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# The Interaction Effects of Boron and Plant Growth Regulators on Pollen Germination of Almond

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**Abstract**

The aim of this study was to determine the effects of different plant growth regulators, NAA (0 mg/l, 50 mg/l and 100 mg/l) and GA3 (0 mg/l, 50 mg/l and 100 mg/l), with various boron concentrations (0 mg/l, 50 mg/l and 100 mg/l) on pollen germination of 'Ṣaba', 'Rabie' and 'Padre' almond cultivars in 10% sucrose and 1% agar medium at 24°C in dark conditions. The results showed that different treatments had significant effects on the percent of germination. The highest pollen germination (average 90.37%) for three almond cultivars was recorded in 100 mg/l boric acid, 10% sucrose and 1% agar medium. The lowest germination percentage (average 3.59%) was found in 50 mg/l NAA, sucrose 10 % and agar 1 % medium for all cultivars. Pollen germination rates significantly decreased with increasing growth regulators in almond cultivars. The pollen germination was greatly inhibited in media with GA3 and NAA or compound from these materials without boric acid. For example, mean pollen germination rates was %15.51 in 100 mg/l GA3+ 10% sucrose + 1% agar, while this value was 82.89% from the 100 mg/l GA3+100 mg/l Br+ 10% sucrose + 1% agar.

**Keywords:** Almond, Growth regulators, Pollen germination.

## Introduction

It is known that most almond varieties in the world and all the major commercial varieties are self-incompatible. Each variety needs to be grown with another variety to ensure pollination. As the commercial part of the fruit is the kernel, pollination and ovule fertilization are of critical importance to obtain optimal yields. The joint planting of at least two inter-compatible and simultaneously blooming cultivars as well as the presence of pollinating insects for pollen transfer are required (Socias i Company *et al.* 1992; Kester *et al.,* 1996). Therefore, kernel setting in almonds depends on transfer by honey bees of viable, compatible pollen between healthy flowers. Pollen germination and pollen tube growth are prerequisites for fertilization and seed development. Due to the involvement of the pistillate tissue in the nature, physiological and biochemical investigations on pollen germination and pollen tube growth *in vivo* are rather difficult. Thus, *in vitro* germination techniques have been used extensively on a variety of pollen systems. *In vitro* pollen germination rates are considered the best indicator of pollen viability (Shivanna *et al.,* 1991). Most research indicates that sucrose solution is the most favorable pollen germination media (Stanley and Linskens, 1974; Martinez-Gomez *et al.,* 2002; Acar *et al.,* 2010; Imani *et al.,* 2011). The effects of chemical material on pollen germination and tube growth of tree fruit crops depends on the species examined and the concentration and compound used in media culture. For example, 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).

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Vitagliano and Viti (1988) found that Siapton 10L, a polypeptide-amino-acid mixture obtained by protein hydrolysis, stimulated in vitro pollen germination of almond, apricot and peach, whereas GA3 was without effect and paclobutrazol was inhibitory. Acar *et al.* (2010) found that pollen germination of pistachio cultivars were greatly reduced with increased GA3 concentration in the germination medium and reached the lowest value at the 100 ppm GA3. Germination was decreased at 25 ppm in $H_3BO_3$ and gradually increased again to 100 ppm. Gibberellic acid had adverse effects on pollen germination of pistachio.

Boron seems to be involved in many processes including sugar transport, cell wall synthesis and maintenance, membrane integrity, and RNA, indole acetic acid (IAA) and phenol metabolism (Loomis and Durst 1992, Dordas and Brown, 2000). Its precise role has not been elucidated. Recently, it was demonstrated that boron is not only essential in pollen germination and style tube formation but has a vital function in fertilization of flowering crops. *In vitro* boron culture of pollen from most species often leads to improved germination and tube growth of pollen (Wang *et al.,* 2003; Holdaway-Clarke and Hepler, 2003). Nyomora *et al.* (2000) showed that foliar-applied boron reduced bursting of tubes during in vitro germination and the addition of B (100 mg/l) to the culture media significantly increased in vitro germination and tube growth of pollen from trees sprayed with 0.8 kg B/ha, decreased germination in pollen from trees sprayed with 1.7 or 2.5 kg B/ha, and had little effect on tube growth of pollen from trees sprayed with 1.7 or 2.5 kg B/ha.

Base on several recent studies (Bhandal and Malik, 1979; Viti *et al.,* 1990; Swamy and Khanna, 1991; Setia *et al.,* 1994; Acar *et al.,* 2010), the effects of GAs on pollen germination and tube elongation *in vitro* has been different. GAs in absence of boric acid and in higher concentrations had inhibitory effects (Acar *et al.,* 2010), while lower concentrations and the presence of boric acid have stimulating effects on pollen germination and tube growth (Bolat *et al.,* 1999; Viti *et al.,* 1990). Base on a study by Korkutal *et al* (2008), gibberellic acid affected the grape berry by killing pollens and causing grape varieties to become seedless.

Although studies have been conducted on the effects of chemical material on pollen germination and pollen tube growth of almond (Kester *et al.,* 1996; Nyomora, *et al.,* 2000; Mussen and Montague, 2004; Tosun and Koyuncu, 2007), very few have been carried out on the interaction of boron and growth regulators on *in vitro* pollen germination for almond.

The objective of this study was to determine the effects of different chemical materials on pollen germination rate of different almond cultivars and to investigate the interaction of various concentrations of boron and growth regulators on *in vitro* pollen germination of almond.

## Materials and Methods

### *Pollen collection*

Branches with closed flowers were cut from trees of three almond cultivars (‘Ṣaba’, ‘Rabie’ and ‘Padre’) growing at the Research Station of Horticulture, Seed and Plant Improvement Institute (SPII)’s orchard of Kamal Shahr in Karaj, Iran. Pollen was collected in large quantities from these cuttings at 24 hours after cutting, and as freshly opened blossoms at room temperature (20–25°C) (Imani *et al.,* 2011). Pollen grains were collected and stored in glass jars with silica pellets and fitted with air-tight caps at 4°C and were used when required.

### *In vitro pollen germination*

To determine the effects of different growth regulators: NAA and GA3 with various boron concentrations on the pollen germination of ‘Rabie,’ ‘Saba’ and ‘Padre’ almond cultivars, 14 different culture media were prepared containing different compositions including different levels of NAA (0 mg/l, 50 mg/l and 100 mg/l); GA3 (0 mg/l, 50 mg/l and 100 mg/l) with various boron concentrations (0 mg/l, 50 mg/l and 100

mg/l) in 10% sucrose and 1% agar medium at 24°C in dark conditions in 2011. Pollen grains were uniformly scattered onto the medium in Petri dishes and blotted to imbibe all the grains. The Petri dishes were covered and placed in a culture chamber and incubated at 24±2°C in darkness for 24 hours. The percentage of pollen germination and pollen tube length was determined under a light transmission Nikon type-2 microscope. Pollen grains were recorded as germinated when the pollen tube was equal to or longer than the diameter of the pollen grain (Henny, 1977). Data on pollen 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 (Imani *et al.*, 2011). The statistical analysis was performed using Microsoft Excel (2007) and SAS software (SAS Institute Inc, 1990) and means were compared using Duncan's Multiple Range Test (DMRT).

## Results

The results of the effects of different growth regulators, including NAA (0 mg/l, 50 mg/l and 100 mg/l); GA3 (0 mg/l, 50 mg/l and 100 mg/l) with various boron concentrations (0 mg/l, 50 mg/l and 100 mg/l), on the pollen germination and tube growth of 'Şaba,' 'Rabie' and 'Padre' almond cultivars in 10% sucrose and 1% agar medium at 24°C in dark conditions are shown in Tables 1-3.

**Table 1. Germination percentage of pollen almond cultivars as affected by the boron ($H_3BO_3$) and growth regulators level of the germination medium.**

| Treatment | Germination (%) | | |
|---|---|---|---|
| | 'Saba' | 'Rabie' | 'Padre' |
| C[1] | 80.65a | 70.34c | 90.76a |
| $B_1$+ C | 56.76b | 70.68c | 80.45b |
| $B_2$+ C | 85.54a | 90.23a | *95.35a* |
| $G_1$+ C | 18.81c | 15.07f | 10.67d |
| $G_2$+ C | 5.37d | 2.23g | 3.56d |
| $N_1$+ C | 5.54d | 2.56g | 4.67d |
| $N_2$+ C | 10.65d | 20.45f | 10.23d |
| $G_1$+ $N_1$ + C | 20.89c | 20.23f | 10.54d |
| $B_1$+ $G_1$+ $N_1$+ C | 80.34a | 75.86c | 90.12a |
| $N_1$ +$G_2$ + C | 22.23c | 10.76g | 10.23d |
| $N_2$ +B2 + C | 80.76a | 80.45b | 80.12b |
| $G_2$ + $B_2$ + C | 85.45a | 85.45b | 80.34b |
| $N_2$ +$G_2$ + C | 15.65c | 30.76e | 27.45c |
| $N_2$ +$G_2$ + $B_2$ + C | 50.05b | 45.67d | 80.23b |

[1]C =10% sucrose and 1% agar (control); $B_1$=50 mg/l boric acid; $B_2$=100 mg/l boric acid; $N_1$=50 mg/l naphthalene acetic acid; $N_2$=100 mg/l naphthalene acetic acid; $G_1$= 50 mg/l gibberellic acid; $G_2$= 100 mg/l gibberellic acid

[*] Means followed by the similar letter(s) are not significantly different by Duncan test (P<0.05).

**Table 2. Average of pollen germination of almond cultivars as affected by the different treatments of the germination medium**

| cultivar | Pollen germination (%) |
|---|---|
| 'Saba' | 45.88 a |
| 'Rabie' | 44.33 a |
| 'Padre' | 48.19 a |

* Means followed by the similar letter(s) are not significantly different by Duncan test (P<0.05).

**Table 3. Average of percent germination of almond cultivars pollen as affected by the boron ($H_3BO_3$) and growth regulators level of the germination medium**

| Treatment | Average germination (%) |
|---|---|
| C | 80.58 b* |
| $B_1$+ C | 70.58 c |
| $B_2$+ C | 90.37 a |
| $G_1$+ C | 15.51 f |
| $G_2$+ C | 3.72 g |
| $N_1$+ C | 4. 25 g |
| $N_2$+ C | 13.77 f |
| $G_1$+ $N_1$ + C | 17.22 f |
| $B_1$+ $G_1$+ $N_1$+ C | 82.10 b |
| $N_1$ +$G_2$ + C | 12.86 f |
| $N_2$ +$B_2$ + C | 80.31 b |
| $G_2$ + $B_2$ + C | 82.89 b |
| $N_2$ +$G_2$ + C | 25.32 e |
| $N_2$ +$G_2$ + $B_2$ + C | 64.045d |

* Means followed by the similar letter(s) are not significantly different by Duncan test ($P<0.05$).

**Discussion**

Most almond varieties in the world and all the major commercial varieties are self-incompatible. The commercial part of the fruit is the kernel. Therefore, kernel setting in almonds depends on the transfer of viable, compatible pollen between healthy flowers by honey bees (Kester *et al*., 1996). *In vitro* pollen germination rates are considered to be the best indicator of pollen viability (Shivanna *et al.*, 1991). The results shown in Table 3 indicated that the highest pollen germination (average 90.37%) for 'Şaba,' 'Rabie' and 'Padre' almond cultivars was recorded at 85.54%, 90.23% and 95.35% in 100 mg/l boric acid, 10% sucrose and 1% agar medium, respectively. The lowest germination percentage (average 3.59%) was found in 100 mg/l GA3, sucrose 10 % and agar 1 % medium for all cultivars.

On the other hand, there were no considerable differences among cultivars in the ability to germinate and pollen tube growth (Table 2). Different media showed significantly different effects with regard to their germination percentage of three almond cultivars pollen grain following in vitro culture (Table 3). It is evident that the pollen germination was harshly inhibited in media with GA3 and naphthalene acetic acid (NAA) or in compound from these materials that lacked the presence of boric acid. For example, the mean pollen germination rate was %15.51 in 100 mg/l GA3+ 10% sucrose and 1% agar medium, while this value was 82.89 in the 100 GA3+100Br+ 10 % sucrose and 1 % agar medium (Table 1). The results are compatible with the study by Gupta and Mutry (1985). In addition, according to the report by Acar *et al.* (2010), *in vitro* pollen germination of pistachio trees was greatly inhibited by increased gibberellic acid concentration, while it was promoted by increasing boron concentration in the germination medium. As shown in Table 1, all treatments had different effects on the percentage of pollen germination, regardless of cultivar. The positive effects of growth regulators on pollen germination percentage were variable and dependent on material concentration and medium compounds.

The highest pollen germination percentage of Padre was recorded as 95.35 % in 100 mg/l boric acid + 10% sucrose +1% agar, whereas the lowest was 3.56% in basic medium without the presence of boric acid. According to the findings of Brewbaker and Majumder (1961), boric acid is known to be crucial for pollen germination and tube growth, and it is required at concentrations of 100 ppm for almond (Kester and Gradziel, 1996; Martinez-Gomez *et al.*, 2002). Additionally, a role of boron has a vital role in flowering and the fruiting process of almond, as demonstrated by Nyomora and Brown (1997). Similar results were reported on the positive effects of supplemented boric acid on sucrose medium, as it increased the germination rate of pistachio pollen (Brown *et al.*, 1994; Acar *et al.*, 2010).

The limited pollen germination in media containing GA3 and naphthalene acetic acid (NAA) and compound of these materials without the presence of boric acid suggested that these materials are necessary for nutrient uptake and pollen metabolism. Culturing of pollen in boron often resulted in improved germination and tube growth of pollen (Wang *et al.*, 2003; Holdaway-Clarke and Hepler, 2003).

The addition of various concentrations of boric acid to the control greatly enhanced pollen germination, especially at 100 mg/l. The optimum concentration for stimulating germination varied and was dependent on the type and concentrations of medium tested. Higher concentrations of GA3 and naphthalene acetic acid (NAA) and compound from these materials were consistently inhibitory. The negative effects of high concentration of GA3 and naphthalene acetic acid (NAA) and their compound on pollen germination suggested that a definite level of endogenous GA3 and NAA was necessary for normal germination of pollen grains. High concentrations appeared toxic and low concentrations may have been inadequate. The results of our study correlated to the report by Bolat *et al.* (1999) and Acar *et al.* (2010).

In conclusion, *in vitro* pollen germination of almond was inhibited by high concentration of GA3 and NAA and their compound, while it was enhanced by adding boron concentration in the germination medium.

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