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DETERMINATION OF POLLEN GRAIN VIABILITY AND GERMINATION LEVELS FOR PISTACHIO AND TEREBINTH IN AYDIN /TURKEY ECOLOGY

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Abstract

With this research, the viability levels, production potentials, and storage periods of pollen of naturally grown four male terebinth types (*Pistacia terebinthus* L.) and four male pistachios (*Pistacia vera* L.) grafted on terebinth trees were determined in Uzunlar Village, Atça District, Aydın Province. For the aim of determining the pollen viability levels, TTC, IKI, and safranine solutions were used. The viability ratio ranged between 88.24% (safranine test) and 70.18% (TTC test) in pistachio trees, between 85.36% (IKI test) and 63.73% (safranine test) in terebinth types. The average amount of pollen within a flower resolved by hemacytometric method was changed between 135 000 and 176 250 depend on species and types. While the germination percentage of pollen of pistachio trees and terebinth types was 71% and 26%, respectively in the beginning of storage, that of pollen in the room conditions after 4th day, in the refrigerator from 10th day and in the deep freezer from 2nd month was decreased to zero.

Introduction

Pistacia genus belongs to a member of *Anacardiaceae* family has 13 or more species (Kafkas, 2007). Among these species, pistachio (*Pistacia vera* L.) occupies an important trade issue due to its edible seeds. The origin center of dioecious pistachio species in the Near East includes the Central Asia and Turkey (Zohary, 1952). Iran and the USA are the leading countries in pistachio production in the world (approximately 65% of the world production) followed by Turkey, Syria and China (Anon., 2007). While all pistachio production is performed in the first class soil and irrigated in Iran and USA, that is performed in dry, infertile, and rocky soil in Turkey and other producing countries. The most common wild *Pistacia* species are *P. terebinthus* L., *P. atlantica* Desf., and *P. eurycarpa* Yalt., in Turkey (Kafkas *et al*., 2002).

Because the edible portion of pistachio is the seed, the pollination and fertilization are obligatory to obtain the fruit. Unfertilized flowers drop or the fruit set as empty inside. Wind pollination is dominated and 20-28 hours after pollination, fertilization takes place. The parthenocarpic fruit production depends on controlled by protoandrious condition and the quality of the pollen grains of pollinizers. Therefore, the viability and germinability ratios of the grown genotypes need to be known. Some researchers stated that macro- and microelements and amino acids of pollen grains have important effects on the germinability of pollen grains (Rashed *et al*., 1995; Afshari *et al*., 2008). Although ecological criteria are suitable in Aydın province, fertilization problems can be seen. In this study, the pollen grain viability and germination level of pistachio grown in Aydın conditions were determined.

Materials and Methods

In this study, 4 terebinth types (*Pistacia terebinthus* L.) naturally grown in Uzunlar village (35º 57.0´ N, 28º 11.0´ E, 865m (a.s.l.)) of Atça district in Aydın province and 4 male pistachios (*Pistacia vera* L.) grafted on terebinth were used as experimental plant material.

To determine the flowering characteristics of the trees, the number of spike on a one-year-old branch, flowering spike length, the amount of pollen production, pollen grain viability ratio, pollen germination ratio and pollen storage were studied. The flower spikes randomly chosen on 16 one-year-old branches from four different sites of the tree were counted and the average number was calculated. The average flower spike length was measured during 70% flowering stage on 20 spikes randomly chosen from four different directions of the tree.

Amount of pollen production: A total of 20 flowers in the stage of just opening but not fully opened were collected from the trees used in the study. These flowers were divided into two groups containing 10 flowers in each. The anthers on individual flowers were counted and placed in small vials. The vials were incubated in their lids open in front of a window receiving sunlight for the anther to dehisce for several days (Eti, 1990). 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. After this suspension was incubated one day, a drop was placed on the a two-counting area containing Thoma slide to where a special cover slip was replaced. The pollen count was conducted on randomly chosen four large squares in each counted area that evaluated as replicates. The average pollen grains amount per anther and flower was determined with this process.

Pollen grain viability tests: The spikes along with the respected branches were cut and brought to the laboratory in the stage of anther dehiscence and to start shedding the pollen grains. After the anthers were incubated at 20-25ºC in the laboratory, the dehisced pollen grains were collected. TTC (2, 3, 5-triphenly tetrazolium chloride), IKI (iodine + potassium iodide) and safranine solution were used in viability tests to determine the viability levels of the pollen grains.

For TTC solution, first 10% stock solution was prepared. From this solution 1 portion was mixed with 9 portion of 60% saccharose solution. Therefore, the amount in the final TTC solution was reached at 1%. The counts were made after two hours of TTC viability test applied (Norton, 1966). IKI solution was prepared as 1g KI + 0.5g I dissolved in 100ml distilled water. The counts were made few minutes after pollen grains were placed on IKI solution. To prepare safranine solution, 1g safranine was dissolved in 40ml 95% alcohol and 100ml stock solution was prepared with 60ml distilled water. The solution used in staining was prepared as mixing 20ml from this solution with 40ml glycerol and 20ml distilled water. The counts were made one hour after pollen grains placed on safranine. The study was conducted with a total of eight replicates as two slides and randomly chosen four areas for each type in three separate tests performed to determine viability levels of pollen grains.

Storage duration of pollen grains: The pollen grains were stored in open-lid vials at +20˚C at ambient condition, in open-lid vials in a desiccator at +10˚C in a refrigerator and in open-lid vials in a desiccator at –20˚C in a deep-freezer. The germination tests were performed every day in pollen grains stored in ambient conditions, every other day

in pollen grains stored in the refrigerator and every month in pollen grains stored in the deep-freezer. The concentrated Petri method containing with 1% agar + 10% saccharose was used as a germination medium (Ak & Kaşka, 1997).

Data analysis: The analysis of variance (ANOVA) was performed with the data obtained from the experiments using with a custom-designed TARİST statistical analysis computer package program with completely randomized design. The least squared difference (LSD) test was used to determine the difference between the means and the means were placed in different groups.

Results and Discussion

The male pistachio and terebinth trees used in this study have an erect growing habit which is a desirable trait in male trees. Therefore, the erect-grown-male trees can provide a fairly good pollination and fertilization in the orchard although their number is limited (Atlı, 1995). The mean number of male flower cluster in a one-year shoot was found as 5.88 in pistachio and 5.81 in terebinth. In the stage of 70% flowering in the experimental trees, the mean length of flower cluster was measured as 5.18cm in pistachio and 4.12cm in terebinth.

Amount of pollen production: The difference between pistachio and terebinth according to the pollen production was low, and it was not significantly important (Table 1).

Among the pistachio trees, the highest and lowest amount of pollen grain per flower were determined as 176 250 and 146 250, respectively. Among the terebinth types, the amount of pollen grain per flower was changed between 165 000 and 135 000, and the types were found statistically different according to the amount of pollen grain production.

Pollen grain viability tests: While pollen viability ratios were close to each other using IKI and safranine tests in two species, that was higher in pistachio using TTC test. The pollen grain viability ratio was obtained the highest in pistachio in safranine 80.91% and the lowest in terebinth in 69.68% (Table 2).

The highest and lowest pollen grain viability ratios were obtained as 88.24% (in safranin test) and 70.18% (in TTC test), respectively, in pistachio types. The highest and lowest pollen grain viability ratios were obtained as 85.36% (in IKI test) and 63.73% (in safranine test), respectively, in terebinth types.

In TTC viability test, Ak (1992) determined that the pollen grain viability was 90% in *P. vera* and 80% in *P. terebinthus*. Atlı (1995) obtained the highest and lowest pollen grain viability of 98.80% and 85.70%, respectively, in *P. vera*. In our TTC viability test, it was found that the mean and highest pollen grain viability were 76% and 79.96, respectively, in pistachio and 70% and 74.92%, respectively, in terebinth types.

The storage duration of pollen grains: Storage of pollen grains in room conditions. In the pollen grain germination experiments stored in room conditions, pistachio gave higher germination ratios than terebinth (Table 3). In pistachio, in the beginning of the experiment, the pollen grain germination ratio was changed between 78.22% and 63.29%. In terebinth, the pollen grain germination ratio was changed between 38.46% and 17.17% in the first day germination.

Table 1. Pollen production amount in pistachio and terebinth trees.

	Mean number of anther per flower	Mean number of pollen grain per flower	Mean number of pollen grain per anther
Pistachio	5.65	159 375	28 182
Terebinth	4.55	155 625	34 215
LSD 0.01		ns	
Pistachio No			
1	5.85	176 250	30 128
2	5.50	146 250	26 591
3	5.60	161 250	28.795
4	5.65	153 750	27 212
LSD 0.01		ns	
Terebinth No			
1	4.60	157 500 a	34 239
2	4.60	165 000 a	35 870
3	4.45	165 000 a	37 079
4	4.55	135 000 b	29 670
LSD 0.01		20 910**	

ns= Non-significant
*: p= Significant at 5% alpha level
**: p= Significant at 1% alpha level

Table 2. The pollen grain viability ratios of pistachio and terebinth types using TTC, IKI and safranine tests.

	TTC viable (%)	IKI viable (%)	Safranine viable (%)
Pistachio	75.68 a	78.30	80.91
Terebinth	69.68 b	78.60	78.33
LSD 0.01	5.31 *	ns	ns
Pistachio No			
1	73.68 ab	79.39 ab	77.14 b
2	78.91 a	85.34 a	88.24 a
3	70.18 b	78.11 ab	78.70 b
4	79.96 a	70.38 b	79.55 b
LSD 0.01	6.99 *	10.37 **	7.89 **
Terebinth No			
1	67.76	82.65 a	82.62
2	68.93	85.36 a	82.19
3	67.12	70.54 b	63.73
4	74.92	75.88 ab	84.76
LSD 0.01	ns	10.27 *	ns

ns= Non-significant
*: p= Significant at 5% alpha level
**: p= Significant at 1% alpha level

Table 3. The pollen grain germination ratios of pistachio and terebinth types stored under room condition.

	Room conditions (1% agar + 10% sucrose)			
	1.day (initial)	**2.day**	**3.day**	**4.day**
Pistachio No				
1	78.22 a	18.11	6.96	1.31
2	72.13 ab	21.49	7.93	1.41
3	68.99 ab	17.32	8.16	1.45
4	63.29 b	17.39	6.65	2.77
LSD_1	10.48 **	ns	ns	ns
Pistachio Mean	70.66 a	18.58 a	7.42 a	1.73
Terebinth No				
1	38.46	7.86	4.37	1.27
2	26.23	10.53	4.34	0.00
3	22.52	9.01	3.86	2.50
4	17.17	6.38	3.47	0.00
LSD_1	ns	ns	ns	Ns
Terebinth Mean	26.10 b	8.45 b	4.01 b	0.94
LSD_2 0.01	8.95 **	6.61 **	2.80**	ns

ns= Non-significant

*: p= Significant at 5% alpha level

**: p= Significant at 1% alpha level, LSD_1 determines the differences among the genotypes (1, 2, 3, 4), LSD_2 determines the differences between means of male pistachio and terebinth

The pollen grain germination ratios were dramatically decreased and reached to near zero starting from the 4th day stored in both species under room conditions. Therefore, after 4th day the storage was ceased. Kuru & Ayfer (1990) reported that the pollen grain germination ratio of *Pistacia* species was decreased below 30% after storage for 1 day in ambient conditions. Atlı (1995) reported that while the initial pollen grain germination ratio was 80%, then that decreased to 30% at the 2nd day, to 10% at the 3rd day, and to zero at the 4th day. In contrast, Vaknin & Eisikowitch (2000) stated that after prehydration, pollen grains stored in room conditions kept their germination characteristics until seven days.

Storage of pollen grains in the refrigerator: While the pollen grain germination ratios was higher in pistachio than terebinth until 4th day of the storage in the refrigerator, that was obtained very close values in two species in the proceeding days of the storage (Table 4). The pollen grain germination ratios were found between 55.83% and 43.26% in pistachio and between 35.64% and 10.60% in terebinth at the 2nd day. In the germination tests conducted every other day, the pollen grain germination ratios started to decrease and reached to zero at the 10th day.

Kuru & Ayfer (1990) reported that the pollen grain germination ratios within the vials whose lids were closed with cotton decreased below 30% after 17 days in the desiccator and 13 days outside the desiccator under 4-9ºC temperature and 50-75% relative humidity conditions in the refrigerator. Atlı (1995) reported that while the pollen grain germination ratio was 80%, that decreased to 30% at the 7th day, to 20% at the 9th day, and to 10% at the 11th day in the desiccator at stored in closed-lid vials at +5ºC in the refrigerator.

Table 4. The pollen grain germination ratios of pistachio and terebinth types stored in the refrigerator.

	Refrigerator conditions (1% agar+10% sucrose)					
	1.day (initial)	**2.day**	**4.day**	**6.day**	**8.day**	**10.day**
Pistachio	70.66 a	48.74 a	33.55 a	4.72	2.57	0.72
Terebinth	26.10 b	25.18 b	8.37 b	4.16	2.51	0.46
LSD 0.01	8.95 **	7.32 **	3.99 **	ns	ns	ns
Pistachio No						
1	78.22 a	55.83	32.71	1.65	1.21	0.00
2	72.13 ab	43.26	31.46	6.57	4.40	1.51
3	68.99 ab	44.24	35.54	3.45	2.16	1.35
4	63.29 b	51.61	34.48	7.20	2.50	0.00
LSD 0.01	10.479 **	ns	ns	ns	ns	ns
Terebinth No						
1	38.46	27.27 a	9.19	5.04	2.44	0.38
2	26.23	27.22 a	8.75	4.63	3.20	0.63
3	22.52	35.64 a	8.30	3.45	2.20	0.40
4	17.17	10.60 b	7.24	3.51	2.20	0.45
LSD 0.01	ns	9.61 **	ns	ns	ns	ns

ns= Non-significant
*: p= Significant at 5% alpha level
**: p= Significant at 1% alpha level

Table 5. The pollen grain germination ratios of pistachio and terebinth types stored in the deep freezer.

	Deep freezer conditions (1% agar+10% sucrose)	
	1.month	**2.month**
Pistachio	12.29	1.09
Terebinth	10.55	1.09
LSD 0.01	Ns	ns
Pistachio No		
1	11.24 b	1.50
2	23.31 a	0.87
3	8.59 b	0.92
4	6.02 b	1.05
LSD 0.01	11.283 **	ns
Terebinth No		
1	15.21 a	0.76
2	12.78 a	0.89
3	11.74 a	1.37
4	2.47 b	1.35
LSD 0.01	5.28 **	ns

ns= Non-significant
*: p= Significant at 5% alpha level
**: p= Significant at 1% alpha level

Storage of pollen grains in the deep freezer: The pollen grains of pistachio and terebinth species showed similar and very low germination ratios under the storage in the deep freezer during one and two months (Table 5). The highest pollen grain germination ratios were 23.31% in pistachio and 15.21% in terebinth at the end of one month storage.

The reason of not to store for pollen grains longer period without spoilage that humidity could not be controlled sufficiently in this study. Atlı (1995) reported that the pollen grain germination ratio whose initial was 80% in the beginning of the storage decreased to 50% after one month, to 30% after two months, and even to zero in some types in -18ºC deep freezer. Polito & Luza (1988a) observed that 'Peters' pollen grains maintained their germination ability after 4 months stored at -20°C, but their germination ability was decreased drastically in the 12th month. While it was stored at -20°C at 33% RH, 'Peters' pollen showed high germination ratio until the end of the 12th month (Polito & Luza, 1988b).

In conclusion, pistachio whose pollen production, viability, and germination ratios are high should be preferred to terebinth in both natural and artificial pollinations studies. Although the anthesis time of terebinth to female pistachio is closer than male pistachio, due to the low germination ability, the male terebinth was observed not to be suitable for pollination and fertilization. More studies are required for the pollen grains to be stored in the refrigerator and deep freezer used in artificial pollination. Sufficient and on-time cultural practices applied in orchards can improve pollen grain quality.

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