African Journal of Agricultural Research Vol. 5(14), pp. 1830-1836, 18 July, 2010
Available online at http://www.academicjournals.org/AJAR
ISSN 1991-637X ©2010 Academic Journals

Full Length Research

Pollen susceptibility of *Pistacia* species to different pH medium

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Accepted 2 June, 2010

This study investigated the effect of different pH levels on *in vitro* pollen germination and pollen tube growth in *Pistacia palaestina*, *Pistacia terebinthus*, *Pistacia atlantica*, *Pistacia khinjuk*, and *Pistacia vera* cv Kaska. Pollen susceptibility of *Pistacia* species to acidity or alkalinity of germination medium (pH 2.0, 3.0, 4.0, 5.0, 6.0, 6.7 (distilled water), 7.0, 8.0 and 9.0) were tested. It was found that pollen germination and pollen tube growth for all species were greatly reduced when grown in the pH 3.0 medium. None of the pollen of the *Pistacia* species could germinate at pH 2.0. With growth mediums between pH 3.0 and 9.0, the amount and pattern of response in pollen germinability and tube growth varied considerably between species. According to mean pollen germination, the highest value was obtained at pH 6.0 (93.29%), while pH 3.0 had the lowest value, with 12.08%. The pollen tube growth of the species ranged from 87.6 μm (pH 3.0) to 1054.6 μm (pH 9.0). The pH of the germination medium also affected differentiation in pollen germination and pollen tube growth between the genotypes.

Key words: *Pistacia*, *in vitro*, pollen germination, pollen tube growth, pH.

INTRODUCTION

In vitro pollen germination is one of the most convenient and reliable methods used to test the viability of fresh or stored pollen. It is a valuable tool to address basic questions in sexual reproduction. The media used for *in vitro* germination of pollen of different species ranges from simple sucrose/boric acid media to complex media (Jayaprakash and Sarla, 2001). As inadequate pollination significantly reduces fruit set, it is important to study the various factors which affect pistachio pollen viability. Germination medium pH has also been shown to affect pollen germination of pistachio (Therios et al., 1985). 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, both male and female trees are required in order to produce nuts. The pollen is spread by wind. There is no self or inter- incompatibility between *Pistacia* species, and all male *Pistacia* trees may pollinate female pistachio trees (Whitehouse et al., 1964; Ayfer, 1964; Ak, 1992; Acar, 2004).

Turkey is the third largest producer of pistachio after Iran and the United States. Over 90% of Turkish pistachio production is based in the Southeast Anatolian Region of Turkey. However, pistachio trees can be grown in 56 provinces of Turkey (Tekin et al., 2001). Considerable levels of acid rain have been determined in different parts of Turkey, but the effects on the nature have not been investigated in detail (Munzuroglu et al., 2005). Acid rain affects a range of processes, such as changes of pollen physiology and ultrastructure, reduced photosynthesis, nutrient loss from leaves, altered water balance, and variation of several enzyme activities (Bellani et al., 1997, Qiu et al., 2005). In addition, pollen grains are known to be extremely sensitive to environmental factors. Pollution causes a number of changes in pollen, such as reduction in starch content, morphological immaturity at pollination time, reduction in enzyme activity, pollen germinability and tube growth (Abraitiene et al., 2003).

The pH of the *in vitro* germination medium is the important factor controlling pollen germination and pollen tube development in different plant species (Therios et al., 1985; Henny, 1977; Bellani et al., 1997; Abraitiene et al.,

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2003; Munzuroglu et al., 2003, 2005; Burke et al., 2004; Qiu et al., 2005; Mbogning et al., 2007). Once released from anthers, pollen grains act as independent functional units and are exposed to the ambient environment. Therefore, environmental conditions during flowering would more severely affect pollen than the deeply seated ovules. Although pH ranges and optimum values are known to vary for pollen germination and pollen tube growth among and within species (Proctor, 1983; Rinallo, 1989; Bellani et al., 1997; Qiu et al., 2005; Munzuroglu et al., 2003), no previous study could be identified which examined the effect of varying substrate pH on *Pistacia* species pollen germination and pollen tube growth. Therios et al. (1985) reported that the optimum incubation period in *P. vera* was 24 h after sowing the pollen on the germination medium, and the best germination percentage recorded at 25°C and pH 6. The aim of the present study was to assess the effect of pH levels of germination medium on the germinability and pollen tube growth of *Pistacia* species tree pollen grains and to determine the susceptibility of differing *Pistacia* pollen genotypes to pH.

MATERIAL AND METHODS

Plant material

This study was carried out on five *Pistacia* species consisting of *P. palaestina, P. terebinthus, P. atlantica*, *P. khinjuk* and *P. vera* cv Kaska, maintained at the Pistachio Research Institute experimental orchards located in Gaziantep, Turkey.

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

In vitro pollen germination and pollen tube growth

Germination media were prepared in distilled water (pH 6.7) and pH was adjusted with 0.1 N HCl or 0.1 N NaOH. A series of solutions of germination media with pH 2.0, 3.0, 4.0, 5.0, 6.0, 6.7 (distilled water), 7.0, 8.0 and 9.0 were prepared. The pH was measure using a digital pH meter. *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 in 20% (w/v) sucrose medium with different pH levels for 24 h at 25°C in dark conditions and then distinct differences were observed in pollen germination and pollen tube growth between the pH levels. For each treatment, germination was recorded in three drops by counting three fields.

Pollen germination (PG) was determined by direct microscopic observation (EUROMEX Bio-medical, Arnhem, Holland). Pollen grains which produced a tube equal to their own diameter were counted as germinated (Henny, 1977). Germination percentage was determined by dividing the number of germinated pollen grains per field of view by the total number of pollen grains per field of view, and was expressed as a percentage. Measurements of pollen tube length (PTL) 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 9 pollen tubes measured from each drop after 24 h. The replicated values on pollen germination and tube length were analyzed using one-way ANOVA (MSTAT, Michigan State University, Lansing). All percentage data were arcsine transformed. Duncan's Multiple Range Test was used for mean separation. Significant differences were determined at $p \leq 0.05$.

RESULTS

In vitro pollen germination and pollen tube growth

Data collected from pollen grain germination and pollen tube growth observations showed that pollen germinated in all pH values tested except for the pH 2.0 medium. None of the pollen of the *Pistacia* species could germinate at the pH 2.0 medium. Statistical analysis revealed that pH value significantly affected the pollen germination percentage and PTL of each *Pistacia* species (Figures 1, 2 and Table 1). The maximum germination rates were obtained from different pH values for each species. The pollen germination percentages were generally high within the pH range of 4.0 to 8.0, and also adequate at pH 9.0, but the pollen tube growth was low in the acidic media and highest in the basic medium (pH 9.0) (Table 1).

Figures 1 and 2 showed the variation in PG and PTL of *Pistacia* species in response to pH value. The pollen germination percentage of *Pistacia palaestina* ranged from 22.84 to 93.38%, and pollen tube length ranged from 143 to 1032 µm, at pH values of 3.0 and 6.0, respectively. There was no significant difference in germination of *P. palaestina* pollen within the pH range of 4.0 - 6.0, but PTL rapidly increased from pH 3.0 to 6.0, and then decreased at pH 7.0. At pH levels above 7.0, PTL started to increase again, while the pollen germination percentage decreased (Figure 1a). Similarly, pollen germination and PTL of *P. terebinthus* increased rapidly to 98.62 % and 1027 µm at pH 6.0, and then decreased at pH 7.0 for PTL and at pH 8.0 for PG; both factors increased again at pH 9.0 (Figure 1b). The pollen of *P. atlantica* required pH-neutral media for maximum pollen germination; Pollen germination percentage was reduced in both acidic and basic media for this species. Pollen tube growth of *P. atlantica* increased rapidly from pH 3.0 to 6.7, decreased at pH 7.0, and then reached maximum PTL (1239 µm) at pH 9.0 (Figure 1c). *Pistacia khinjuk* required acidic media for optimum pollen germination. The highest germination rate was obtained from pH 4.0 and 6.0; germination decreased gradually from pH 6.0 to 9.0, while the lowest value was observed for pH 3.0. The PTL was also reached maximum value at pH 6.0, then decreased in distilled water, and slowly increased again up to pH 9.0 (Figure 2a). The results for *P. vera* were different from the other *Pistacia* spp. used in the experiment. Germination percentage and PTL of *Pistacia* species were drastically reduced at pH 3.0, and that of *P. vera* cv Kaska stopped at pH 3.0. Pollen germination was

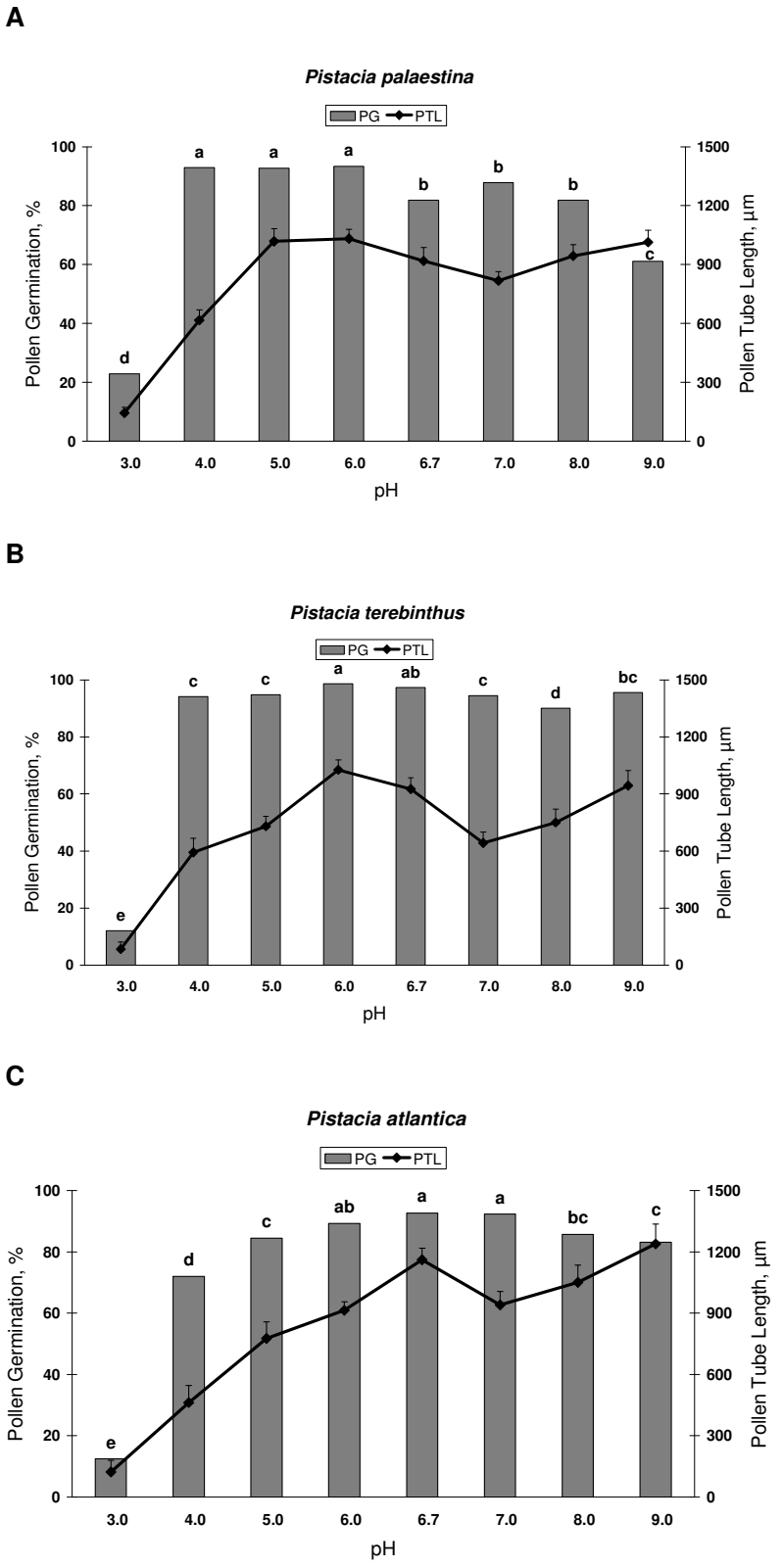

Figure 1. The response of pollen germination and pollen tube growth of *P. palaestina* (a), *P. terebinthus* (b) and *P. atlantica* (c) to different pH values of germination media. Differences in letters above the bars indicate lineages that differ significantly at $p \leq 0.05$. Standard deviations of pollen tube length were given.

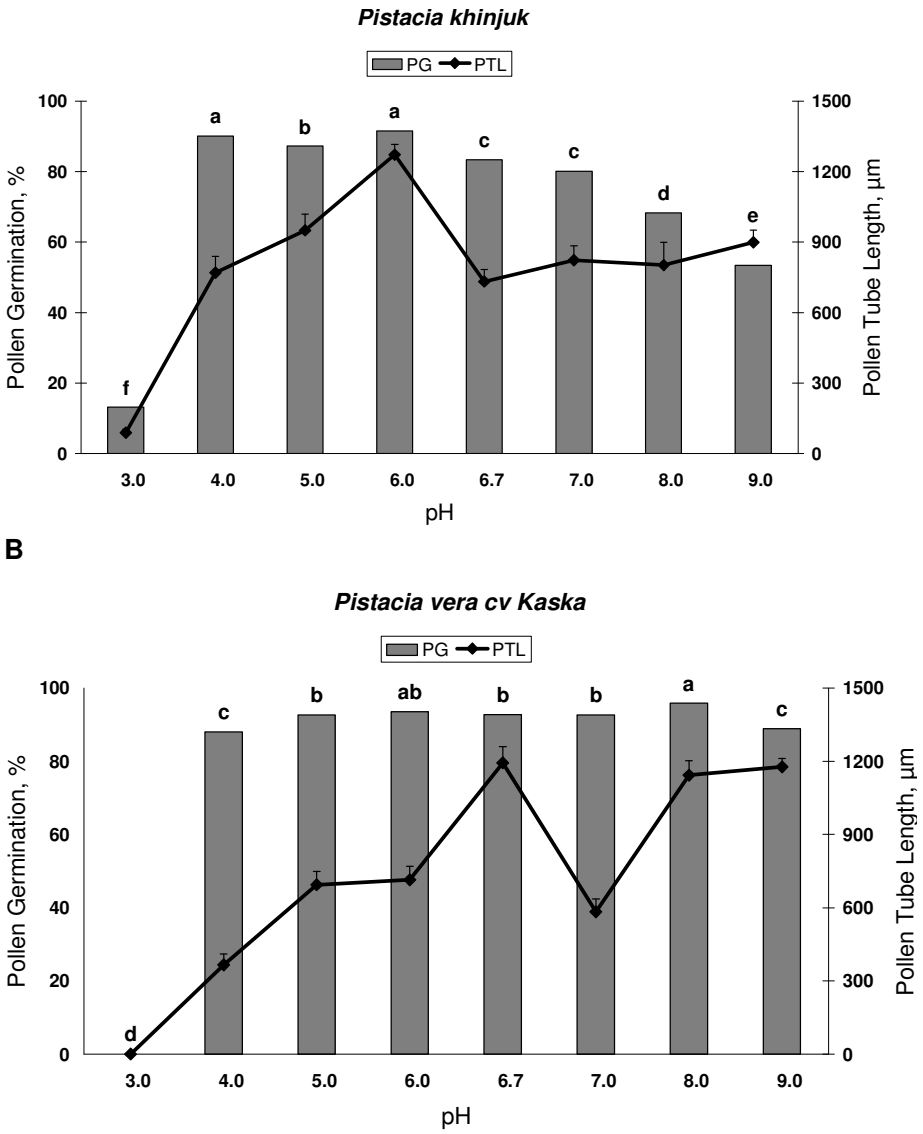

Figure 2. The response of pollen germination and pollen tube growth of *P. khinjuk* (a) and *P. vera* cv Kaska (b) to different pH values of germination media. Differences in letters above the bars indicate lineages that differ significantly at $p \leq 0.05$. Standard deviations of pollen tube length were given.

Table 1. Mean pollen germination percentage and pollen tube growth of *Pistacia* species in the different pH value of the *in vitro* germination medium.

	pH value of germination medium							
	3.0	**4.0**	**5.0**	**6.0**	**6.7**	**7.0**	**8.0**	**9.0**
Pollen germination (%)	12.08 c*	87.43 ab	90.41 a	93.29 a	89.60 a	89.38 a	84.39 ab	76.42 b
Pollen tube length (µm)	87.6 d	561.4 c	833.6 ab	991.8 a	986.2 a	761.2 bc	937.8 ab	1054.6 a

*Letters next to numbers indicate different groups determined by Duncan's test ($p \leq 0.05$).

generally high within the pH range of 4.0 to 9.0 for *P. vera* cv Kaska, and reached 95.90% at pH 8.0. The maximum PTL (1194 µm) was obtained from distilled water (pH 6.7), which then decreased sharply to 583 µm at pH 7.0, and increased again up to pH 9.0 (Figure 2b).

Average pollen germination rate and PTL of *Pistacia* spp. were calculated and compared in respect of pH value of germination media (Table 1). There was no

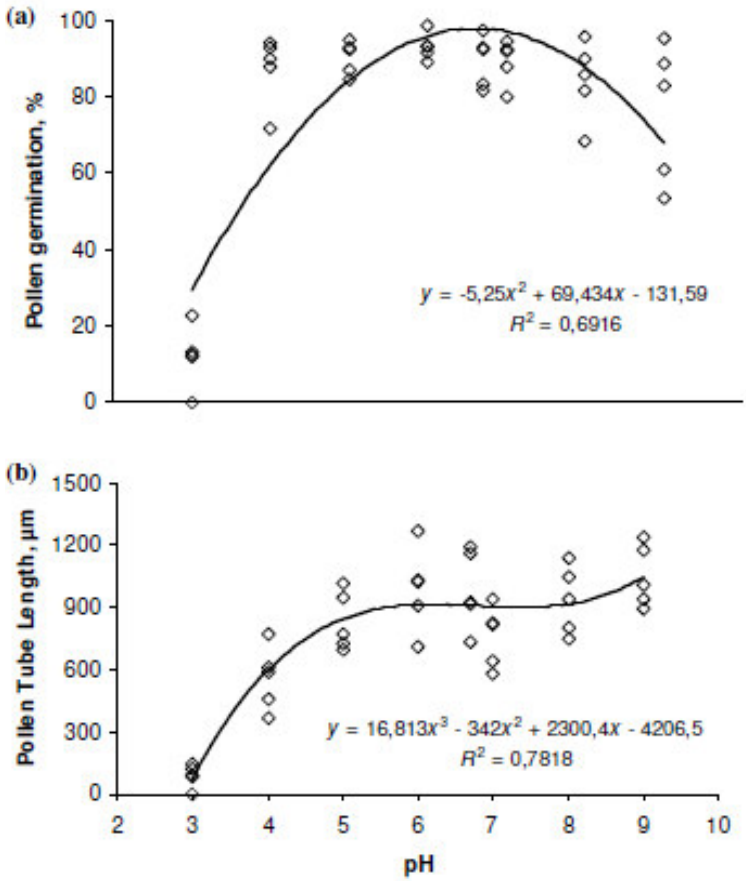

Figure 3. Relationship between pH value and pollen germination percentage (correlation = 0.4829), and pH value and pollen tube length (correlation = 0.7478). Pollen germination in response to pH value of germination media based on Quadratic regression (a), and pollen tube length in response to pH based on Cubic regression (b) of five *Pistacia* spp.

significant difference in mean pollen germination within the pH range of 5.0 to 7.0. For mean germination of *Pistacia* species used in the study, the highest germination percentage of 93.29% was obtained from pH 6.0, while the lowest value, 12.08% was obtained from pH 3.0. The germination percentage gradually decreased when the pH value of germination media increased from pH 6.0 to 9.0. There was a positive correlation between the pollen germination percentage (y) and the pH value of media (x) (correlation = 0.4829, and $y = –5.25x^2 + 69.43x – 131.59$, $R^2 = 0.6916$) (Figure 3a). The average values of PTL ranged between 87.6 µm and 1054.6 µm for pH 3.0 and pH 9.0, respectively. Similar to pollen germination, PTL increased up to pH 6.0, then decreased to pH 7.0, and increased again from pH 7.0 to pH 9.0 (Table 1). Pollen tube length was positively correlated with the pH value of germination media (correlation = 0.7478, and $y = 16.81x^3 – 342x^2 + 2300.4x – 4206.5$, $R^2 = 0.7818$) (Figure 3b). Similarly, pollen germination was also positively correlated with pollen tube length (correlation = 0.7513, and $y = 9.3127x + 51.436$, $R^2 = 0.5644$).

DISCUSSION

Formal pollination is essential for pistachio trees, because the marketable part is the seed; to obtain a good fruit set, pollination and fertilization are required. Previous studies reported that the yield and quality of nuts were influenced by pollen performance in pistachio (Ak, 1992; Acar, 2004). The processing is also another factor affecting the pistachio nut quality (Aktas and Polat, 2007; Bilim and Polat, 2008). Pollen viability depends not only on its quality, but is also related to temperature, mineral nutrient and different plant growth regulators etc. in the germination environment (Qiu et al., 2005). The pollen tube is a highly specialized cell type that delivers the sperm cells to the ovule for fertilization. Pollen tube growth is thus crucial for the process of plant sexual reproduction and food production; it is also of fundamental

interest because it is one of the fastest growing plant cell types known (Holdaway-Clarke and Hepler, 2003).

The validity of the *in vitro* evaluation of pollen germination is a predictor of *in vivo* behavior (Hormaza and Herrero, 1999). The results obtained from the present study indicated that the acidity of germination media is one of the important factors related to pollen performance of *Pistacia* spp. PG and PTL dropped with reduced pH value of germination media (Table 1). The pH of the germination medium has been shown to affect pollen germination of several plant species (Henny, 1977; Bellani et al., 1997; Abraitiene et al., 2003; Munzuroglu et al., 2003; Burke et al., 2004; Qiu et al., 2005; Mbogning et al., 2007). Therios et al. (1985) reported that the pollen germination percentage of pistachio peaked at pH 6.0; germination ratio decreased when the pH of the germination media was higher or lower, with germination percentage at pH 9.0 of only 7%. In the present study, the acidity or alkalinity of germination media had an effect on pollen performance, both on pollen germination and on pollen tube growth. This is the first study on the effect of a wide range of pH on pollen germination and pollen tube growth of different *Pistacia* spp. Pollen from the different species tested in this work behaved differently, and they were obviously affected by pH value of germination media. According to Kwack (1964), the optimal pH for pollen germination of several species was rather narrow, with the best results generally at pH 7.3 or 8.3 and the poorest results at pH 5.3. Qiu et al. (2005) reported that injury symptoms could be observed in pollen treated at pH 3.5, and pollen germination was severely reduced. The corrosive-like structure was observed on pollen wall when incubated in acidic medium that had pH 3.5 and 2.5. These results suggested that pollen is damaged and its germination reduced by acidity.

The present study also investigated the response of different genotypes to variations in pH value (Figures 1 and 2). *P. palaestina*, *P. terebinthus* and *P. khinjuk* had the maximum pollen germination and pollen tube length at pH 6.0. *P. atlantica* had the highest value for pollen germination at pH 6.7 and for PTL at pH 9.0, while in *P. vera* cv Kaska the optimum pH value was 8.0 for pollen germination and 6.7 for PTL. *In vitro* pollen germination and pollen tube growth of all species were severely reduced under low pH conditions. The average pollen germination percentage of *Pistacia* spp. was between 84.39 and 93.29% within the pH range of 4.0-8.0, with 89.60% germination in distilled water (Table 1). The results of the present study higher ranged than those reported by Henny (1977), Therios et al. (1985), Munzuroglu et al. (2003), Abraitiene et al. (2003), Qiu et al. (2005), Burke et al. (2004) and Mbogning et al. (2007). Among the *Pistacia* spp. used in the present study, the most pH tolerant species within the pH range of 3.0 - 9.0 were *P. terebinthus* for pollen germination and *P. atlantica* for pollen tube length. Therefore, the observed differences in pollen germination and pollen tube length in the present study were a reflection of genotype variability. There were obvious correlations between pH and pollen tube length, and pH and pollen germination in *Pistacia* spp. (Figure 3). On the other hand, correlation was also observed between pollen germination and pollen tube length. We infer from these results that the pH level of *in vitro* germination media is the important factor controlling pollen germination and pollen tube growth for *Pistacia* spp. In conclusion, *Pistacia* spp. pollen was generally more tolerant of a basic medium than acidic; however, their pollen was adaptable to a wide range of pH levels. Those species with higher pollen germination and pollen tube growth *in vitro* can be evaluated as wide pH range tolerant.

ACKNOWLEDGMENT

The authors thank Kamil Sarpkaya for advice on statistics.

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