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Stimulation of 1-aminocyclopropane-1-carboxylic acid-dependent ethylene production in detached rice leaves by methyl jasmonate

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Effects of methyl jasmonate (JA-Me) on ethylene production in detached rice leaves were investigated. Without 1-aminocyclopropane-1-carboxylic acid (ACC) pretreatment, JA-Me had no effect on ethylene production. However, JA-Me markedly stimulated ethylene production in detached rice leaves pretreated with a saturating concentration of ACC. JA-Me stimulated ACC-dependent ethylene production within 2 h of its application, a result suggesting that JA-Me enhances ACC-dependent ethylene production directly. $CoCl_2$ at 1 mM inhibited ACC-dependent ethylene production stimulated by JA-Me, suggesting that ethylene production from ACC is mediated by the ethylene-forming enzyme. Cycloheximide also inhibited ACC-dependent ethylene production induced by JA-Me, indicating that sustained synthesis of the ethylene-forming enzyme is required. JA-Me stimulated ACC-dependent ethylene production in detached leaves that had been aged for 1, 2 and 3 days. However, aged leaves were less responsive to JA-Me in terms of ACC-dependent ethylene production. Abscisic acid was found to inhibit ACC-dependent ethylene production stimulated by JA-Me.

Key words: abscisic acid; 1-aminocyclopropane-1-carboxylic acid; ethylene; methyl jasmonate; *Oryza sativa*

Introduction

Methyl jasmonate (JA-Me) and jasmonic acid are endogenous substances which have been identified in many plants [1–3]. JA-Me and jasmonic acid were found to be powerful promoters of leaf senescence [2,4–6]. Ethylene has been shown to be involved in the regulation of leaf senescence [7–9]. Recently, Saniewski et al. [10–11] reported that JA-Me stimulated ethylene biosynthesis in tomato fruits. However, this is the only plant system so far that has been used to examine the effect of JA-Me on ethylene biosynthesis. It is not known whether other plant or tissue systems also show a similar effect of JA-Me on ethylene production. For this reason we investigated the effect of JA-Me on ethylene production in detached rice leaves.

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Abbreviations: ABA, abscisic acid; ACC, 1-aminocyclopropane-1-carboxylic acid; JA-Me, methyl jasmonate.

Materials and Methods

Seedlings of rice (*Oryza sativa* L. cv. Taichung Native 1) seedlings were grown in hydroponic culture as described previously [12]. Unless otherwise indicated, ACC-dependent ethylene production was determined. For the determination of ACC-dependent ethylene production, 10 leaf segments, weighing 45 mg, were pretreated with a saturating concentration of ACC (10 mM) in water and then treated with various compounds in water for various periods of time as indicated in the figure and table legends. Pretreatment and treatment were carried out at 27°C in darkness. At the time indicated, leaf segments were placed vertically in test tubes which were closed with rubber stoppers and incubated in darkness at 27°C for

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1 h. The ethylene in the gas phase of the closed tubes was determined by analysis of 1 ml of sample withdrawn with a hypodermic syringe as described elsewhere [9]. Absolute levels of ethylene varied among experiments because of seasonal effects. However, the patterns of responses to JA-Me were reproducible. Each treatment had 4 replicates. All experiments described here were repeated at least twice. Similar results and identical trends were obtained each time. The data reported here were from a single experiment.

Results and Discussion

The effects of the concentration of JA-Me on ACC-dependent ethylene production in detached rice leaves in darkness are presented in Fig. 1. JA-Me markedly promoted ACC-dependent ethylene production in detached rice leaves. Increasing JA-Me concentration from 0.45 to 45 μM progressively enhanced ACC-dependent ethylene production by detached rice leaves. However, ACC-dependent ethylene production declined at a JA-Me concen-

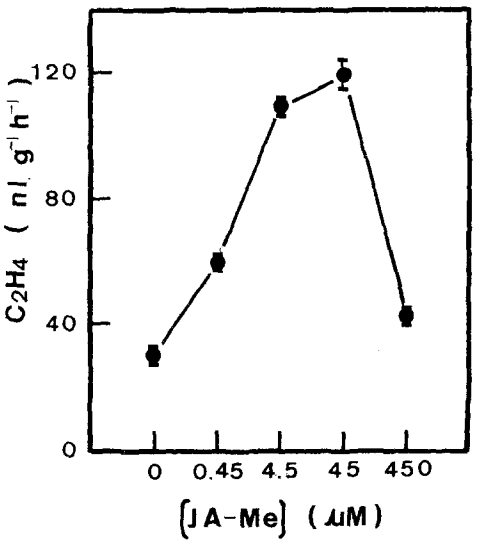

Fig. 1. Effect of JA-Me concentration on ACC-dependent ethylene production in detached rice leaves. Detached rice leaves were pretreated with 10 mM ACC in water for 2 h and then treated with various concentrations of aqueous JA-Me in darkness. Ethylene production was assayed after 6 h of treatment. Bars indicate standard errors.

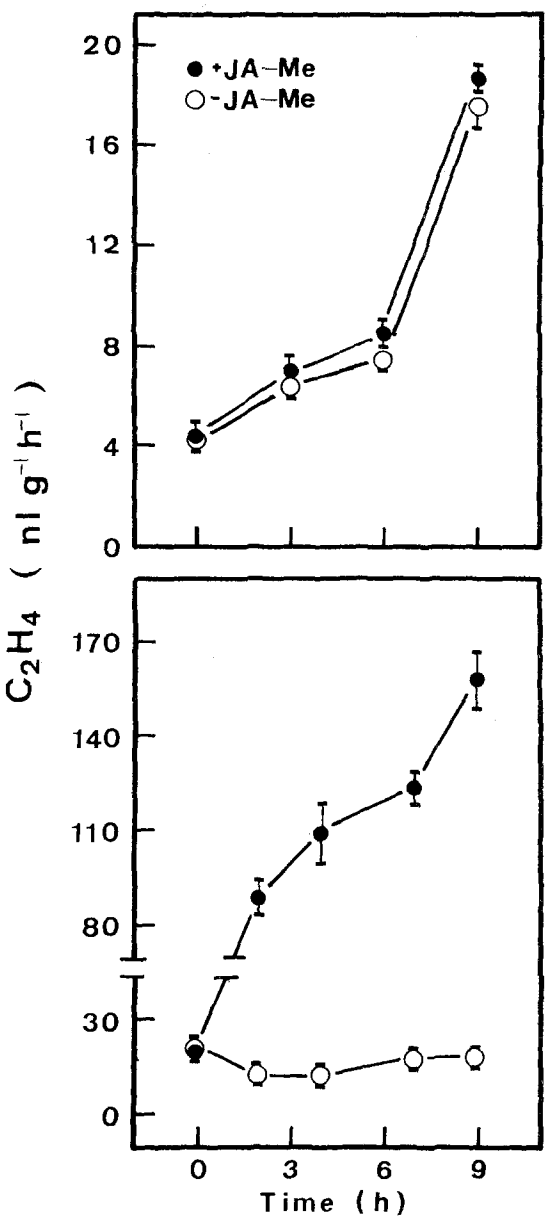

Fig. 2. Changes with time in rates of basal (upper) and and ACC-dependent (lower) ethylene production in detached rice leaves treated with JA-Me. For basal ethylene production, detached leaves were treated with either water or 45 μM JA-Me in water in darkness. To determine ACC-dependent ethylene production, detached rice leaves were pretreated with a saturating concentration of ACC (10 mM) in water for 2 h and then treated with either water or 45 μM JA-Me in water in darkness. Ethylene production was assayed at the times indicated. Bars indicate standard errors.

tration of 450 μM. The ability of JA-Me to stimulate ACC-dependent ethylene production was also observed when illumination (16.7 μmol/m^2 per s, photosynthetic photon flux, provided by Gro-lux fluorescent lamp) was used (data not shown).

Ethylene production was stimulated in tomatoes by JA-Me in the absence of exogenous ACC [10–11]. However, we could not detect any effect of JA-Me on ethylene production without ACC pretreatment (Fig. 2, upper).

JA-Me stimulated ACC-dependent ethylene production was detected within 2 h of its application (Fig. 2, lower). Increasing duration of treatment with JA-Me from 2 to 9 h progressively enhanced ACC-dependent ethylene production by detached rice leaves. The observation that the stimulation of ACC-dependent ethylene production could be detected immediately after application of JA-Me suggests that JA-Me directly enhances ACC-dependent ethylene production.

In order to determine if ethylene production from ACC is mediated by ethylene-forming enzyme in detached rice leaves treated with JA-Me, the effect of 1 mM $CoCl_2$, which inhibits the conversion of ACC to ethylene [12], was studied (Table I). Ethylene formation was inhibited by $CoCl_2$ in detached rice leaves treated with JA-Me, indicating that detached rice leaves treated with JA-Me formed ethylene from ACC through ethylene-forming enzyme. Cycloheximide also inhibited ACC-dependent ethylene production by JA-Me (Table I), suggesting that sustained synthesis of the ethylene-forming enzyme is required for JA-Me-stimulated ACC-dependent ethylene production.

Table I. Effects of $CoCl_2$ and cycloheximide (CHI) on ACC-dependent ethylene production induced by methyl jasmonate (JA-Me) in detached rice leaves. Detached rice leaves were pretreated with ACC (10 mM) in water for 2 h and then treated with aqueous JA-Me (45 μM) in the absence or presence of $CoCl_2$ (1 mM) or CHI (50 μM). Ethylene was assayed after 3 h of treatment in darkness. Means ± S.E. are shown.

Treatment	Ethylene (nl/g per h)
No Additions	23.0 ± 1.4
+ JA-Me	93.2 ± 2.3
+ JA-Me + $CoCl_2$	29.1 ± 2.4
+ JA-Me + CHI	35.7 ± 0.7

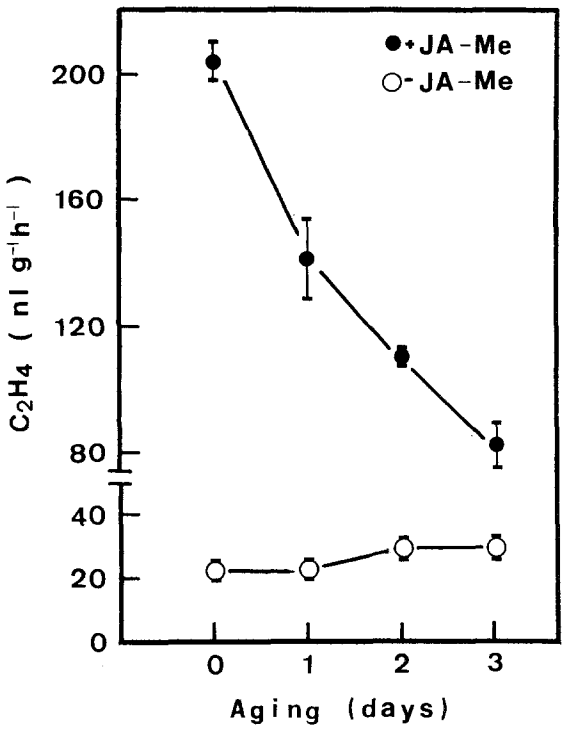

Fig. 3. Effect of aging on methyl jasmonate (JA-Me)-stimulated ACC-dependent ethylene production in detached rice leaves. Detached rice leaves, aged for 1, 2, or 3 days in water in darkness, were pretreated with 10 mM ACC in water and then treated with either water or 45 μM JA-Me in water in darkness. Ethylene production was assayed after 6 h of treatment. Bars indicate standard errors.

To study the effects of aging, segments of rice leaves were incubated in water in darkness for 1, 2, or 3 days before the start of the experiment. Segments of rice leaves aged for 1, 2 or 3 days all exhibited JA-Me-stimulated ACC-dependent ethylene production (Fig. 3). However, aged leaf segments were less responsive to JA-Me in terms of ACC-dependent ethylene production. Increasing the duration of aging resulted in a decrease of ACC-dependent ethylene production induced by JA-Me. Untreated segments of leaves produced low levels of ACC-dependent ethylene regardless of the duration of aging (Fig. 3).

Jasmonic acid is similar to ABA both chemically

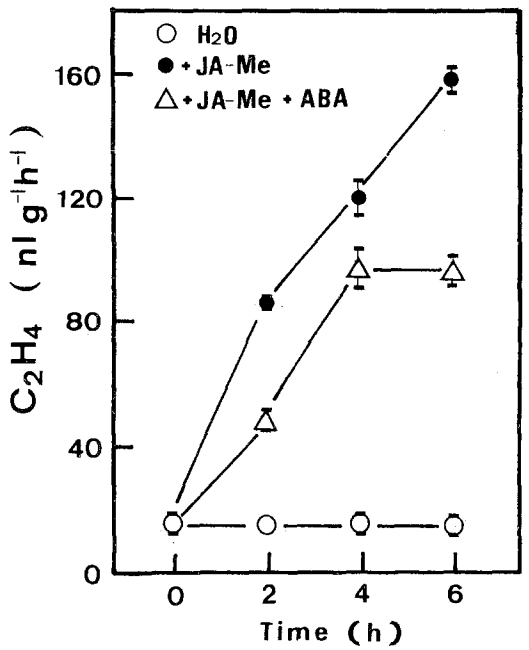

Fig. 4. Effect of methyl jasmonate (JA-Me) in the absence or presence of ABA on ACC-dependent ethylene production in detached rice leaves. Detached rice leaves were pretreated with 10 mM ACC in water and then treated with either water, JA-Me (45 μM) in water or JA-Me (45 μM) + ABA (45 μM) in water in darkness. Ethylene production was assayed at the times indicated. Bars indicate standard errors.

and biologically [13]. Both compounds are keto-acids with similar molecular weights, solubility properties and pKs and in some tissues, jasmonic acid causes an inhibition of growth that often mimics the effect of ABA [14]. To determine whether JA-Me interacts with ABA in regulating ACC-dependent ethylene production, the effect of JA-Me in the absence or presence of ABA on ACC-dependent ethylene production was examined. ACC-dependent ethylene production was found to be significantly inhibited by ABA (Fig. 4). This inhibition is probably not due to the inhibition of JA-Me uptake by ABA, because detached rice leaves pretreated with ABA also resulted in an inhibition of ACC-dependent ethylene production stimulated by JA-Me (data not shown). ABA has also been reported to inhibit stress-induced ethylene production [15–17].

It has been shown that ABA levels increase during aging of segments of oat leaves [18]. If ABA levels increase similarly in aged rice leaves, then the decrease in ACC-dependent ethylene production stimulated by JA-Me in aged rice leaves could result from ABA-mediated inhibition.

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