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Gum Formation by Methyl Jasmonate in Tulip Shoots is Stimulated by Ethylene

M. Saniewski,[1] K. Miyamoto,[2] and J. Ueda[2,*]

[1]Research Institute of Pomology and Floriculture, Pomologiczna 18, 96-100 Skierniewice, Poland; and [2]College of Integrated Arts and Sciences, Osaka Prefecture University, 1-1 Gakuen-cho, Sakai, Osaka 599-8531, Japan

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Abstract. The promotive effect of methyl jasmonate (JA-Me) on the induction of gum in tulip shoots (*Tulipa gesneriana* L. cvs. Gudoshnik and Apeldoorn) was studied in the presence of ethylene. Gum formation in the stem and the basal part of the leaves was induced by JA-Me (1% w/w in lanolin) and stimulated strongly by the simultaneous application of 1 or 5 mM 1-aminocyclopropane-1-carboxylic acid (ACC). JA-Me at a concentration of 0.1% did not induce gum, but that together with ACC at a concentration of 1 or 5 mM induced it substantially. Although JA-Me stimulated ethylene production substantially in the stem of intact tulips, ethephon (1% w/w) or ACC (1 or 5 mM) did not induce gum formation in tulip shoots. JA-Me induced gum formation in tulip shoots even in the presence of aminooxyacetic acid or cobalt ions. Moreover, gum formation was also observed in the cut shoot applied with JA-Me as a solution at concentrations of 0.23 mM or more. These results strongly suggest that JA-Me is required for gum formation in tulip shoots, and ethylene probably makes the tissues of shoots sensitive to JA-Me.

Key Words. 1-Aminocyclopropane-1-carboxylic acid—Ethephon—Ethylene—Gum formation—Jasmonic acid—Methyl jasmonate—Tulip shoots

Jasmonic acid (JA) and its related compounds are distributed widely in the plant kingdom together with multiple physiological effects, suggesting that these compounds are putative plant hormones. One of the characteristic physiological effects of methyl jasmonate (JA-Me) is gum formation in plant tissues as reported in bulbs, leaves, or stems of tulips (Saniewski 1989, Saniewski and Puchalski 1988, Saniewski and Węgrzynowicz-Lesiak 1994). Gummosis, which is the process of the accumulation and exudation of gum from plants, has been considered to be regulated by ethylene induced in response to injury by insects and pathogens, wounding, and some stresses in many plants, especially in the species of stone-fruit trees and their fruits (Abeles 1973, Boothby 1983). In tulip bulbs, infection by *Fusarium oxysporum f. sp. tulipae* and the application of ethylene or the ethylene-generating compound ethephon has been found to induce gum formation (De Hertogh et al., 1980, De Munk and Saniewski 1989, Kamerbeek and De Munk 1976, Kamerbeek et al., 1971, Moe and Hagness 1975). JA-Me has also been reported to stimulate ethylene production in various plants such as preclimacteric apples (Saniewski et al. 1986); tomatoes (Saniewski and Czapski 1985, Saniewski et al. 1987); rice leaves (Chuo and Kao 1992); olive leaves (Sanz et al. 1993); flowers of *Dendrobium*, *Petunia*, and *Phalaenopsis* (Porat et al., 1993, 1995); and tendrils of *Bryonia dioica* (Weiler et al. 1993). These facts raise the possibility that JA-Me induces gum formation in tulip shoots in relation to the promotion of ethylene production. In this paper we report that the effect of JA-Me on gum formation in tulip shoots is stimulated substantially by ethylene and that there is a possibility that JA-Me is essential for gum formation in tulip shoots. A possible mode of action of the stimulating effect of ethylene on gum formation by JA-Me is discussed.

Materials and Methods

Plants

The experiments were performed with the bulbs of tulip (*Tulipa gesneriana* L. cvs. Apeldoorn and Gudoshnik) from commercial stocks. After lifting, the bulbs with a circumference of 10–12 cm were stored at

Abbreviations: JA, jasmonic acid; JA-Me, methyl jasmonate; ACC, 1-aminocyclopropane-1-carboxylic acid; AOA, 1-aminooxyacetic acid; NAA, naphthaleneacetic acid.

*Author for correspondence.

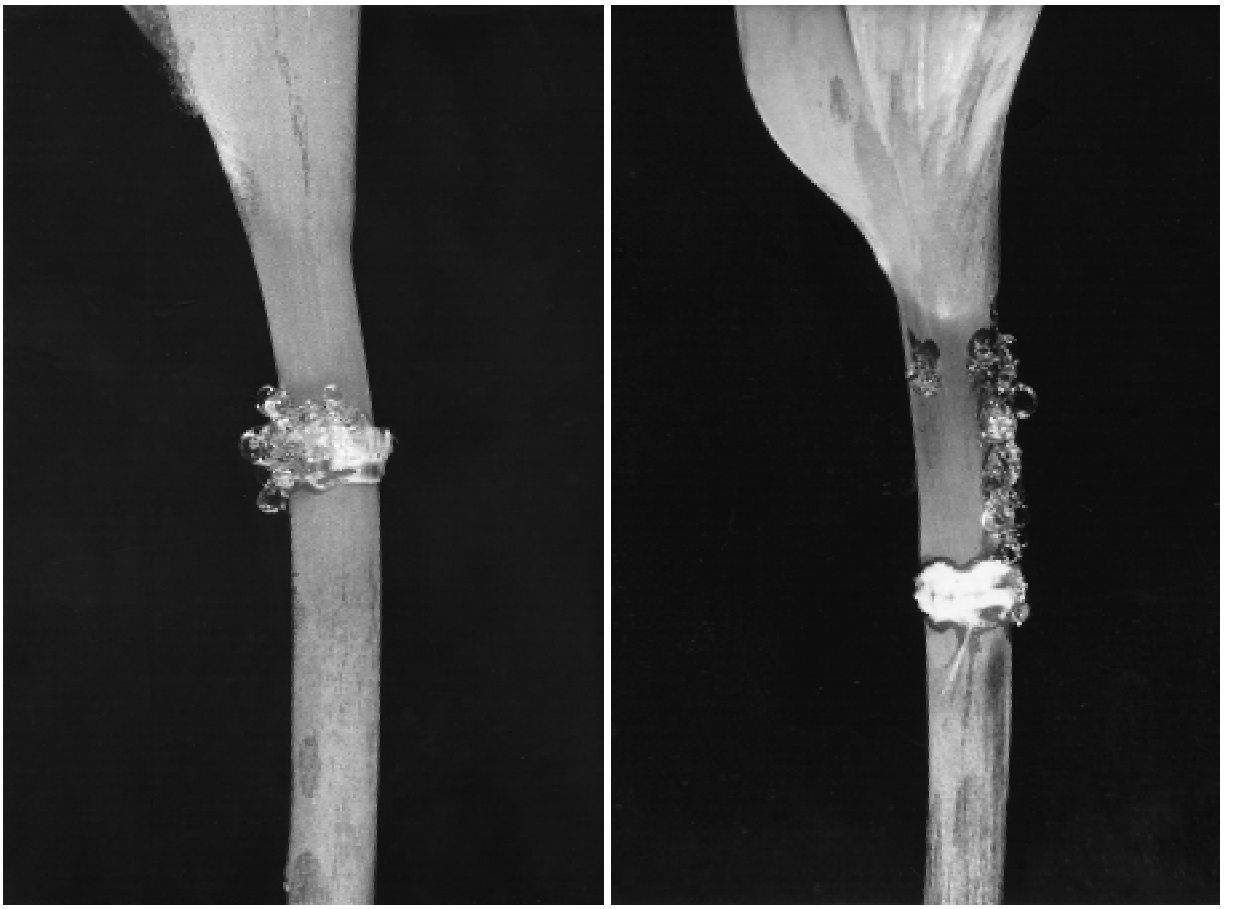

Fig. 1. Effect of JA-Me with and without ACC on gum formation in stem of intact tulips. Pictures were made 9 days after treatment. *Left*, 1% JA-Me; *right*, 1% JA-Me + 5 mM ACC.

18–22°C until October 15 and then dry cooled at 5°C for a minimum of 12 weeks. After removal of the tunics from the bulbs, the bulbs were planted in pots individually and cultivated at a temperature of 17–20°C under natural daylight condition in a greenhouse. Tulip plants at the age of 3–4 days before flowering were used for the following experiments. In intact tulips, JA-Me (0.1% or 1% w/w) or ethephon (1% w/w) in lanolin paste as a ring (about 2–3 mm in width) was applied to the first or second internode at 2 cm below the node. ACC (1 mM or 5 mM), AOA (1 mM), or $CoCl_2$ (1 mM) soaked with cotton wick was applied to the middle part of the stem or the first leaf sheath.

In some experiments, JA-Me (0.23, 0.45, or 0.90 mM) in the presence or absence of ACC (1 or 5 mM) was applied as a solution to the cut shoot of tulips that were cut at the level of soil in a pot.

After appropriate incubation, gum formation was observed optically. A lot of 7–10 plants was used in each treatment.

Determination of Ethylene Production

Ethylene production was measured above the place of treatments in a segment about 1.0 cm long according to the method reported previously (Saniewski and Węgrzynowicz-Lesiak 1994). The segments were cut longitudinally into two parts, and one half of the segment was used for the measurement of ethylene production. For the analysis, stem samples were placed in 10-mL vials, and then the vials were sealed. After 2 h, 1 mL of gas was withdrawn and analyzed by gas chromatography.

Results and Discussion

We have found already that JA-Me induced gum formation in the stem of intact tulip in cultivars Apeldoorn and Gudoshnik (Saniewski 1989, Saniewski and Puchalski 1988, Saniewski and Węgrzynowicz-Lesiak 1994). We could confirm the same results in this study. Applying JA-Me at a concentration of 1% w/w in lanolin paste to the stem induced infiltration, which is associated with the appearance of liquid gums inside the tissues, resulting in the exudation of the gum onto the surface of the tissue, mostly near the place of the application 5–6 days after the treatment (Fig. 1). JA-Me induced gum formation in cut shoots of tulips 4 days after the treatment, especially in the basal part of the leaves, when it was applied as a solution at concentrations of 0.23 mM, or higher (Fig. 2).

Fig. 2. Effect of JA-Me or ACC on gum formation in the basal part of the leaves in the cut shoot of tulips. Pictures were made 5 days after treatment. From *left* to *right*, control, 0.45 mM JA-Me, 0.9 mM JA-Me, and 5 mM ACC.

Table 1. Gum formation in intact tulip shoots. Gum formation was observed 6 days after the treatments and quantified as relative amounts. −, no gums; + to +++, increasing degree of gum production.

Treatments	Gum formation
Control	−
JA-Me (0.1%)	−
JA-Me (1%)	+
Ethephon (1%)	−
ACC (1 mM)	−
ACC (5 mM)	−
JA-Me (0.1%) + ACC (1 mM)	+
JA-Me (0.1%) + ACC (5 mM)	+
JA-Me (1%) + ACC (1 mM)	+++
JA-Me (1%) + ACC (5 mM)	+++
AOA (1 mM)	−
JA-Me (1%) + AOA (1 mM)	+
$CoCl_2$ (1 mM)	−
JA-Me (1%) + $CoCl_2$ (1 mM)	+

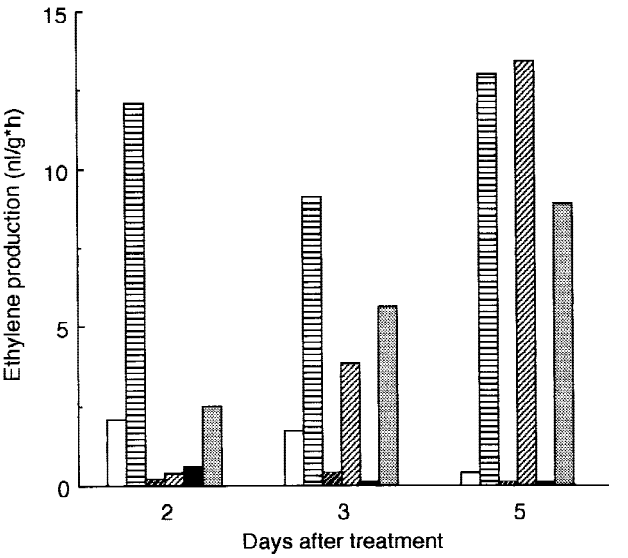

Fig. 3. Effects of JA-Me on ethylene production in the presence or absence of AOA or cobalt chloride in tulip stems. Ethylene production in the segments of the second internode excised from intact tulips treated with JA-Me in the presence or absence of AOA or cobalt chloride for 2, 3, or 5 days was determined. □, control; ▭, 1% JA-Me; ▨, 1 mM AOA; ▨, 1% JA-Me + 1 mM AOA; ■, 1 mM $CoCl_2$; ▩, 1% JA-Me + 1 mM $CoCl_2$.

The simultaneous application of JA-Me at 1% together with ACC at a concentration of 1 or 5 mM shortened the onset of the infiltration or gum formation substantially. The expanded areas of infiltration and gum formation were observed 2 days after that treatment (Table 1 and Fig. 1). This observation about the stimulating effect of ethylene on gum formation was quite clear at the low concentration of JA-Me (0.1%) because the low concentration of JA-Me (0.1%) did not induce gum formation, but that concentration together with ACC at a concentration of 1 or 5 mM induced it substantially (Table 1). The application of JA-Me together with ACC at a concentration of 1 or 5 mM, as a solution, to cut shoots of tulips also accelerated gum induction substantially; the first symptoms of gummosis were observed 1 day after the treatment (data not shown). The synergistic effect between JA-Me and ethylene has already been reported in various phenomena such as gum formation in peach shoots (Saniewski et al. 1998), the gene expression of pathogen-related proteins in tobacco (Xu et al. 1994), the breakdown of cell integrity and cell membrane in sunflower cotyledons (Emery and Reid 1996), and genetic expression of proteinase inhibitor in wound tissues (O'Donnell et al. 1996, Seo et al. 1997).

JA-Me promoted ethylene production substantially in tulip stem segments. As shown in Figs. 3 and 4, although ethylene production in control stem tissues was very low, JA-Me enhanced ethylene production in stem tissues. Furthermore, the simultaneous application of $CoCl_2$ or AOA, both of which are inhibitors of ethylene biosynthesis (Amerhein and Wenker 1979, Lau and Yang 1976), with JA-Me did not affect JA-Me-induced gum formation in tulip shoots, whereas the ethylene production induced by JA-Me was reduced substantially by AOA or $CoCl_2$ (Fig. 3). The stimulating effect of JA-Me on ethylene production has been found already in various plants such as preclimacteric apple fruits (Saniewski et al. 1986, 1987), tomato fruits (Saniewski and Czapski 1985, Saniewski et al. 1987), olive leaves (Sanz et al. 1993), detached rice leaves (Chou and Kao 1992), flowers of *Dendrobium*, *Petunia*, or *Phalaenopsis* (Porat et al. 1993, 1995), and tendrils of *B. dioica* (Weiler et al. 1993).

The application of ACC produced a large amount of

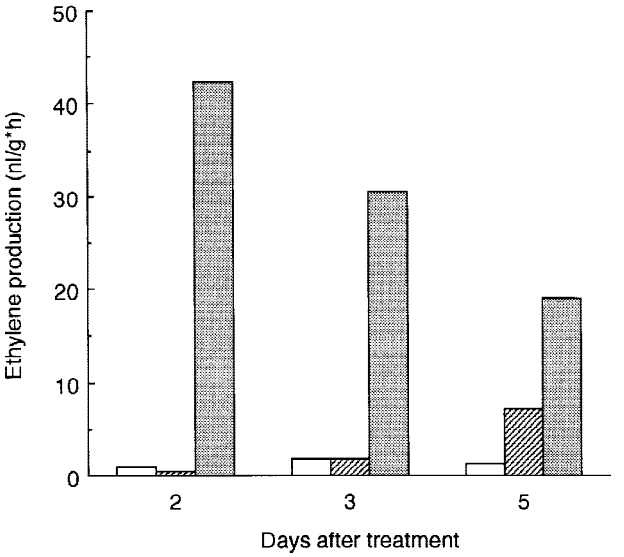

Fig. 4. Effects of JA-Me or ACC on ethylene production in tulip stems. Ethylene production in the segments of the first internode excised from intact tulips treated with JA-Me or ACC for 2, 3, or 5 days was determined. □, control; ▨, 1% JA-Me; ■, 5 mM ACC.

ethylene compared with the control (untreated) and JA-Me treatment (Fig. 4). However, gum formation was not induced by ACC (Table 1). An ethylene-generating compound, ethephon, had no effect on gum formation in the stem of intact tulips (Table 1). Applying high concentrations of IAA or naphthaleneacetic acid (NAA), which induced ethylene, did not induce gum formation in the stem of tulip (Saniewski et al., 1990). These results suggest that JA-Me, but not ethylene, is essential for gum formation in tulip shoots, although the process of gummosis is considered to be regulated by ethylene in tulip bulbs (De Hertogh et al. 1980, De Munk and Saniewski 1989, Kamerbeek and De Munk 1976, Kamerbeek et al. 1971, Moe and Hagness 1975). In tulip bulbs, ethylene induced gum (Saniewski and Puchalski 1988), as well as JA-Me. The reason for this discrepancy in gum formation of tulip between bulbs and shoots has been unclear yet. Recently it has been suggested that one of the ethylene actions in wound response is the regulation of JA levels (O'Donnell et al. 1996, Seo et al. 1997). Dathe (1992) has suggested that JA increases the sensitivity to ethylene for the tiller production in spring barley. The promotion of senescence induced by JA-Me in detached rice (Tsai et al. 1996) or detached maize leaves (Hung and Kao 1996) has been suggested to be mediated through an increase in the sensitivity of tissues to ethylene. Similar explanations might apply to gum formation in tulip shoots. The susceptibility to ethylene after treatment with JA-Me or the effectiveness of ethylene to affect the endogenous level of JA-Me seems to be involved in the differential effect on gum formation between bulbs and shoots in tulips.

It has been reported that gum pocket initials originated through the differentiation of cells of newly formed xylem; and through enlargement and fusion of gum pocket the ducts were formed, resulting in the exudation of gum (Abeles 1973, Bradley et al. 1969, Saniewski and Dyki 1997). Recently, JA-Me or JA has been reported to promote the degradation of cell wall polysaccharides in the petioles of *Phaseolus vulgaris* (Miyamoto et al. 1997, Ueda et al. 1996). Thus, JA-Me, possibly with JA-related compounds, seems not only to induce gum but also to affect the formation of gum pockets or ducts for enhancement of gum secretion by affecting cell wall polysaccharide metabolism.

In conclusion, JA-Me is essential for gum formation in tulip shoots. JA-Me induces gum formation in a way in addition to the stimulation of ethylene production, whereas ethylene probably makes the tissue of shoots sensitive to JA-Me.

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