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Full Length Research Paper

Effects of boron and gibberellic acid on *in vitro* pollen germination of pistachio (*Pistacia vera* L.)

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This study was conducted on male pistachio cultivars which consisted of Uygur, Atli, Kaska, Sengel and Kavak to study the influence of boron and gibberellin on pollen germination *in vitro*. Pollen was sown in germination media that included 20% sucrose, 10, 25, 50, 75 and 100 ppm boric acid (H_3BO_3) and gibberellic acid (GA_3), separately. It was found that pollen germination for all cultivars were greatly reduced with increased GA_3 concentration in the germination medium and reached the lowest value at the 100 ppm GA_3 whereas germination was decreased up to 25 ppm in H_3BO_3 and gradually increased again to 100 ppm. With the mediums of boric acid and gibberellic acid ranging from 0 - 100 ppm, the amount and pattern of response in pollen germinability varied among cultivars considerably. Pollen germination was severely inhibited by GA_3 and slightly promoted by boron. The results suggest that gibberellic acid had adverse effects on pollen germination of pistachio.

Key words: Pistachio, *Pistacia vera*, *in vitro*, pollen germination, boron, gibberellic acid.

INTRODUCTION

Pistachio is a dioecious and wind pollinated fruit species; the pistillate and staminate flowers are formed on different trees. Both the staminate and pistillate inflorescences form panicles with up to several hundred individual flowers (Crane and Iwakiri, 1981). Pollination is important for this species; the marketable part is the seed and to obtain a good fruit set, adequate numbers of suitable male trees have to be interplanted in the orchards, located according to wind and rain conditions (Acar and Eti, 2008). Inadequate pollination significantly reduces fruit set in pistachio trees; therefore, it is important to study the various factors that affect the pollen viability.

In vitro pollen germination rates are considered the best indicator of pollen viability (Shivanna et al., 1991). Most researchers advise on the use of sucrose solution as pollen germination media. Besides boric acid, calcium, gibberellic acid, agar, etc. may be used as media (Johri and Vasil, 1961). Acar and Ak (1998) achieved the high germination rate in pistachio pollens by using 15 and 20% sucrose solutions. Brewbaker and Kwack (1964) reported that calcium and boron induced pollen germination *in vitro*. According to Acar (2004), the optimum *in vitro* pollen germination conditions for pistachio trees were 20% (w/v) sucrose medium, hanging drop germination method and 25°C temperature in dark conditions for 24 h. In nature, water, sugar and amino acids are supplied by the style to nourish the growing pollen tube. For many species, boron and calcium are also required for pollen tube growth. Boron, which is provided by stigmas and styles, facilitates sugar uptake and has a role in pectin production in the pollen tube (Richards, 1986). Boron is an essential element for plant growth and must be present in adequate amounts to ensure optimal plant growth and productivity. Boron plays a role in flowering and fruiting process in pistachio (Brown et al., 1994) and its deficiency results in low pollen viability, poor pollen germination and reduced pollen tube growth (Nyomora and Brown, 1997). Boron takes part in pollen germination and style tube formation and therefore has a vital function in fertilization of flowering crops. Boron added in the form of boric acid, is also essential for the *in vitro* culturing of pollen from most species; for example, it is well appreciated that elimination of boric acid from the culture medium often leads to tube bursting (Holdaway-Clarke and Hepler, 2003).

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Table 1. Percent germination of male *Pistacia vera* L. cultivars pollen as affected by the boron (H_3BO_3) level of the germination medium.

H_3BO_3 (%)	Pollen germination (%, ±SD)					
	Uygur	**Atli**	**Kaska**	**Sengel**	**Kavak**	**Mean**
Control	90.16±5.2 ab*	88.65±3.6 cd	96.18±3.0 a	86.38±5.4 ab	74.49±9.3 b	87.17±8.0
10	88.19±3.9 bc	91.99±4.6 bc	89.86±4.1 b	83.18±3.1 b	79.79±6.7 ab	86.60±5.0
25	83.63±4.4 c	86.16±4.2 d	87.43±4.7 b	79.95±11.1 b	52.15±12.1 c	77.86±14.7
50	85.33±4.4 c	88.69±6.1 cd	94.10±3.4 a	86.27±5.0 ab	58.81±10.5 c	82.64±13.8
75	88.72±3.8 bc	93.73±4.3 ab	94.19±2.9 a	89.14±4.2 a	81.04±5.4 ab	89.37±5.3
100	93.02±4.4 a	95.72±4.0 a	94.37±3.4 a	90.97±3.2 a	83.94±6.9 a	91.60±4.6

*The letters following the numbers indicate different groups determined by Duncan's test ($p \leq 0.05$).

Gibberellins (GAs) are essential endogenous regulators of plant growth and development. GAs are involved in many aspects of plant development, including seed germination, trichome development, stem and leaf elongation, flower induction, anther development and fruit and seed development (Pharis and King, 1985; Ross et al., 1997; Yamaguchi et al., 1998; Kamiya and Garcia-Martinez, 1999; Hedden and Phillips, 2000). GAs also, are present in developing pollen after anthesis and numerous studies have reported the effect of GA application on pollen tube growth *in vivo* or *in vitro* (Singh et al., 2002). Depending on the species examined and the concentration used, GAs can promote, inhibit, or have no effect on pollen germination and tube elongation *in vitro* (Bhandal and Malik, 1979; Viti et al., 1990; Setia et al., 1994).

The influence of boron and gibberellin on *in vitro* pollen germination has not been determined for use in male pistachio cultivars and types. The objective of this study is to determine the effect of boron and gibberellic acid on *in vitro* pollen germination of pistachio.

MATERIALS AND METHODS

Plant Material

The present study was carried out on male pistachio (*Pistacia vera* L.) cultivars (cvs) which consisted of Uygur, Atli, Kaska, Sengel and Kavak, maintained at the experimental orchards of the Pistachio Research Institute in the Gaziantep province of Turkey.

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

Pollen germination

Pollen germination *in vitro* was performed with the hanging drop method. The germination was 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, supplemented with 10, 25, 50, 75 and 100 ppm of boric acid (H_3BO_3) and gibberellic acid (GA_3) separately for 24 h at 25°C in dark conditions. On the other hand, 20% sucrose medium was used in the experiment as control. For each treatment, germination was recorded in three drops by counting three fields.

Pollen germination was determined by using a light microscope. 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. In separate experiments, the effect of boric acid and gibberellic acid on pollen germination percentage was determined.

The replicated values of pollen germination were analyzed using the one-way ANOVA procedure. 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

The effects of boron and gibberellin on the *in vitro* pollen germination of five male cultivars and types were evaluated and expressed as the percentage of germinated pollen grains.

The percentage of germinated pollen in different concentrations of H_3BO_3 is shown in Table 1. The statistical analysis revealed that H_3BO_3 level of the germination medium significantly affected the pollen germination percentage at all pistachio male cultivars used in the experiment. Pollen germination was decreased up to 25 ppm, and gradually increased again to 100 ppm. The highest pollen germination was observed on 100 ppm H_3BO_3 for Uygur, Atli, Sengel and Kavak cvs. Significant differences were not observed between control, 50, 75 and 100 ppm for Kaska cultivar. The highest pollen germination (96.18%) was obtained from control and followed by 100, 75 and 50 ppm in Kaska, respectively. The lowest values were obtained from 25 ppm boric acid for all cultivars used in the study (Table 1). Germination percentages of the pistachio pollen were higher with the addition of boron than the 20% sucrose alone (control). Higher concentration (100 ppm) of boric acid had the greatest effect on pollen germination with the average of 91.60% value, while the lowest germination occurred at 25 ppm H_3BO_3 (Table 1). There was no obvious correlation

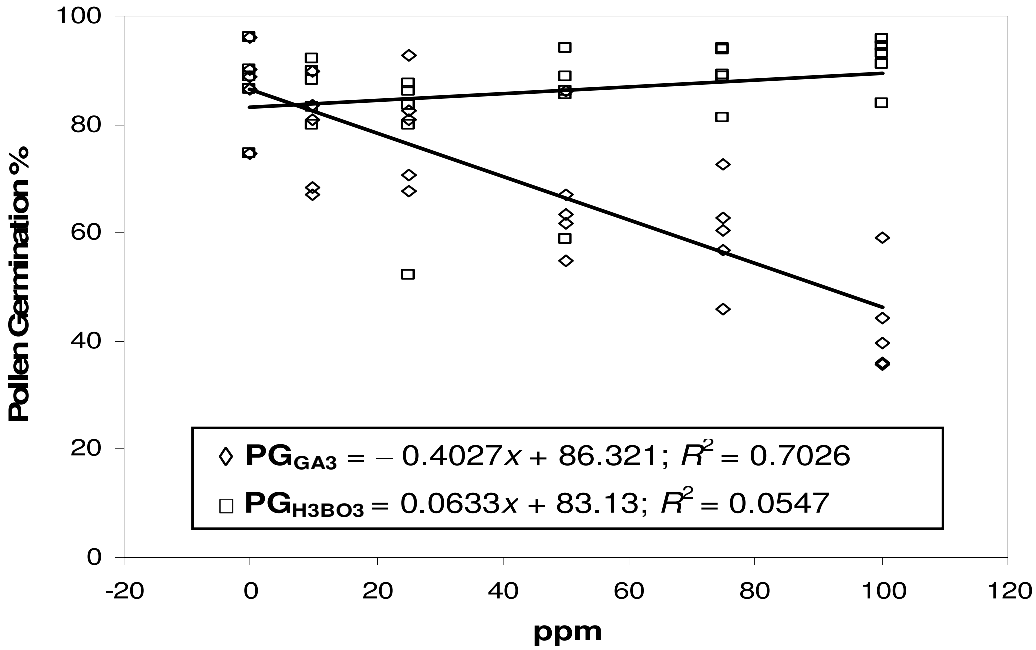

Figure 1. Relationship between pollen germination percentage (PG) and GA_3 and H_3BO_3 contents of germination media.

Table 2. Percent germination of male *Pistacia vera* L. cultivars pollen as affected by the gibberellic acid (GA_3) level of the germination medium.

GA_3 (%)	Pollen germination (%, ±SD)					
	Uygur	**Atli**	**Kaska**	**Sengel**	**Kavak**	**Mean**
Control	90.16±5.2 [a*]	88.65±3.6 [a]	96.18±3.0 [a]	86.38±5.4 [a]	74.49±9.3 [a]	87.17±8.0
10	89.65±4.7 [a]	67.02±8.8 [b]	83.57±4.1 [b]	81.00±5.3 [a]	68.44±6.1 [ab]	77.94±9.8
25	92.87±3.4 [a]	67.67±9.5 [b]	80.83±7.4 [b]	82.60±11.7 [a]	70.59±9.9 [ab]	78.91±10.1
50	86.14±8.7 [a]	54.68±4.8 [c]	63.40±6.5 [c]	66.93±8.1 [b]	61.71±7.7 [bc]	66.57±11.8
75	72.54±8.5 [b]	45.81±6.0 [d]	62.83±5.9 [c]	60.27±14.8 [b]	56.88±12.7 [c]	59.66±9.7
100	39.57±14.0 [c]	36.13±4.6 [e]	59.16±3.4 [c]	44.27±6.1 [c]	35.73±7.2 [d]	42.97±9.7

*The letters following the numbers indicate different groups determined by Duncan's test ($p \leq 0.05$).

of H_3BO_3 and pollen germination percentage (correlation = 0.2339) (Figure 1).

Table 2 shows the variation for pollen germination percentages of male pistachio cvs in response to GA_3 level. At higher GA_3 concentrations, the response of pollen germination revealed differences among cultivars. The pollen germination percentages of control were higher than that of GA_3 added mediums, except for 25 ppm application in Uygur cultivar. There were no significant differences in pollen germination of Uygur and Sengel cvs within the GA_3 range of 0 - 50 ppm, and 0 - 25 ppm, respectively. On the other hand, the highest germination was obtained from the control treatment in Atli, Kaska and Kavak cvs. The pollen germination was severely inhibited with the higher GA_3 concentrations. According to mean pollen germination rates, the highest value (87.17 %) was obtained from the control and the lowest value (42.97 %) obtained from the 100 ppm GA_3 concentration (Table 2). The pollen germination was negatively correlated with the GA_3 (correlation = -0.8382) (Figure 1). These results suggest that gibberellic acid had adverse effects on pollen germination of pistachio.

DISCUSSION

Pollination and pollen performance are the main factors that are effective in pistachio productivity, because the marketable product is the seed. Pollen germinations were performed on sucrose medium containing different concentrations of boric acid and gibberellic acid. Results showed that pollen germination of male cultivars and types in boric acid medium were generally higher than that of control, except for Kaska (Table 1). The highest pollen germination percentage for boric acid application was obtained from Kaska-control (96.18%) and followed by Atli-100 ppm H_3BO_3 (95.72%), while Kavak-25 ppm had the lowest value with 52.15% (Table 1).

Boric acid is known to be crucial for pollen germination and tube growth and it is required at concentrations of 100 ppm, for most species (Brewbaker and Majumder, 1961). It is known that, supplemented boric acid on sucrose medium had increased germination rate of pistachio pollen (Therios et al., 1985; Ak et al., 1995; Acar and Ak, 1998) and with increased concentration of boric acid in germination media, pollen germination increased up to 10 ppm for clone A and 20 ppm for clone B; and germination ratio remained constant from 20 to 200 ppm and reduced at higher concentrations (Therios et al., 1985). According to Brown et al. (1994), boron (B) plays a role in flowering and fruiting process in pistachio. Inadequate or excessive levels of B can result in decreased pollen viability, increased flower drop, reduced yields and nut quality in pistachio. For virtually all crops, boron has vital importance for pollen tube growth and pollen viability and thereby for seed and fruit development. The optimal concentration of boric acid required in culture media is 100 ppm for pollen germination and growth for many species (Shorrocks, 1997). In the present experiment, the highest pollen germination values were obtained from 100 ppm boric acid for all cultivars except for Kaska, however, there were no significant differences between the germination percentage of Kaska in control, 100, 75 or 50 ppm boric acid concentrations (Table 1). The germination percentage obtained in the present study was supported with the results of previous studies (Brewbaker and Majumder, 1961; Therios et al., 1985; Brown et al., 1994; Ak, et al., 1995; Shorrocks, 1997; Acar and Ak, 1998). Therefore, it may be suggested that 100 ppm of boric acid had a stimulant effect on pollen germination of pistachio trees.

Pollen germination percentages were severely reduced with increased GA_3 concentration in the germination medium for all cultivars and reached the lowest value at the 100 ppm GA_3 (Table 2). The highest pollen germination percentages were observed on control (20% sucrose alone) for all cultivars except for Uygur. The highest germination value of Uygur was obtained from 25 ppm GA_3 as 92.87%. The response of male pistachios for pollen germination was different among the cultivars. Distinct differences of pollen germination were observed between 75 - 100 ppm GA_3 for Uygur, Sengel and Kavak, whereas it was observed between control and 10 ppm for Atli and between 25 - 50 ppm for Kaska. The lowest germination percentages were observed for 100 ppm GA_3 with mean of 42.97% (Table 2). A significant linear correlation was found between the pollen germination percentage and GA_3 level of germination media (Figure 1).

Pollen viability differs among species, genotypes of the same species, or even among years in the same genotype (Kakani et al., 2005). Stanley and Linskens (1974) reported that, various germination test and media as well as culture conditions may affect the germination results of a given cultivar. Depending on the examined species and the used concentration, GAs can promote, inhibit, or have no effect on pollen germination and tube elongation *in vitro* (Bhandal and Malik, 1979; Viti et al., 1990; Setia et al., 1994). According to Tosun and Koyuncu (2007), 10 ppm gibberellic acid was determined as promoter in pollen germination and tube growth of sweet cherries and lower concentrations (0.05 ppm) of GA_3 and boric acid also stimulated pollen germination and tube growth in apricot, while the higher concentrations had inhibitory effects (Bolat et al., 1999). Besides, 50 ppm GA_3 inhibited pollen germination of some olive cultivars *in vitro* (Viti et al., 1990).

The observed inhibition of pollen germination of pistachio males by high concentrations of GA_3 is consistent with the result of previous studies (Sidhu et al., 1986; Viti et al., 1990; Swamy and Khanna, 1991; Bolat et al., 1999; Singh et al., 2002; Tosun and Koyuncu, 2007) in which high GA concentrations have been shown to inhibit pollen germination and pollen tube growth.

In conclusion, *in vitro* pollen germination of pistachio trees was greatly inhibited by increased gibberellic acid concentration, while it was slightly promoted by increased boron concentration in the germination medium. The results of this study revealed adverse effects of GA_3 and stimulant effects of boron on *in vitro* pollen germination of pistachio.

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