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Morphological and qualitative study of pistachio (*Pistacia vera L.*) pollen grains and effect of different temperatures on pomological traits

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Abstract

The viability, pollen tube growth and morphological characters associated with pistachio pollen quality are important for plant breeders and growers. Different kind of pollens of fruit trees has shown different morphological and pomological characteristics. Germination rate and growth of dry and wet pollen requires to be studied if confronted to low temperatures. This research was conducted, in order to investigate the effect of macro and microelements, protein content, morphological characteristics, in natural and artificial conditions, on germination of dry and wet pollens of four mid-season flowering male genotypes of pistachio. A factorial experiment in a completely randomized design (CRD) was applied with 33 treatments and 3 replications. Size of measured pollens was different from 20 to 24 µm; most of them were circular shaped with different surface characteristics. The highest and lowest germination rate of fresh pollens was 85% and 53% for type R28 and N2 and after 3 days, the germination was decreased to 16% and 8% respectively. The highest contents of B and Ca were measured in pollens with the highest percentages of germination as 4.2mg/100g and 386mg/100g respectively. Keeping wet pollens more than 4 hours or shorter under 0°C caused reduction in percentage of germination. Long storage of pollen grains under temperatures lower than 0°C in dried condition was possible, but 7 days after drying of pollen grains and keeping under −20°C, the percentage of germination were decreased to 30%. This descending trend continued quickly toward day 30 under −20°C and after that with lesser intensity.

Keywords: Boron, Calcium, Germination, Pistachio, Pollen, Temperature

Introduction

The pistachio tree is a dioecious plant with male and female flowers that are growing on separated trees (Acar and Ak 2001). Pistachio flowers has no petal, therefore, there is no honey bee-attraction to facilitate indirect pollination. Thus, pollination usually performs by wind. To achieve a desirable and the best economic production, pollination and fertilization is necessary. Providing a large amount of pollens is so critical in pistachio growing areas, but most producers are not aware of that on performance of pistachio (Ak et al, 1995). Viability and pollen tube growth and morphological characterization of pollen grains are recognized as important characteristics of plants. Genetic improvement is able to modify these

Table 1. Comparison among macro, micro elements and protein content in pollen of 4 pistachio genotypes (Mean values)

Pollen type	K (mg/100g)	P (mg/100g)	N (mg/100g)	Fe (mg/100g)	B (mg/100g)	Mg (mg/100g)	Ca (mg/100g)	Protein (mg/100g)
N2	607 [d]	187 [a]	61 [c]	153 [c]	2.8 [b]	173.9 [bc]	188.4 [d]	381.2 [b]
N16	883 [a]	160 [b]	95 [a]	582 [c]	4 [a]	329.1 [a]	305.2 [b]	593.7 [a]
R27	771 [c]	169 [ab]	88 [b]	228 [b]	2.9 [b]	168.5 [c]	291.7 [c]	550 [ab]
R28	861 [b]	148 [c]	88 [b]	239 [b]	4.2 [a]	192.9 [b]	386.3 [a]	550 [ab]

*Means within a column followed by the same letter are not significantly different at $P \leq 0.05$.

vital specifications in pistachio easily. We need some simple breeding methods such as selection of pollinators to improve potential of new pistachio orchards (Bolat and Pirlak 1999; Martinez and Herrero 1994). Several methods have been applied yet in order to distinguish the differences between pistachios varieties. Methods of identification are usually founded based on pollen shape and external features (Moore and Webb 1978). Fogle (1977) suggested that morphology of the pollen grain, including pollen shape, pores, size and exine pattern, are relevant criteria for identification of cultivars (Moore and Webb 1978). There are significant differences between various species of a particular genus, in size, shape and surface features of pollen (Marthens and Fretz 1980; Davarynejad et al, 1995). Other scientists have reported that size and shape of pollens are different and unstable within species and even cultivars (Talaei and Imani, 1998). Acar and Ak (2001) achieved to the high germination rate, in pistachio pollens, around 94% and 72% by using 10 – 20% and 15% sucrose solution respectively. Several researchers have studied the effect of macro-microelements, and amino acids on pollen germination (Davarynejad et al, 1995; Rashed et al, 1998). They suggested that there are some relationships between variables such as macro-microelements and pollen germination. The pollen grains of pear trees that were sprayed with B and Ca have shown the highest percentages of germination (Wol 2001).

In this research, correlations between vital variables such as pollen shape and percentage of germination in different medium and temperatures were investigated in four dominant male genotypes in order to determine the best condition for pollen storage and reproduction.

Materials and Methods

This research was conducted in 2005 at Iranian pistachio research institute- Rafsanjan and Islamic Azad university-Tehran branch.

Plant Materials

Pollen grains were collected from four different male genotypes of pistachio trees N2, N16, R27, and R28. The cluster of male flower attached to the branch, was excised slightly before full blooming and quickly transferred to laboratory and immersed in the jar of water. Then, full blooms were bundled and placed over a white fabric and pollens screened through a small resource mesh. Pollens were then collected in a separate damp bottle.

Temperature treatments

The damped bottles were sealed and incubated in three separate incubators at 0°C, –2°C and –4°C with three different incubation periods of (2hr, 4hr and 6hr). A portion of collected pollens were placed in open and dry bottles in three different desiccators containing ($CaCl_2$) at four different temperatures of 0°C, –2°C, –4°C and –20°C for about 7 and 30 days . All temperature treated along fresh pollen grains (as control) were examined for germination in room temperature (24°C). Fresh pollen grains usually have the life period of 24hr, 48hr and 72hr.

Medium

Two different medium were used for germination testing. First medium contained just 15% Sucrose, 1% Agar and second medium consisted of 15% sucrose, 1% Agar, 0.03% $CaNO_3$ and 20 ppm boric acid with pH=7 (AK et al, 1995).

Table 2. Comparison among storage time and type of pollen in natural condition on germination rate of 4 pistachio cultivars (Mean values)*

	Time of storage			
Pollen type	**Fresh Pollen (%)**	**24 hours (%)**	**48 hours (%)**	**72 hours (%)**
N2	**53** ^{d}	**45** ^{e}	**23** ^{g}	**8** ^{i}
N16	**71** ^{b}	**62** ^{c}	**41** ^{ef}	**14** ^{h}
R27	**62** ^{c}	**51** ^{d}	**36** ^{f}	**14** ^{h}
R28	**85** ^{a}	**75** ^{b}	**52** ^{d}	**16** ^{h}

*Means within a column followed by the same letter are not significantly different at $P \leq 0.05$.

Pollen grains were place on medium petri dishes by a small brush after each different temperature treatments and incubated at 24°C. After 24 hours, 100 pollens from each treatment were examined visually under binocular and then germinated pollen grains having pollen tubes were counted. Micrographs were captured by SEM electronic microscope from the treated by –20°C pollens. Pollen grains re-dried in the room temperature and placed on aluminum small plates to facilitate covering with gold particles. Then, plates were placed 3 minutes in coater container under vacuum condition and pollen grains were covered with gold particles and observed by LEO electronic microscope model 44oi with magnification of X4000, X1200 and X10,000 3 times each.

Macro-Micro element and protein studies

Some part of the pollen grains was used to measure the macro and micro elements and protein contents. Most of these elements such as Ca, Mg, B, Fe were determined by an atomic absorption method. The potassium content was determined by flame photometry (Tekin et al, 1995). The protein contents of pollen grain samples were measured by Bradford method (Romangoli et al, 2003). The used terminology is according to Walker and Doyle (1976) and Moore et al., (1991). All treatments were tested by four replicates and experiment was carried out as a factorial in a completely randomized design. The data was analyzed by MSTATC software and compared by Duncan test.

Results

Pollen size and shape

The surface of 4 genotypes was different with the exception of two genotypes of R27 & N16 which have shown slight similarity with each other (Fig 1). The average size of pollens was about 20–24µ. The Exine pattern of Genotype N2 was circular to prolate-shaped, without pores and nearly in reticulates. Pollens of genotypes R27 & N16 were distinguished circular, perforate and reticulate on surface. Pollen of R27 contained plenty of light stains on ridges. Pollen shape of N16 was circular and high perforates. Pollens of R28 were circular with little pores and obscurely reticulate.

Relationships between macro- micro elements and germination of pollen

The results of comparisons between 7 elements (macro & micro) and protein has shown in Table 1. According to analysis of variance, the effect of cultivars and elements at levels of 1% and 5% were significantly different. The top amount of N was determined in pollens of N16 (95 mg/l00g) while the least belong to N2 and the same ratio was found in content of proteins as well. Genotypes N16, R27, R28 compare to N2 showed higher protein content (Table 1). The highest content of B and Ca was found in pollen grains of R28, N16, R27 and N2, respectively.

Table 3. Comparison among time of storage, temperature and type of pollen maintained in wet container on percentage of germination (Mean values)*

	Time(hours) in 0°C			Time(hours) in - 2°C			Time (hours) in - 4°C		
Pollen type	**2**	**4**	**6**	**2**	**4**	**6**	**2**	**4**	**6**
N2	**45 ghi**	**61 d**	**20 l**	**46 gh**	**35 jk**	**20 l**	**40 hi**	**37 ghij**	**20 l**
N16	**70 bc**	**72 b**	**58 def**	**63 bcd**	**50 fg**	**46 gh**	**60 d**	**39 ghij**	**27 ij**
R27	**60 d**	**71 b**	**45 ghi**	**62 cd**	**44 ghij**	**20 l**	**59 d**	**39 ghij**	**28 kl**
R28	**81 a**	**82 a**	**60 d**	**81 a**	**65 bcd**	**50 efg**	**59 d**	**50 efg**	**44 ghi**

*Means within a column followed by the same letter are not significantly different at $P \leq 0.05$.

The highest percentage of germination in fresh pollen grains belong to R28, N16, R27 and N2 respectively (Table 2). The pollens viability also reduced within few days. Pollen grains have shown higher germination rate in second growth media which contained Boron and Calcium (Table2).

Evaluation of germination at temperatures below 0°C in dry and wet conditions

Duration and temperature of storage, type of pollen, interactions between duration and temperature, Duration and type of pollen, temperature and type of pollen showed significant differences at 1% and 5% levels. The highest and lowest percentage of germination at 0°C were about 82% and 20% in R28 (4 hours storage) and N2 (6 hours storage) respectively (Table3).

The highest percentage of germination in dry conditions was recognized on R28 (46%) under effect of storage in different conditions.

R28 and R27 showed the highest percentage of germination corresponds to interactions among type of pollen, temperature and storage time (Table 4) (cv = 4.05%).

Discussion

Pollen traits

The sculpturing features showed different exine structures in different genotypes. These kinds of differences are very important to identify cultivars of fruit trees (Davarynejad et al, 1995; Marcuci et al, 1984; Talaie et al, 1998). In addition to surface sculpture, number of exine pores could be another index for identification of cultivars (Bolat and Pirlak, 1999). In this research N2 genotype had almost no hole and R28 genotype had some little holes. Pollens of N16 and R27 have large holes due to genetic variability (Romangoli et al., 2003). Study on 4 male genotypes showed that they are obviously different in size and surface (exine) which should be because of disorders in mitosis (Martens and Fretz, 1980). In addition, wet and immature pollen grains showed weak and unstable construction whilst dry pollen grains were quite rigid, stable and suitable for SEM microphotography. In general, the morphological characteristics of pollen grains could be appropriate index for identification of male trees and suitable cultivars, particularly when they jointly show well physiological and qualitative characteristics.

Relationship of macro- microelements, and germination

Existence of Ca and B elements in pollen grains and role of them in germination rate have been discussed by researchers (Nyomora et al., 2000; Parre and Geitman 2004; wol et al., 2001). All pollen samples showed the highest percentage of germination in first day (fresh pollens) and then gradually decreased in following days. Storage of pollen grains on fresh air caused quick reduction in germination rate. For example, Pollen grains of cultivar R28 showed the highest percentage (85%) in first day and then 75%, 52% and 16% in the following days and therefore faced a major drop and reduction (CV: 6.46%)(Acar and Ak 2001; Crane et al, 1974; Martinez and Herrero 1994). Some proteins in pollen grains probably are enzymes that control growth of pollen

Table 4. Comparison of germination rate among different dried pollens after 7 and 30 days of storage*

	Time of maintenance (day)	
Pollen type	**7 days**	**30 days**
N2	**30 e**	**21 f**
N16	**49 a**	**40 cd**
R27	**45 b**	**36 d**
R28	**50 a**	**43 b**

*Means within a column followed by the same letter are not significantly different at $P \leq 0.05$.

tube and fertilization. Size, amount of proteins in pollen grains and distance between stigma and ovary are important characters (Roulston et al., 2000). The role of Boron and Calcium elements in proper settlement of pectin and callus on the pollen tube is obvious. Pollen grains with insufficient Boron contain have extra phenolic acid, high carboxylic and saturated esters compare to normal pollens.
Furthermore, inadequate Boron causes gathering of pectic acids alongside of pollen tube walls which could be associated to increase in volume of carboxylic acids and dead of pollen grains (Wang et al 2003). Optimum Calcium content is also important in elasticity of cell membrane and pectin's structure. Calcium concentrations between 300 ppm to 500 ppm cause proper germination of pollen grains, but low concentration of Calcium led to imbalance germination and finally incompatibility (Brewbaker and Kwack 1963; Parre and Geitman 2004).

Effects of temperatures below 0°C on germination of pollen at dry and wet conditions

Severe reduction, up to 20%−30% was occurred in germination rate at temperatures below −20°C with passing of time (Table 3). Storage of pollen grains for 4 hours in 0°C and − 4°C reduced the germination rate to 82% and 50% respectively, which is evidence of damage to pollen grains because of freezing condition (Table 3).
Resistance of pollen to low temperatures is different depends on cultivar and blooming time. The pollen of different pistachio trees with early blooming shows more resistance to low temperatures (Polito et al., 1988). One of critical points in germination of fresh pollen grains is their ability to completion of membrane structure in the time of water absorption (Heslop 1974). Weakness of water absorption in low temperatures probably causes incompletion of membrane structure. This could be due to loss of elasticity in time of water absorption in low temperatures (Hoekstra 1984). Pollens showed the higher rate of tube growing when they were culturing in higher density on Petri dishes. A visual experience to detecting higher growing rate of tubes is the random and unusual scattering of pollen grains upon transplanting by small brush to media.
Enrichment of media with nutrients could greatly help to ability of pollens to make more ovules that are fertile.

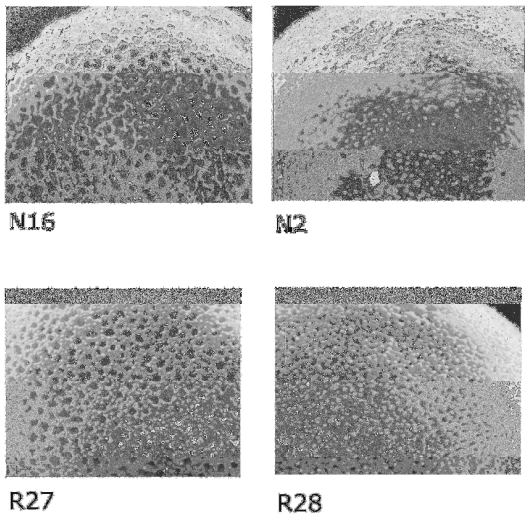

Fig 1. Comparison of four Scan Electron micrographs of different pollen genotypes of pistachio (10000 X)

Pollens begin to compete in the rich conditions, which can be useful, to making more fertile grain's mixture during germination period (Brewbaker and Kwack 1963).
Effects of pollen grains with each other might be due to exudation of dissolved materials from pollen grains that contains growth elements like Calcium (Pasonen and Kapyla 1988).

Conclusion

This investigation revealed huge differences in pollen morphological features among genotypes, which can potentially be used for cultivar identification. Pomological investigations into cultivated male genotypes also showed that wet pollen grains are damaged by temperatures below zero.

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