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The effects of temperature on *in vitro* pollen germination and pollen tube growth of *Pistacia* spp.

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ABSTRACT

The pollen germination and pollen tube growth among nine *Pistacia* genotypes was quantified in order to identify differences in the tolerance of pollen to temperature variations. The effect of temperature on *in vitro* pollen germination and pollen tube growth was investigated in *Pistacia vera* (Uygur, Atli, Kaska, Sengel, Kavak), *P. atlantica*, *P. khinjuk*, *P. terebinthus* and *P. palaestina*. When pollen was incubated in a germination medium for 24 h in darkness, distinct differences were observed in pollen germination and pollen tube growth at different temperatures.

Pollen was collected at anther dehiscence and was exposed to temperatures from 5 to 40 °C at 5 °C intervals. There were some differences between the percentage of pollen germination and pollen tube growth at different temperatures. *Pistacia* species and cultivars were found to range from most tolerant to most susceptible, depending on pollen characters.

Pollen germination of the genotypes ranged from 83% to 97% and pollen tube length ranged from 697 to 1270 μm. A modified bilinear model best described the response to temperature of pollen germination and pollen tube length. Genotype variation was found for cardinal temperatures (T_{min}, T_{opt} and T_{max}) of pollen germination percentage and pollen tube growth. Mean cardinal temperatures averaged over nine genotypes were 6.7, 23.9 and 41.1 °C for maximum percentage pollen germination and 6.4, 23.9 and 39.8 °C for maximum pollen tube length.

1. Introduction

Temperature is the important factor controlling plant growth and development. Fruit set in many crops is sensitive to high temperature. Pollen grains, once released from anthers, act as independent functional units and are exposed to the ambient environment. Therefore, high temperature during flowering would more severely affect pollen than the deeply seated ovules (Kakani et al., 2005).

The genus *Pistacia* is a member of the *Anacardiaceae* family and consists of at least eleven species (Zohary, 1952) including *Pistacia vera* L., the cultured pistachio, which has edible nuts and considerable commercial importance. The pistachio tree is dioecious, meaning the male flowers are borne on one tree and the female flowers on another. Therefore, the production of nuts requires both male and female trees. *Pistacia* pollen is spread by wind. There is no self or cross incompatibility between the *Pistacia* species, and all *Pistacia* male trees may pollinate females (Ayfer, 1964; Ak, 1992; Acar, 2004). Fertilization occurs between 24 and 48 h after pollination, due to slow growth of the pollen tube (Ayfer, 1967; Shuraki and Sedgley, 1994; Acar, 2004).

Pollen performance, which includes pollen germination, pollen tube growth rate and pollen competition, is an important component of fertilization success in seed-producing plants. Pollen performance is clearly affected by the genotype of the pollen (Snow and Spira, 1991). Temperature is one of the most important environmental factors that could affect pollen performance during the progamic phase (Hedhly et al., 2005). It has been shown that temperature affects pollen germination (Elgersma et al., 1989; Shivanna et al., 1991), and pollen tube kinetics in the style (Jefferies et al., 1982; Elgersma et al., 1989). Temperature ranges and optimum temperature values for pollen germination and pollen tube growth were studied for different fruit species, including jojoba (Lee et al., 1985), pears (Mellenthin et al., 1972; Vasilakakis and Porlingis, 1985), papaya (Cohen et al., 1989), cherimoya (Rosell et al., 1999), mango (Sukhvibul et al., 2000), *Prunus mume* (Wolukau et al., 2004), almond (Godini et al., 1987), apricot (Vachun, 1981; Egea et al., 1992; Austin et al., 1998; Pirlak, 2002), sour cherry (Cerovic and Ruzic, 1992), and sweet cherry (Pirlak, 2002). The optimum temperature required for pollen germination was about 15 and 20 °C for apricot, sour cherry and sweet cherry (Vachun, 1981; Egea et al., 1992; Cerovic and Ruzic, 1992; Austin et al., 1998; Pirlak, 2002). Thus, temperature effects on pollen germination are incon-

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sistent and seem to be cultivar or species dependent (Song et al., 1999; Sukhvibul et al., 2000).

Although optimum temperature for pollen germination and pollen tube growth is known to vary among and within species, there is no extensive study, within the literature, on the response of pollen germination and pollen tube growth to germination temperature ranges in *Pistacia* species. Only limited studies have been published on the effects of temperatures on pollen germination of pistachio (Therios et al., 1985). The cardinal temperatures (T_{min}, T_{opt} and T_{max}) for maximum percentage pollen germination and maximum pollen tube length have not been reported for pistachios. Therefore, this is the first study of the effect of temperature on pollen germination and pollen tube growth of *Pistacia* spp. The objective of this study was to quantify the response of *in vitro* pollen germination and pollen tube growth to temperatures from 5 to 40 °C, and to determine the cardinal temperatures (T_{min}, T_{opt} and T_{max}) for *Pistacia* spp.

2. Materials and methods

2.1. Plant material

The present study was carried out on nine *Pistacia* spp. consisting of *P. atlantica*, *P. khinjuk*, *P. terebinthus*, *P. palaestina* and Uygur, Atli, Kaska, Sengel and Kavak genotypes of *P. vera*, maintained at the experimental orchards of the Pistachio Research Institute in the Gaziantep province of Turkey.

Pollen from the *Pistacia* genotypes was obtained at the beginning of blooming from inflorescences collected randomly from male trees. Inflorescences that had some flowers with dehiscent anthers were removed from the male trees, transferred to the laboratory, and spread over tissue paper. Pollen that was shed overnight was sieved and collected in laboratory conditions.

2.2. In vitro pollen germination and pollen tube growth

In vitro germination was assessed with the hanging drop method. Pollen germination and pollen tube growth were determined by placing a small drop of germinating media on a cover glass; pollen grains were sown on the drops with a clean brush, and the cover glass was then inverted and rested on the cavity slide. Pollen was incubated under dark conditions in 20% (w/v) sucrose medium for 24 h from 5 to 40 °C at 5 °C intervals. Following this incubation period, distinct differences were observed in pollen germination and pollen tube growth between the groups incubated at different temperatures. For each treatment, germination was recorded in three drops by counting three fields. A pollen grain was considered germinated when pollen tube length was at least equal to or greater than the grain diameter (Kakani et al., 2002). Germination percentage was determined by dividing the number of germinated pollen grains per field of view by the total number of pollen per field of view and expressed as percentage.

Measurements of pollen tube length were recorded directly by an ocular micrometer fitted to the eyepiece of the microscope. Mean pollen tube length was calculated as the average length of nine pollen tubes measured from each drop after 24 h. The replicated values on maximum pollen germination and tube length were analyzed using the one-way ANOVA procedure (SAS Institute, 1997).

2.3. Curve fitting procedures and determination of cardinal temperatures

The maximum pollen germination and pollen tube lengths recorded after 24 h were analyzed by linear and non-linear regression models commonly used to quantify developmental responses to temperatures (Kakani et al., 2002). The fit of each regression equation describing the response of pollen germination and pollen tube lengths to temperatures was compared for the amount of variation accounted for by R^2 and root mean square deviation (RMSD) for observed and fitted values. The highest R^2 and lowest RMSD for pollen germination and pollen tube lengths were obtained using quadratic and modified bilinear models, respectively. Accordingly, the cardinal temperatures were calculated from the fitted equations for all the genotypes.

The non-linear regression procedure PROC NLIN (SAS Institute, 2004) was used to estimate the parameters of the quadratic and modified bilinear equation. For the quadratic model, minimum (T_{min}), optimum (T_{opt}), and maximum (T_{max}) temperatures were estimated by Eqs. (1)–(3) as follows:

$$\text{Pollen germination} = a + bT - cT^2$$

$$T_{opt} = \frac{-b}{2c} \quad (1)$$

$$T_{min} = \frac{-b + (\sqrt{b^2 - 4ac})}{2c} \quad (2)$$

$$T_{max} = \frac{-b - (\sqrt{b^2 - 4ac})}{2c} \quad (3)$$

where, T is actual treatment temperature, and a, b and c are genotypic-specific constants generated using PROC NLIN in SAS. For the modified bilinear equation, T_{opt} was generated by fitting the model (4) by SAS, and T_{max} and T_{min} were determined by Eqs. (5) and (6) (Kakani et al., 2002) as shown below:

$$\text{Pollen tube length} = a + b_1(T - T_{opt}) + b_2 \times ABS(T_{opt} - T) \quad (4)$$

$$T_{min} = \frac{a + (b_2 - b_1) \times T_{opt}}{b_1 - b_2} \quad (5)$$

$$T_{max} = \frac{a - (b_2 + b_1) \times T_{opt}}{b_1 + b_2} \quad (6)$$

3. Results

3.1. Pollen germination

The effect of eight constant temperature regimes (from 5 to 40 °C at 5 °C intervals) on the *in vitro* pollen germination of nine *Pistacia* genotypes was evaluated and expressed as the percentage of germinated pollen grains (Table 1). There was no pollen germination observed for the *Pistacia* genotypes at a temperature of 40 °C

Table 1
Maximum pollen germination percentage and cardinal temperatures for pollen germination of nine *Pistacia* genotypes in response to temperature.

Genotype	Maximum pollen germination (%)	Cardinal temperatures (°C)		
		T_{min}	T_{opt}	T_{max}
Uygur	88.7	8.4	26.7	45.0
Atli	97.4	5.2	23.0	41.0
Kaska	93.8	5.0	23.0	41.1
Sengel	92.7	5.8	22.5	39.2
Kavak	91.6	6.7	24.0	41.4
Pistacia atlantica	96.0	7.2	24.3	41.5
Pistacia khinjuk	83.3	7.2	23.7	40.1
Pistacia terebinthus	89.0	7.3	24.1	41.0
Pistacia palaestina	92.0	7.6	23.7	40.0
Mean	91.6	6.7	23.9	41.1
s.e.d.	5.17*	0.23**	0.32**	0.47**

* Significant at $P < 0.05$ level.
** Significant $P < 0.01$ level.

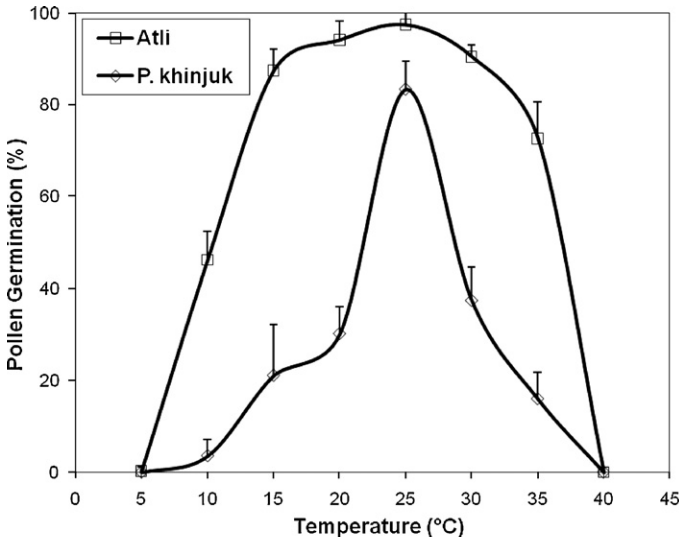

Fig. 1. Pollen germination of two pistachio genotypes (*P. khinjuk* and Atli) in response to temperature. Genotypes with variation for maximum pollen germination are presented for clarity. Error bars indicate ± SD.

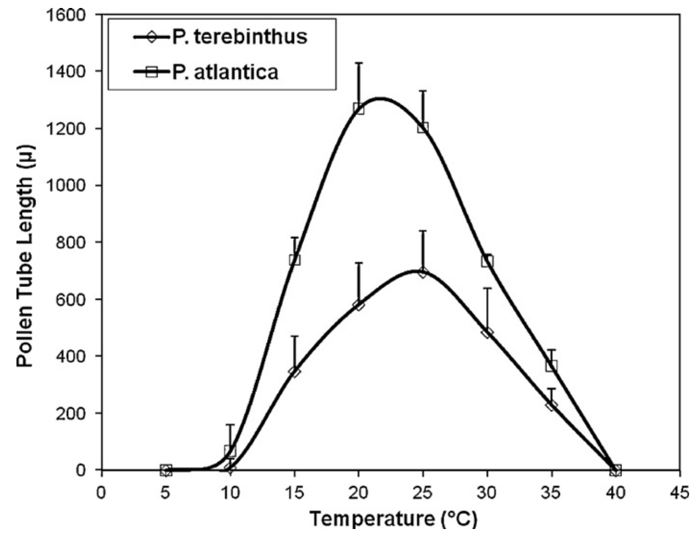

Fig. 2. Pollen tube length of two pistachio genotypes (*P. terebinthus* and *P. atlantica*) in response to temperature. Genotypes with variation for maximum pollen tube length are presented for clarity. Error bars indicate ± SD.

(Fig. 1); only the pollen grains of four genotypes (Atli, Kavak, Sengel and *P. palaestina*) germinated at 5 °C, and the germination rates were below 1%.

Fig. 1 shows the variation for pollen germination in response to temperature of two *Pistacia* genotypes for clarity. There was considerable variability in the cardinal temperatures for pollen germination among the genotypes. Maximum germination percentage ranged from 83.3% (*P. khinjuk*) to 97.4% (Atli), with a mean of 91.6% (Table 1). Cardinal temperatures for pollen germination differed among genotypes. Values of T_{min} ranged from 5.0 °C (Kaska) to 8.4 °C (Uygur) with an average of 6.7 °C. Optimum temperature (T_{opt}) ranged from 22.5 °C for Sengel to 26.7 °C for Uygur with an average of 23.9 °C. The T_{max} values ranged from 39.2 °C for Sengel to 45.0 °C for Uygur with an average of 41.1 °C (Table 1).

3.2. Pollen tube growth

Genotypes differed significantly in pollen tube length at optimum temperatures (Table 2). Pollen tube length ranged from 697 μm for *P. terebinthus* to 1270 μm for *P. atlantica*, with an average of 1013 μm (Table 2).

Similar to pollen germination, the variation in pollen tube length of two *Pistacia* genotypes in response to temperature is shown for clarity (Fig. 2). The modified bilinear model fit is shown for *Pistacia* genotypes that had high variation in pollen tube length and cardinal temperatures for pollen tube growth (Table 2). The T_{min} ranged from 4.0 °C for Atli to 8.7 °C for Uygur with an average of 6.4 °C. The T_{opt} ranged from 22.0 °C for Sengel to 26.4 °C for Uygur with an average of 23.9 °C. Values of T_{max} ranged from 38.0 °C for *P. palaestina* to 40.7 °C for Kavak with an average of 39.8 °C (Table 2).

On the other hand, it was determined under the microscope that pollen tubes of Uygur and Kaska cultivars were convoluted at 30 and 35 °C, respectively, whereas the tubes of *P. terebinthus* were shortened and often swollen at the tips at 35 °C.

Table 2
Maximum pollen tube length and cardinal temperatures for pollen tube length of nine *Pistacia* genotypes in response to temperature.

Genotype	Maximum pollen tube length (μm)	Cardinal temperatures (°C)		
		T_{min}	T_{opt}	T_{max}
Uygur	852	8.7	26.4	40.3
Atli	1069	4.0	23.1	40.3
Kaska	1194	5.6	23.6	39.9
Sengel	1042	5.2	22.0	39.0
Kavak	1096	6.1	24.4	40.7
Pistacia atlantica	1270	6.7	22.3	39.8
Pistacia khinjuk	792	6.7	24.8	40.0
Pistacia terebinthus	697	6.9	24.5	40.0
Pistacia palaestina	1109	7.7	24.2	38.0
Mean	1013	6.4	23.9	39.8
s.e.d.	78.92*	0.63**	1.33**	0.57**

* Significant at $P < 0.05$ level.
** Significant at $P < 0.01$ level.

4. Discussion

Temperature is among the most important environmental factors affecting plant reproductive processes such as pollen germination, pollen tube growth and fruit set (Kakani et al., 2005), and had also an effect on pollen performance, both on pollen germination and on pollen tube growth of *Pistacia* spp.

The current study, which examined pollen response to temperature from 5 to 40 °C, shows very clearly that pollen germination and pollen tube growth processes can also be described in terms of temperature. All nine genotypes had clear temperature optima, above and below which pollen germination and maximum pollen tube length were reduced, and this response was well-described by a modified bilinear regression model. Pollen from the different genotypes tested in this work responded differently. *In vitro* pollen germination and pollen tube growth of all genotypes were severely reduced under both high and low temperature conditions. The optimum value for both pollen germination and pollen tube length was 23.9 °C for the *Pistacia* genotypes used in the study.

The effects of temperature on pollen germination and pollen tube growth have been reported by several researchers. Pistachio pollen germination was slow at low temperature and increased linearly, and reaching its maximum value at 25 °C; beyond that degree, germination percentage decreased (Therios et al., 1985). Low temperature did not inhibit *in vivo* pollen germination of apricot at 10 °C (Austin et al., 1998), and that of sour cherry at 15 °C (Cerovic and Ruzic, 1992). The optimum temperature required for *in vitro* pollen germination of apricot was between 15 and 20 °C, and degrees above 25 °C caused a decrease in pollen germination rates (Vachun, 1981; Egea et al., 1992). The lowest pollen germination and pollen tube length of apricot and sweet cherry were obtained at 5 °C, and the best results were observed between 15 and 20 °C (Pirlak, 2002). It was also reported that degrees lower than 15 °C reduced pollen germination in pears (Mellenthin et al., 1972; Vasilakakis and Porlingis, 1985).

The validity of the *in vitro* evaluation of pollen germination is a predictor of *in vivo* behavior (Hormaza and Herrero, 1999). There were significant differences among the *in vitro* pollen performances of different male genotypes at each temperature. The *Pistacia* pollen germination percentage was between 83% and 97% with a mean of 92% (Table 1), which was much higher than that observed by Therios et al. (1985), Atli et al. (1995), Caglar and Kaska (1995), Kuru (1995), Acar and Ak (1998), Acar (2004) and Kamiab et al. (2006). The pollen tube length was between 697 and 1270 μm with an average of 1013 μm (Table 2).

Kaska cultivar had the lowest T_{min} value of 5 °C and the pollen germination was 94%. Atli had the highest pollen germination of about 97% and had a T_{max} of 41 °C. Uygur cultivar had the highest T_{min} (over 8 °C) and the highest T_{max} (45 °C), and its pollen germination was 89%. The average cardinal temperatures for pollen germination were 7 °C (T_{min}), 24 °C (T_{opt}) and 41 °C (T_{max}) (Table 1). Atli cultivar had the lowest T_{min} (4 °C), and had an average pollen tube length (1069 μm), while Kavak had the highest T_{max} (about 41 °C), and had 1096 μm for pollen tube length. The widespread cultivar, Uygur, also had the highest T_{min} of 9 °C and a high T_{max} of over 40 °C, and the average pollen tube length was 852 μm. The average cardinal temperatures for pollen tube growth were 6 °C (T_{min}), 24 °C (T_{opt}) and 40 °C (T_{max}) (Table 2). Values obtained from the *Pistacia* spp. were similar to those reported for jojoba (Lee et al., 1985), pear (Vasilakakis and Porlingis, 1985), papaya (Cohen et al., 1989), cherimoya (Rosell et al., 1999), mango (Sukhvibul et al., 2000) and *P. mume* (Wolukau et al., 2004). According to Sukhvibul et al. (2000), the effects of temperature on pollen germination are inconsistent and seem to be cultivar or species dependent. Therefore, the differences in pollen germination and pollen tube length observed in the present study were a reflection of genotype variability.

In conclusion, this research showed that *in vitro* pollen germination and pollen tube growth of different *Pistacia* genotypes varied with temperature. The maximum percentage pollen germination and pollen tube length of genotypes, and T_{min} and T_{max} were the most important parameters describing genotypic tolerance to low and high temperatures.

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