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Methyl Jasmonate Influences of Pollen Germination and Pollen Tube Growth of Apricot (*Prunus armeniaca* L.)

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Abstract : In this study, the effect of concentrations of methyl jasmonate (MJ) on pollen germination (PG) and pollen tube growth (PTG) was investigated in two apricot (Bebeco and Kabaasi) cultivars. MJ led to decrease in PG and PTG of these cultivars. While PG was 63.8% in Bebeco and 52.9% in Kabaasi for control treatment, they were recorded as 11.9% in Bebeco and 32.5% in Kabaaşı by diminising with the application of 1 mM MJ. In addition, 0.5 mM and higher of MJ had an inhibiting effect on PTG. The relationships between PG and PTG were high with r=0.93 and r=0.97 for Bebeco and Kabaasi cultivars.

Key words: Methyl jasmonate, Pollen germination, Apricot.

Kayısıda (*Prunus armeniaca* L.)'da Polen Çimlenmesi ve Polen Tüp Gelişimi Üzerine Metil Jasmonatın Etkileri

Özet: Bu çalışmada, methyl jasmonatın farklı konsantrasyonlarının Bebeco ve Kabaaşı kayısı çeşidinde polen çimlenmesi ve polen tüp uzunluğu üzerine etkileri araştırılmıştır. Methy jasmonat her iki çeşitte polen çimlenmesi ve polen tüp uzunluğunun azalması üzerine etki etmiştir. Kontrolde polen çimlenmesi Bebeco çeşidinde %63.8 ve Kabaası çeşidinde %52.9 olarak belirlenirken, 1 mM methyl jasmonat uygulanması polen çimlenmesini Bebeco çeşidinde %11.9'a ve Kabaası çeşidinde %32.5'e düşürmüştür. Bunun yanında 0.5 mM ve daha yüksek oranlarda methyl jasmonat uygulamaları, polen tüp uzunluğu üzerine engelleyici etkiye sahip olmuştur. Polen çimlenmesi ve polen tüp uzunluğu arasında yüksek ilişkiler r=0.93 (Bebeco) ve r=0.97 (Kabaasi) kaydedilmiştir.

Anahtar kelimeler: Metil jasmonat, Polen çimlenmesi, Kayısı

Introduction

One of influential growth regulators in the plants, jasmonates (JAs) originate from linolenic acid via a pathway consist of many enzymatic steps. The plants contain jasmonic acid (JA), its methyl ester (JAME), certain L-amino acid conjugates, glucose ester and hydroxylated forms (Sembdner and Parthier 1993; Andrea et al. 2005). Jasmonic acid regulates the expression of plant genes and influences specific aspect of disease/pest resistance traits and plant growth and development (Staswick 1992; Lee and Back 2005). Jasmonates (JAs) modulate many advance plant processes such as inhibition of seed germination and shoot and root growth (Sembdner and Parthier 1993; Yıldız and Yılmaz 2002), fruit ripening and development of strawberry (Yılmaz et al. 2003), prevention of chlorophyll and carotenoid formation (Saniewski and Czapski 1983) and reduction of photosynthetic (Popova et al. 1988). They have not only effects on leaf abscission, leaf senescence (Wilen et al. 1991), inhibition of root elongation in *Arabidopsis thaliana* (Staswick et al. 1992), reproductive structures of fruits and flowers, and anther and pollen formation (Sanders et al. 2000; Stinzi and Browse 2000), but also play an active role for plant defense mechanism against unfavorable environmental stress, like drought, low temperature and salinity (Wasternack and Parthier; 1997Wasternack and Hause 2002).

On the other hand, while jasmonates are involved in diverse processes including, stimulating ethylene production in tomato and strawberry fruits (Perez et al. 1997; Saniewski 1987; Sembdner and Parthier 1993; Yılmaz et al. 2003), they inhibit ethylene production in rice seedlings (Yeh et al. 1995). It is believed that crosstalk between jasmonate and ethylene pathways enables plants to optimize their defense

strategies more efficiently and economically and variety of biological activities (Baldwin 1998; Parthier 1990).

It is known that plant hormones play many roles in plant growth and development. Ethylene which is a plant hormone, has an important role in seed and pollen germination. It has been considered that jasmonates might affect seed and pollen germination via ethylene (Sembdner and Parthier 1993). In fact, it was reported that jasmonates have inhibitory effects on the germination of nondormant seeds, for example, lettuce (Yamane et al. 1981), amaranth (Kepczyenski and Bialecka 1994), apple (Ranjan and Lewak 1995; Yıldız et al. 2007), pear (Yıldız et al. 2008) seeds. However, exogenous jasmonates seem to have a effect on breaking of the seed dormancy (Ranjan and Lewak 1995).

In particular, many studies indicated that ethylene production increased in the pistil after pollination and it is correlated with post-pollination events in flowers (Burg and Dickman 1967; O'Neill 1997). The studies on effects of jasmonates on pollen germination are limited. But it is known that jasmonates interact with ethylene. Many research reported that ethylene has important effects on pollen germination and jasmonates might affect pollen germination (Sembdner and Parthier 1993).

The aim of this study was to determine the effect of Methyl Jasmonate on the pollen germination and pollen tube growth in two apricot cultivars.

Materials and Metods

The flowers were collected from 12 years old trees of Bebeco and Kabaasi apricot cultivars. Before petals opened, they were kept at the room temperate for 24 hours. Pollens were germinated in liquid media containing 10% sucrose and 0.01% boric acid (Brewbaker and Kwack 1963; Munzur and Gür 2000). Sterile 3 microscopic slides were used for each treatment. MJ solutions and culture medium at the same volumes were used. At first, 50 µl liquid media were dropped on microscopic slides. Then, 50 µl MJ solution and were dripped on to slides. MJ solutions prepared with distilled water were 0.1 mM, 0.25 mM, 0.50 mM and 1 mM. For control group, 50 µl distilled water was dropped instead of MJ solutions.

Pollens were shed in this liguid culture media under Olympus light microscope with aid a of hygiene pin. The microscopic slides on which was shed pollen were placed in petri dishes with a moist filter paper lining the lower plate, serving as an improvised humidity chamber. Then, the petri dishes were settled in incubator at 22±2°C for 3 hours under dark conditions.

After incubation, a few drop of 10% ethanol was added to the microscopic slides for fixations and then lamella was closed. Germination percentages of pollens were determined by examining grain and tube lengths of pollens were measured by using an ocular micrometer under Olympus light microscope (Shivanna and Rangaswamy 1992).

In the study, a completely randomized design with three replications was used. The statistical package program Minitab release 10.2 for Windows was used for the analysis of variance. The means were compared using Duncan's Multiple Range Test, and significant differences were found at $P<0.05$. Using Excel package program, correlation coefficients were computed to conclude relationships between pollen germination (PG) and pollen tube growth (PTG) in apricot cultivars.

Results and Discussion

The percentages of pollen germination and tube growth varied to apricot cultivars. In control, the highest PG and PTG were determined in Bebeco (63.8% - 398.4µm) followed by Kabaasi (52.9% - 300.1 µm). In the treatment of 0,1 mM of MJ, the highest PG and PTG were recorded as 68.4% and 385.7 µm for Bebeco, followed by Kabaasi (67.7% - 342.1 µm). In the treatment of 0,25 mM of MJ, PG and PTG were 67.8% and 359.3 µm for Bebeco, 65.8% and 377.2 µm for Kabaasi (Table 1 and 2). While PG and PTG in 'Kabaasi' and PTG in 'Bebeco' differed ($P<0.001$) statistically by MJ treatments, PTG of 'Bebeco' was not influenced by MJ treatments (Table 3).

Table 1. Pollen germination (PG) percentages of two apricot cultivars treated with MJ concentrations.

PG	Control	0.1 mM	0.25 mM	0.50 mM	1 mM	Mean
Bebeco	63.8 a***	68.4ns	67.8 a***	61.8 a***	11.9 b***	54.7
Kabaasi	52.9 b	67.7	65.8 b	35.2 b	32.5 a	50.8
Mean	58.3	68.03	66.77	48.5	22.2	52.7

ns: non-significant. *** P<0.001.

Table 2. Pollen tube growth (PTG) µm of two apricot cultivars treated with MJ concentrations.

PTG	Control	0.1 mM	0.25 mM	0.50 mM	1 mM	Mean
Bebeco	398.4 a	385.7 ns	359.3 ns	336.5 a***	241.4 a***	344.3
Kabaasi	300.1 a	342.1	377.2	217.1 b	164.9 b	280.4
Mean	349.6	363.8	368.2	276.8	203.2	312.3

ns: non-significant. *** P<0.001.

Table 3. Differences between MJ treatments concerning pollen germination (PG) and pollen tube growth (PTG) in two apricot cultivars.

Treatments of MJ	Bebeco		Kabaasi	
	PG (%)	PTG (µm)	PG (%)	PTG (µm)
Control	63.8 a***	398.4 ns	52.9 ab***	300.1 ab**
0.1 mM	68.4 a	385.7	67.7 a	342.1 a
0.25 mM	67.8 a	359.3	65.8 a	377.2 a
0.50 mM	61.8 a	336.5	35.2 bc	217.1 bc
1 mM	11.9 b	241.4	32.5 c	164.9 c

ns: non-significant. ** P<0.01, *** P<0.001.

The values of PG and PTG in the concentrations of 0.1 mM and 0.25 mM of MJ were close with those of the control. Both PG and PTG showed a significant decrease when the culture media was treated with 0.50 mM and 1mM of MJ (Figure 1 and 2).

In the treatment of 0.50 mM of MJ, PG and PTG were (61.8% - 336.5 µm) in Bebeco, and (35.2% - 217.1 µm) in Kabaasi. In the treatment of 1 mM of MJ, PG and PTG were recorded as 11.9% and 241.4 µm for Bebeco and 32.5% - 164.9 µm for Kabaasi (Table 1 and 2). In addition, the relationships between PG and PTG were high with r=0.93 and r=0.97 for Bebeco and Kabaasi cultivars (Figure 3 and 4).

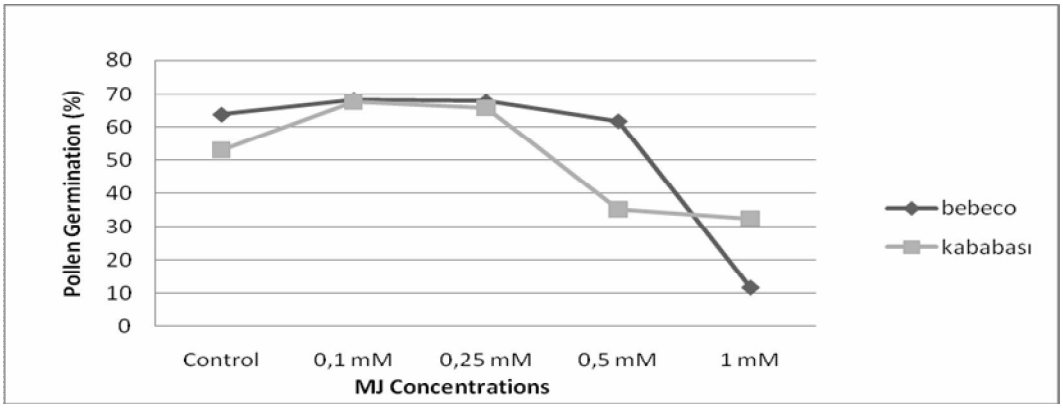

Figure 1. Pollen germination (PG) percentages of two apricot cultivars treated with MJ concentrations.

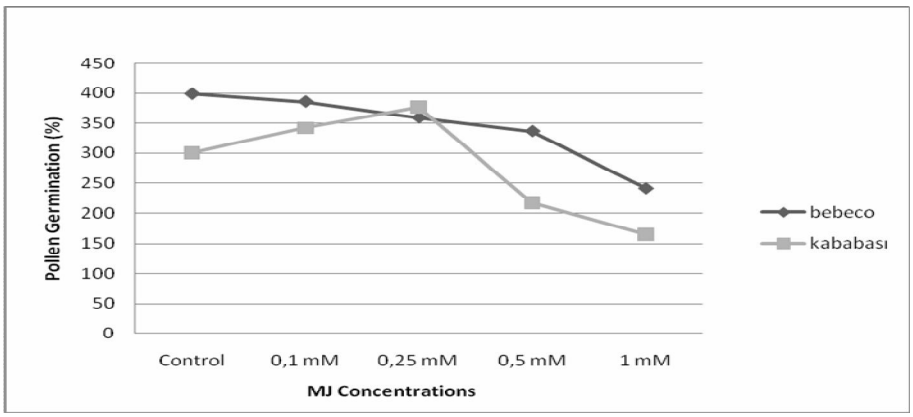

Figure 2. Pollen tube growth (PTG) of two apricot cultivars treated with different concentrations of MJ.

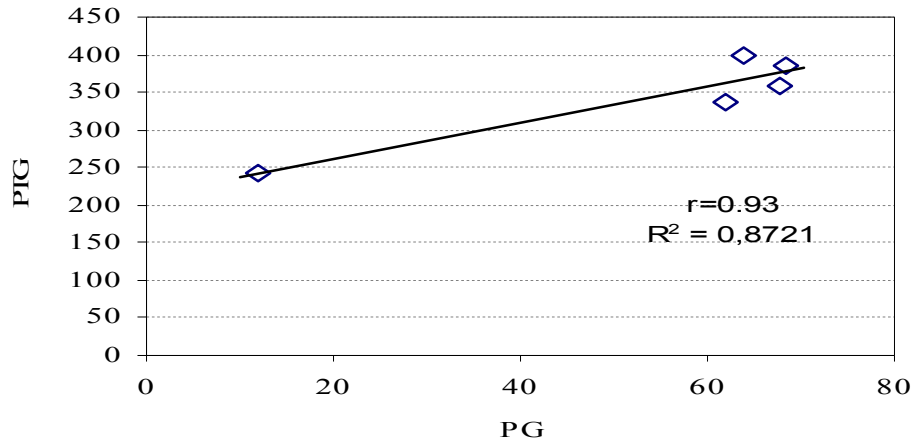

Figure 3. Relationship between pollen germination (PG) and pollen tube growth (PTG) in Bebeco.

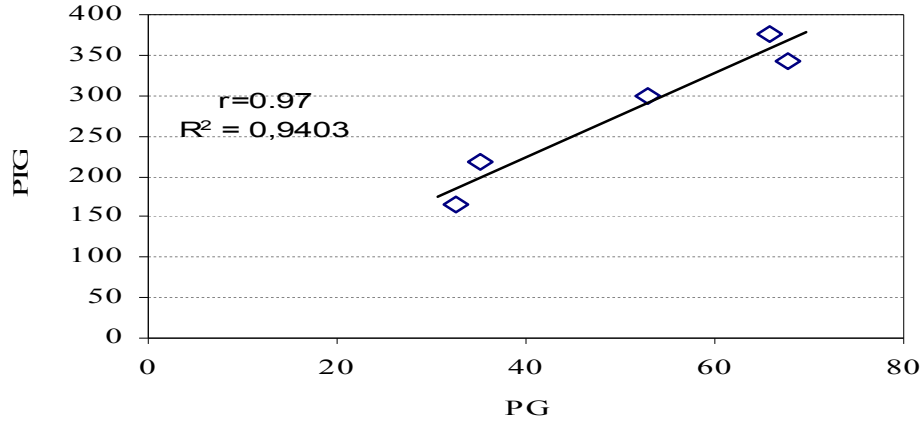

Figure 4. Relationship between pollen germination (PG) and pollen tube growth (PTG) in Kabaasi.

Investigating the pollen germination and pollen tube length in apricot cultivars, Bolat and Pırlak (1999) determined pollen germination as 45.6% in Hasanbey, 41.8% Salak, 39.6% in Karacabey and 35.9% in Sekerpare. They recorded pollen tube length as 295µm in Hasanbey, 306 µm in Salak, 251 µm in Karacabey and 268 µm in Sekerpare with 10% sucrose concentration using hanging-drop test. These results had similarities with our results. In addition, they also stated that 20-25% sucrose concentrations have an inhibitory effect on pollen germination and pollen tube length.

On the other hand, jasmonates interact with other phytohormones. They are thought play an important role in senescence, stress, fruit growth and seed germination by affecting endogenous ethylene production (Parthier 1990; Nojavan-Asgharian and İshizawa 1998; Sembdner and Parthier 1993). It has been reported that ethylene stimulates the germination of pollen grains in different species (Song et al. 1998). This situation brings to mind that jasmonates may also affect pollen germination. However, studies concerning effects on pollen germination of jasmomates are very limited.

Our studies showed that exogenous MJ applications in two apricot cultivars caused to inhibition of pollen germination and pollen tube growth. Especially 0.5 mM and 1 mM concentrations of MJ highly inhibited pollen germination and pollen tube growth while 0.1 mM and 0.25 mM treatments of MJ slightly increased pollen germination and pollen tube growth.

Yıldız and Yılmaz (2002) reported that JA significantly inhibited the germination of strawberry pollen and inhibition of the germination by JA was reduced by exogenously applied ethephon (an ethylene releasing compound) and ACC (a precursor of ethylene). Many researchers claim that ethylene affects seed germination of Amaranthus (Kepczynski and Bialdecka 1994) and (Nojavan-Asghari and Ishizawa 1998) Cocklebour. The production of ethylene is required for the inhibition of pollen germination by JA (Yıldız and Yılmaz 2002; Saniewski et al. 1997). Jasmonic acid and its methyl ester methyl jasmonate was identified in pollens and anther of *C sinensis, C. japonica* and *C. Sasanqua* by Yamane et al. (1981). These researchers reported that jasmonic acid shows a nontoxic inhibitory effect, but MJ has not effect on pollen germination.

In view of the result of this study, we suggest that jasmonates may have an important role in pollen germination. A more detailed investigation of this subject would seem appropriate.

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