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Effects of Methyl Jasmonate (MeJA) on the Dark-Induced Senescence in Oat (*Avena sativa* L.) Leaf Segments

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To investigate the relationship between methyl jasmonate (MeJA) and ethylene in leaf senescence, we studied the effects of MeJA on ethylene production and ethylene biosynthetic enzyme activities in oat (*Avena sativa* L.) leaf segments incubated in darkness. MeJA promoted dark-induced senescence judged from the contents of chlorophyll and protein, and increased ethylene production 6 times of the control. MeJA also increased the activities of ethylene biosynthetic enzymes, 1-aminocyclopropane carboxylic acid (ACC) synthase and ACC oxidase as compared to control. In MeJA-treated leaf segments, ACC synthase activity reached its maximum level at 24 h of incubation and ACC oxidase activity peaked at 6 h of incubation. Aminoethoxyvinylglycine (AVG) and Co^{2+}, inhibitors of ACC synthase and ACC oxidase respectively, reduced MeJA-induced ethylene production. They also delayed leaf senescence that was promoted by the treatment of MeJA. From these results, we can suggest that MeJA increased the activities of ACC synthase and ACC oxidase, these increased activities lead to increase in ethylene production and this increased ethylene production might promote dark-induced leaf senescence.

Keywords: methyl jasmonate, leaf senescence, ethylene production, ACC synthase, ACC oxidase, cobalt ion, AVG

Jasmonic acid (JA) and its methyl ester, methyl jasmonate (MeJA), have been found ubiquitously in plant, and known as plant growth regulators which play their roles in the physiological processes such as wounding or senescence (Ueda *et al.*, 1981; Yamane *et al.*, 1981; Vick and Zimmerman, 1984; Staswick, 1992). They have been generally considered as a senescence accelerator. They promoted leaf senescence in oat, rice and barley (Ueda and Kato, 1980; Weidhase *et al.*, 1987a; Chou and Kao, 1992a), and inhibited cytokinin-induced growth in soybean callus cells and radish cotyledon (Ueda and Kato, 1982). They also inhibited photosynthesis via coercion of O_2 evolution in thylakoid membrane (Maslenkova *et al.*, 1990), coercion of synthesis and promotion of degradation of rubisco, and affecting the stomatal closure (Weidhase *et al.*, 1987a,b; Sanz *et al.*, 1993).

Ethylene, a gaseous phytohormone, regulates many aspects of plant growth and development. It, especially, plays an important role in fruit ripening, senescence and stress physiology (Yang and Hoffman, 1984). In carnation flowers the onset of senescence is associated with a sharp increase in ethylene production and induction of the expression of senescence-related genes(Wang and Woodson, 1991). The roles of ethylene in leaf senescence, however, are not established well, yet, though it has been reported that ethylene fostered leaf senescence in oat, rice and parsley (Kao and Yang, 1983; Preger and Gepstein, 1985; Meir *et al.*, 1992).

Exogenously applied MeJA increased ethylene biosynthesis in several plants. It enhanced flower senescence through the elevation of ethylene production in *Petunia* and *Dendrobium* (Porat and Halevy, 1993). It stimulated ethylene production in immature tomato mesophyll cells (Saniewski *et al.*, 1987; Czapski and Saniewski, 1992) and increased ACC oxidase activity in rice (Chou and Kao, 1992b). Although it is likely that MeJA may increase the synthesis of ACC oxidase and/or its activity (Czapski and Saniewski, 1992), the mechanism of increase in ethylene production by the treatment of

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MeJA has not been well established.

So, the content of this paper is the study on the effects of MeJA on ethylene biosynthesis and on the possibility of ethylene mediating in MeJA-induced senescence of oat leaves.

MATERIALS and METHODS

Plant Material

Seeds of oat (*Avena sativa* L., cv AWP-4) were first soaked in tap water for 24 h, then sown in prewashed vermiculite and were grown in the greenhouse. The apical 3-cm segment of the first leaf of eight-day-old seedling was excised and used for this experiment. A group of 5 segments was floated in a flask containing 20 mL of distilled water or of test solution. The flask containing 5 leaf segments was capped with silicon rubber cap and incubated at 26°C in darkness for a different periods of time, with the starting day designated as day 0.

Treatment of Chemicals

MeJA (Aldrich, USA) was diluted with ethanol (Merck, Germany) and treated into 20 mL of distilled water. AVG (Sigma, USA) and $CoCl_2$ (Junsei, Japan) was dissolved in distilled water with high concentration and diluted into 20 mL of solution. 100 μL of ethanol was added to the control.

Quantification of chlorophyll

Chlorophyll extracted and quantified as described by Martin and Thimann (1972).

Quantification of protein

Leaf segments were homogenized with 50 mM potassium phosphate buffer, pH 7.5, using pestle and mortar at 4°C. The homogenate was centrifuged at 15,000 rpm for 30 min, and the resulting supernatant was used for quantification of soluble protein as described by Lowry *et al.* (1951).

Ethylene measurement

Leaf segments were enclosed in a 20 mL gas tight glass container and incubated at 26°C in darkness for 30 min. A one mL sample of gas was withdrawn and analyzed with a gas chromatograph (GC-3BF; Shimadzu, Japan) equipped with an activated alumina column and a flame-ionization detector at 100°C.

ACC synthase assay

ACC synthase activity was analyzed as described by Woodson *et al.* (1992). The enzyme sources were partially purified by a Sephadex G-50 column (0.7 cm × 7 cm). ACC synthase activity was assayed by incubating 0.4 mL of enzyme source with 0.1 mL of 500 μM SAM at 30°C for 15 min. The produced ACC was quantified by chemical conversion of ACC into ethylene.

in vivo ACC oxidase assay

Assay of ACC oxidase activity was performed *in vivo* as described by Wang and Woodson (1989). ACC oxidase activity was determined by ethylene production from leaf segments infiltrated with 2 mM ACC.

RESULTS and DISUCUSSION

Exogenously applied MeJA was reported to increase the activity of ACC oxidase in rice leaves (Chou and Kao, 1992b) and to increase free ACC content and ACC oxidase activity in olive leaves (Sanz *et al.*, 1993). To study the effect of MeJA on senescence in oat leaves, we measured ACC oxidase activity in leaf segments treated with various concentrations of MeJA. ACC oxidase activity increased with the increased concentration of MeJA to 100 μM and decreased with the treatment of higher concentration than 100 μM (data not shown). Hence, in our experiments 100 μM of MeJA was treated to leaf segments.

Effects of MeJA on the senescence of leaf segments

We measured the contents of chlorophyll and protein, as the leaf senescence indicators, during 4-day incubation in darkness (Fig. 1a and b). The contents of chlorophyll and protein in MeJA-treated leaf segments decreased more rapidly than those of control from the 1st day, to about 60% of control on the 2nd day. After the 2nd day, the content of protein continued to decrease more rapidly than that of control to the 4th day. In MeJA-treated leaf segments, however, the decreasing rate of chlorophyll content lessened and on the 4th day, the content of chlorophyll is somewhat higher than that of control. This reversion on the 4th day might not have some physiological significance due to the overdamage of the leaf segments incubated for 4 days. This results indicated that MeJA accelerated dark-induced senescence of oat leaves. This acceleration of leaf senescence by MeJA is well consistent with the former reports (Ueda and Kato, 1980; Ueda *et al.*, 1981;

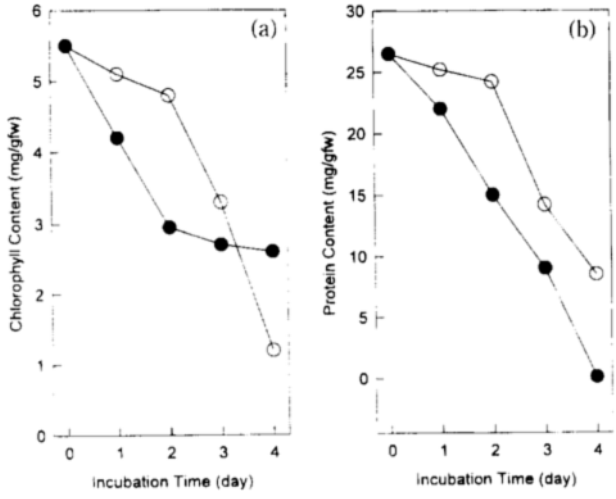

Fig. 1. Changes in the levels of chlorophyll and protein content in oat leaf segments floated on water (○—○) or 100 μM of methyl jasmonate (●--●) at 26°C in darkness. MeJA was treated as described in MATERIALS and METHODS. a): chlorophyll content; b): protein content; The values are the means of 6 independent experiments.

Chou and Kao, 1992a).

Effects of MeJA on ethylene biosynthesis

Ethylene has been reported to play a role in the senescence of many plants (Yang and Hoffman, 1984). It was also reported to participate in dark-induced senescence of oat leaf (Gepstein and Thimann, 1981; Preger and Gepstein, 1985). So, we examined whether the acceleration of dark-induced senescence by MeJA was accompanied by the increase of ethylene production or not (Fig. 2a). Ethylene production from control decreased to 12 h of incubation, increased thereafter and decreased slowly after 24 h of incubation. However, the fluctuation of ethylene production from control was immaterial, compared to that of MeJA-treated leaf segments. Ethylene production from MeJA treated leaf segments decreased to 12 h of incubation and increased thereafter to 24 h of incubation similar to that of control. However, the rate of ethylene production from MeJA-treated leaf segments abruptly increased after 12 h of incubation and reached its maximum level of 11.6 nL/gfw/h, at 24 h of incubation, 6 times as that of control. The rate decreased thereafter. It has been reported also in the leaves of olive, the petals of *Petunia* and *Dendrobium*, and the mesophyll cells of immature tomato that exogenously applied MeJA increased ethylene production (Saniewski *et al.*, 1987; Czapski and Saniewski, 1992; Porat and Halevy,

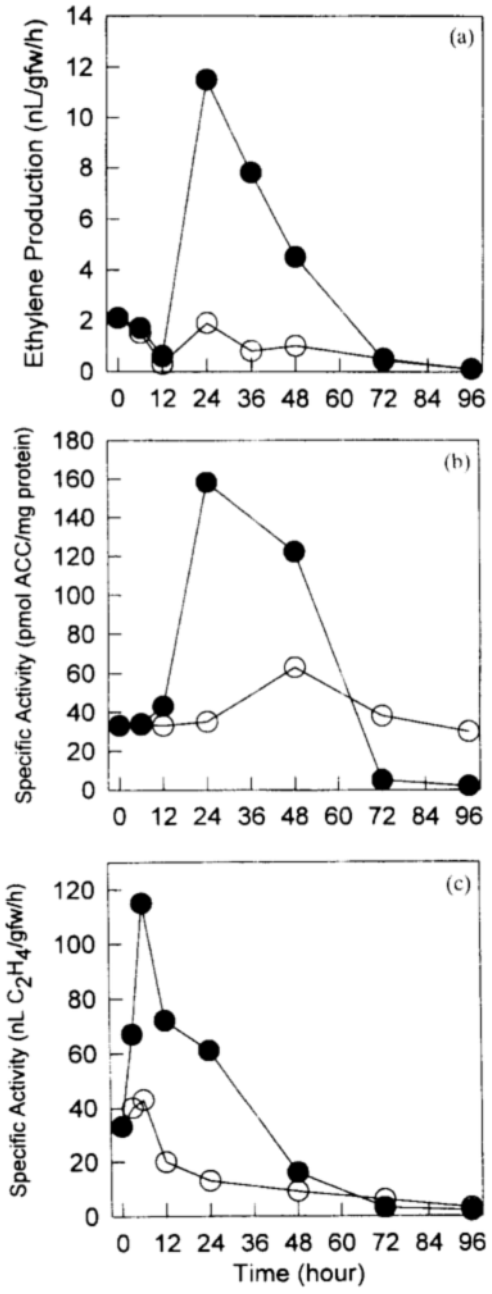

Fig. 2. Effects of MeJA on ethylene biosynthesis from oat leaf segments. Oat leaf segments were enclosed in a gas tight glass container containing distilled water (○--○) or 100 μM MeJA solution (●--●), and incubated at 26°C in darkness. At designated time, oat leaf segments were collected. The rate of ethylene production (a), ACC synthase activity (b) and *in vivo* ACC oxidase activity (c) were measured as described in MATERIALS and METHODS. The values in a) and c) are the means of 6 independent experiments, and the values in c) are the means of 3 independent experiments.

1993; Sanz *et al.*, 1993). However the role of this increased ethylene production has not been studied

well. Considering our result, it is suggested that MeJA could accelerate dark-induced senescence via increase in ethylene production.

The biosynthetic pathway for the plant hormone ethylene has been established as S-adenosylmethionine → ACC → ethylene (Adams and Yang, 1979; Yang and Hoffman, 1984). In this biosynthetic pathway, ACC synthase and ACC oxidase catalyze each step. In higher plants, ethylene production is regulated by the regulation of these two enzymes (Yang and Hoffman, 1984). So, we measured the activities of these two enzymes to determine which enzyme was responsible for the increased ethylene production by MeJA (Fig. 2b and c). The activity of ACC synthase in control was increased after the 1st day of incubation and peaked on the 2nd day. However, ACC synthase activity in MeJA treated leaf segments increased rapidly after 12 h of incubation to peak on the 1st day, which was earlier than control by one day, and was 2.6 times higher than that of control at peak. The change of ACC synthase activity was paralleled to the change of ethylene production (Fig. 2a and b). MeJA also increased the activity of ACC oxidase (Fig. 2c). Treatment of MeJA caused abrupt increase of ACC oxidase activity early in incubation time, which reached its maximum level at 6 h of incubation and decreased rapidly thereafter, whereas ACC oxidase activity in control increased slightly to 6 h of incubation and decreased gradually thereafter. Chou and Kao (1992b) have reported that MeJA increased ACC oxidase activity in rice leaf. From our result, MeJA increased ACC synthase activity as well as ACC oxidase activity, and increase in ethylene production by MeJA is caused by the increase of both enzyme activities. However, the fact that the change of ethylene production was paralleled more to the change of ACC synthase activity than that of ACC oxidase activity suggests that the increase of ethylene production by MeJA is caused mainly by the increase of ACC synthase activity. However, the role of ACC oxidase in ethylene production cannot be underestimated. We can think that MeJA might increase the activity of ACC oxidase first as Chou and Kao's report (1992b) and this increased activity might affect the activity of ACC synthase via certain components.

Effects of AVG and Co^{2+} on the acceleration of dark-induced senescence by MeJA

To confirm that the acceleration of dark-induced senescence in oat leaf segments by MeJA is mediated by the increase of ethylene production, we examined the effects of AVG, an inhibitor of ACC synthase, on the senescence of MeJA-treated leaf segments (Fig. 3). This inhibitor effectively inhibited ethylene production from oat leaves at the 1st day (Fig. 3a). Treatment of AVG with MeJA diminished the effects of MeJA on chlorophyll content, but the content of protein in MeJA-treated leaf segments was not sustained fully as the control level with the

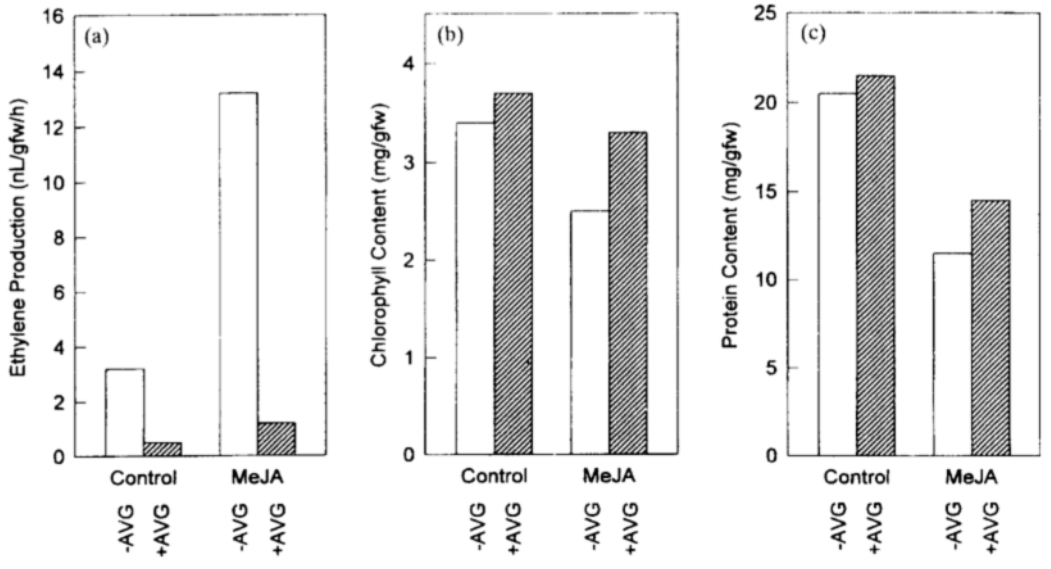

Fig. 3. Effects of AVG on ethylene production (a), chlorophyll content (b) and protein content (c) in MeJA treated leaf segments. Oat leaf segments were treated with 20 μM AVG in the abscence or presence of 100 μM MeJA for 3 days in darkness. The values are the means of 3 independent experiments.

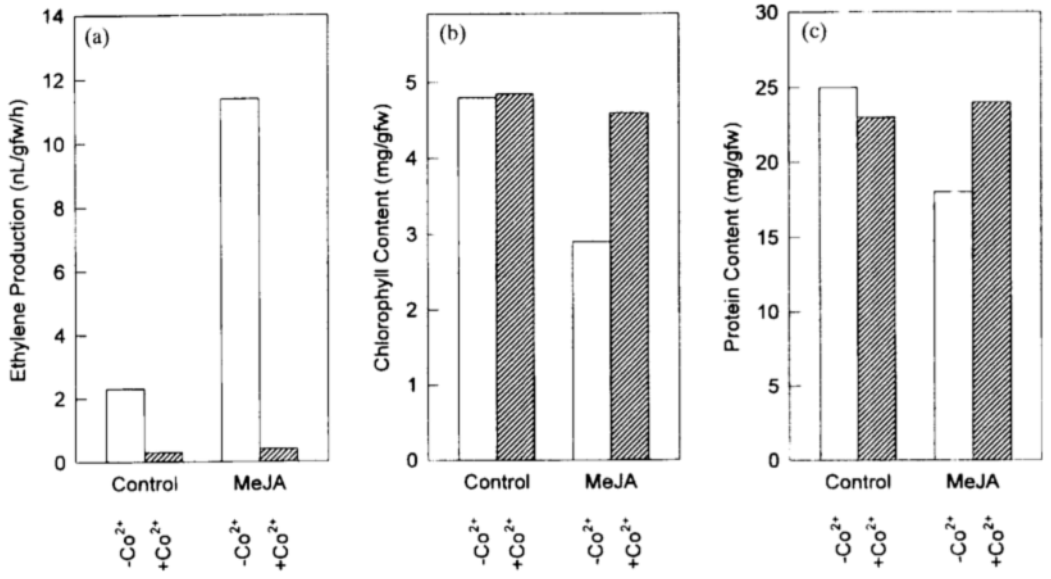

Fig. 4. Effects of Co^{2+} on ethylene production (a), chlorophyll content (b) and protein content (c) in MeJA treated leaf segments. Oat leaf segments were treated with 100 μM $CoCl_2$ in the abscence or presence of 100 μM MeJA for 2 days in darkness. The values are the means of 3 independent experiments.

treatment of AVG. From these AVG effects, it is not certain whether the acceleration of dark-induced senescence in oat leaf segments by MeJA is mediated by the increase of ethylene production. So, we also examined the effects of Co^{2+}, an inhibitor of ACC oxidase. Co^{2+}, also, inhibited effectively ethylene production from oat leaves (Fig. 4a). This inhibitor diminished fully the effects of MeJA on the contents of chlorophyll and protein (Fig. 4b and c). It has been already reported that dark-induced senescence of oat leaves was related with preceding increase of ethylene production and then MeJA accelerated dark-induced senescence of oat leaves (Gepstein and Thiman, 1981; Preger and Gepstein, 1985). However, no direct evidence that MeJA-acceleration of dark-induced senescence was related with increase of ethylene production has been available. Based on our results, it could be linked to the increase of ethylene production. MeJA might accelerate the dark-induced senescence of oat leaves via increase of ethylene production. This increase of ethylene production was caused by increase of the activities of ACC synthase and ACC oxidase.

On the other hand, exogenously applied MeJA has been known to accelerate senescence in many plants through the increase of respiration rate, the inhibition of photosynthesis and the regulation of several gene expression (Melan *et al.*, 1993; Sanz *et al.*, 1993; Sembdner and Parthier, 1993; Reinbothe *et al.*, 1994). MeJA also induces the expression of lipoxygenase gene that participates in the biosynthesis of MeJA (Bell and Mullet, 1993). Lipoxygenase has been taken to accelerate senescence through breaking up fatty acid of phospholipid in cell membranes (Siedow, 1991). So, we could not conclude that only ethylene mediated MeJA-acceleration of dark-induced senescence, but that ethylene played some roles in MeJA-acceleration of dark-induced senescence in oat leaves.

LITERATURE CITED

Adams, D.O. and S.F. Yang. 1979. Ethylene biosynthesis: Identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. *Proc. Natl. Acad. Sci. USA* **76**: 170-174.

Bell, E. and J.E. Mullet. 1993. Characterization of an *Arabidopsis* lipoxygenase gene responsive to methyl jasmonate and wounding. *Plant Physiol.* **103**: 1133-1137.

Chou, C.M. and C.H. Kao. 1992a. Methyl jasmonate, calcium, and leaf senescence in rice(*communication*). *Plant Physiol.* **99**: 1693-1694.

Chou, C.M. and C.H. Kao. 1992b. Stimulation of 1-aminocyclopropane-1-carboxylic acid-dependent ethylene production in detached rice leaves by methyl jasmonate. *Plant Science* **83**: 137-141.

Czapski, J. and M. Saniewski. 1992. Stimulation of ethylene production and ethylene-forming enzyme activity

in fruits of the non-ripening nor and rin tomato mutants by methyl jasmonate. *J. Plant. Physiol.* **139**: 265-268.

Gepstein, S. and K.V. Thimann. 1981. The role of ethylene in the senescence of oat leaves. *Plant Physiol.* **68**: 349-354.

Kao, C.H. and S.F. Yang. 1983. Role of ethylene in the senescence of detached rice leaves. *Plant Physiol.* **73**: 881-885.

Lowry, O.H., N.J. Roseborough, A.C. Farr and R.J. Randall. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**: 265-275.

Martin, C. and K.V. Thimann. 1972. The role of protein synthesis in the senescence of leaves. I. The formation of protease. *Plant physiol.* **49**: 64-71.

Meir, S., S. Philosoph-Hadas and N. Aharoni. 1992. Ethylene-increased accumulation of Fluorescent lipid-peroxidation products detected during senescence of parsley by a newly developed method. *J. Amer. Soc. Hort. Sci.* **117**: 128-132.

Melan, M.A., X. Dong, M.E. Endara, K.R. Davis, F.M. Ausubel and T.K. Peterman. 1993. An Arabidopsis thaliana lipoxygenase gene can be induced by pathogens, abscisic acid, and methyl jasmonate. *Plant Physiol.* **101**: 441-450.

Porat, R. and A.H. Halevy. 1993. Enhancement of petunia and dendrobium flower senescence by jasmonic acid methyl-ester is via the promotion of ethylene production. *Plant Growth Regulation* **13**: 297-301.

Preger, R. and S. Gepstein. 1985. Regulation of ethylene biosynthesis during senescence of oat leaf segments. *Physiol. Plant.* **65**: 163-166.

Reinbothe, S., B. Mollenhauer and C. Reinbothe. 1994. JIPs and RIPS: The regulation of plant gene expression by jasmonates in response to environmental cues and pathogens (*Review article*). *Plant Cell* **6**: 1197-1209.

Saniewski, M., J. Nowacki and J. Czapski. 1987. The effect of methyl jasmonate on ethylene production and ethylene-forming enzyme activity in tomatoes. *J. Plant. physiol.* **129**: 175-180.

Sanz, L.C., J.C. Fernandez-Maculet, E. Gomez, B. Vioque and J.M. Olias. 1993. Effect of methyl jasmonate on ethylene biosynthesis and stomatal closure in olive leaves. *Phytochemistry* **33**: 285-289.

Sembdner, G. and B. Parthier. 1993. The biochemistry and the physiological and molecular actions of jasmonates. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **44**: 569-589.

Siedow, J.N. 1991. Plant lipoxygenase: structure and function. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **42**: 145-148.

Staswick, P.E. 1992. Jasmonate, genes, and fragrant signals (*Update on signaling*). *Plant Physiol.* **99**: 804-807.

Ueda, J. and J. Kato. 1980. Isolation and identification of a senescence-promoting substance from wormwood (*Artemisia absinthium* L.). *Plant Physiol.* **66**: 246-249.

Ueda, J. and J. Kato. 1982. Inhibition of cytokinin-induced plant growth by jasmonic acid and its methyl ester. *Physiol. Plant.* **54**: 249-252.

Ueda, J., J. Kato, H. Yamane and N.Takahashi. 1981. Inhibitory effect of methyl jasmonate and its related compounds on kinetin-induced retardation of oat leaf senescence. *Physiol. Plant.* **52**: 305-309.

Vick, B.A. and D.C. Zimmerman. 1984. Biosynthesis of jasmonic acid by several plant species. *Plant Physiol.* **75**: 458-461.

Wang, H. and W.R. Woodson. 1989. Reversible inhibition of ethylene action and interruption of petal senescence in carnation flowers by norbornadiene. *Plant Physiol.* **89**: 434-438.

Wang, H. and W.R. Woodson. 1991. A flower senescence-related mRNA from carnation shares sequence similarity with fruit ripening-related mRNAs involved in ethylene biosynthesis (*Plant Gene Register*). *Plant Physiol.* **96**: 1000-1001.

Weidhase, R.A., H-M. Kramell, J. Lehmann, H-W. Liebisch, W. Lerbs and B. Parthier. 1987a. Methyljasmonate-induced changes in the poly-peptide pattern of senescing barley leaf segments. *Plant Science* **51**: 177-186.

Weidhase, R.A., J. Lehmann, H. Kramell, G. Sembdner, and B. Parthier. 1987b. Degradation of ribulose-1,5-bisphosphate carboxylase and chlorophyll in senescing barley leaf segments triggered by jasmonic acid methylester, and counteraction by cytokinin. *Physiol. Plant.* **69**: 161-166.

Woodson, W.R., K.Y. Park, A. Drory, P.B. Larsen and H. Wang. 1992. Expression of ethylene biosynthetic pathway transcripts in senescing carnation flowers. *Plant Physiol.* **99**: 526-532.

Yamane, H., H. Takagi, H. Abe, T. Yokota and N. Takahashi. 1981. Identification of jasmonic acid in three species of higher plants and its biological activities. *Plant Cell Physiol.* **22**: 689-697.

Yang, S.F. and N.E. Hoffman. 1984. Ethylene biosynthesis and its regulation in higher plants. *Ann. Rev. Plant Physiol.* **35**: 155-189.

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