53.35^{m}
Garrigues	28.85^{g}	35.70^{k}	58.10$^{a..e}$	66.40^{c}	62.70^{f}
Drake	23.40^{k}	37.60^{i}	50.45^{efg}	53.35^{n}	61.85^{g}
Yaltinski	32.15^{c}	41.85^{e}	52.60$^{b..g}$	70.30^{b}	70.60^{a}
Picantili	34.20^{a}	40.05^{fg}	54.40$^{a..g}$	53.80^{i}	57.15^{k}
D.M. Largueta	29.00^{g}	38.30^{h}	49.50^{fg}	58.75^{m}	55.50^{l}
Lauranne	31.45^{d}	36.45^{j}	50.55^{efg}	60.65^{h}	62.45^{f}
Ayles	31.70^{cd}	37.20^{i}	51.30$^{c..g}$	62.50^{ef}	61.45^{gh}
Felisia	20.60^{l}	35.85^{k}	59.45^{ab}	67.10^{c}	64.35^{e}
Masbovera	33.50^{b}	44.30^{b}	57.40$^{a..f}$	57.05^{k}	59.20^{j}
Bertina	29.75^{f}	39.80^{fg}	60.80^{a}	63.20^{e}	65.80^{d}
Glorieta	25.15^{i}	35.90^{k}	58.70$^{a..d}$	65.35^{d}	64.75^{e}
Super Nova	30.60^{e}	42.55^{d}	58.85$^{a..d}$	62.30^{fg}	60.90^{i}
Guara	30.40^{e}	44.30^{b}	55.10$^{a..g}$	56.25^{l}	53.15^{m}
False Barese	28.20^{h}	39.80^{fg}	48.25^{g}	59.90^{h}	57.10^{k}
Ne Plus Ultra	24.30^{j}	38.75^{h}	53.35$^{a..g}$	61.65^{g}	61.30^{hi}

Means represented with the same letter in a column are not significantly different at P = 0.001.

Table 5. The germination percentages of pollen by “agar in petri dish” tests in almond cultivars.

Cultivar	1% agar+ 10% sucrose	1% agar + 15% sucrose	1% agar + 20%sucrose
Ferragnes	48.25^{de}	63.05^{f}	61.40$^{b..e}$
Ferraduel	52.60^{a}	59.35^{l}	58.15^{ghi}
Nonpareil	49.70^{cd}	68.80^{c}	57.10^{hij}
Cristomorto	53.40^{a}	71.80^{a}	61.05^{cbe}
Tuano	51.80^{ab}	60.15^{hi}	54.40^{k}
Texas	47.65^{e}	65.30^{e}	62.75^{bc}
Garrigues	43.50^{hi}	63.45^{f}	53.80^{k}
Drake	38.50^{j}	57.40^{j}	44.70^{n}
Yaltinski	46.80^{ef}	60.55^{gh}	58.95^{fgh}
Picantili	52.75^{a}	67.50^{b}	60.10^{def}
D.M. Largueta	44.15^{ghi}	65.55^{e}	47.20^{m}
Lauranne	42.90^{i}	56.60^{jk}	56.40^{ij}
Ayles	42.70^{i}	61.40^{g}	51.35^{l}
Felisia	45.55^{fg}	63.15^{f}	59.55^{efg}
Masbovera	39.45^{j}	56.10^{k}	53.60^{k}
Bertina	48.30^{de}	70.20^{b}	64.70^{a}
Glorieta	45.20^{fgh}	72.25^{a}	63.15^{ab}
Super Nova	43.20^{i}	63.30^{f}	50.20^{l}
Guara	46.65^{ef}	60.45^{ghi}	56.35^{ij}
False Barese	47.70^{e}	59.80^{hi}	55.40^{jk}
Ne Plus Ultra	50.40^{bc}	68.75^{c}	61.50^{bcd}

Means represented with the same letter in a column are not significantly different at P = 0.001.

genotypes obtained by two tests are summarized in Tables 4 and 5. Since satisfactory germination levels were only obtained by using “agar (1%) in petri dish” with sucrose concentration of 10, 15 and 20%, and by using “hanging drop” with sucrose concentration of 0, 5, 10, 15 and 20%, we pay attention only to the pollen germination with these concentrations.

In “hanging drop” test, the sucrose concentrations affected the pollen germination. Higher sucrose concentration (15%), in general, improved the germination rates of almond pollen. The highest pollen germination (72.80%) was found in Cristomorto cultivar and followed by Yaltinski cultivar (70.60%) (Table 4). For “agar in petri dish” test (1% agar, 10% sucrose), the highest pollen germination (72.25%) was obtained from Glorieta cultivar 15% sucrose concentration. Drake cultivar had the lowest germination with (38.50%) 10% sucrose concentration.

Eti et al. (1996) reported that the highest pollen germination rated was obtained using 10 or 15% sucrose solution.

Based on the results obtained from viability and germination tests, we may conclude that the almond cultivar used in this study can be used as pollinators to those that bloom in the same period. However, It should be stressed that the high germination levels found *in vitro* does not always indicate a good *in vivo* pollination level for each cross combination since overlapping of blooming period for the monocious almond flowers have priority.

Conclusion

On the basis of results from pollen viability, germination percentage and pollen production rate, we may conclude that all the cultivars tested in this study showed sufficient characteristics as good pollinators. On the other hand, in establishing an almond orchard with these cultivars, overlapping of flowering, ecological conditions and cultural practices should be appropriate in order to obtain higher yield with high quality nuts.

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