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Effect of Methyl Jasmonate on Senescence of Broccoli Florets

Kazushi Watanabe, Tomoko Kamo, Fumie Nishikawa and Hiroshi Hyodo*

Department of Biological Sciences, Faculty of Agriculture, ShizuokaUniversity, Ohya, Shizuoka 422-8529

Summary

Broccoli (*Brassica oleracea* L. cv Italica) florets senesced rapidly after harvest at ambient temperatures. During senescence at 20 ℃, the rate of ethylene production of florets significantly increased, concurrent with a rapid yellowing of sepals ascribed to chlorophyll degradation. 1-Aminocyclopropane-1-carboxylic acid (ACC) oxidase activity in florets also rapidly increased to a peak; the enzyme activity then declined sharply, paralleling the increased pattern of ethylene production. Treatment of florets with 1 mM methyl jasmonate (MJ) significantly promoted ethylene production and ACC oxidase activity during senescence, attaining a higher peak faster than the control. Chlorophyll loss and a concurrent enhancement of ACC synthase activity by exogenous 1 mM MJ were accelerated to a greater extent than those of the control. The rise in ethylene production was reduced and delayed by treatment with 10 mM diethyldithiocarbamate (DIECA) that is thought to be an inhibitor of jasmonate biosynthesis. These results suggest that jasmonates may be associated with promoting senescence by enhancing ethylene production in broccoli florets.

Key Words: *Brassica oleracea*, diethyldithiocarbamate, ethylene production, methyl jasmonate, senescence.

Introduction

Harvested broccoli florets senesce rapidly at ambient temperatures accompanied by the degradation of ascorbic acid and chlorophyll that lead to yellowing of sepals. It has been shown that endogenous ethylene is involved in postharvest senescence of broccoli florets (Lieberman and Hardenburg, 1954; Ku and Wills, 1999). During senescence of broccoli, the rate of ethylene production in broccoli florets significantly increased to a maximum, then declined, almost paralleling the pattern of ACC oxidase activity. Chlorophyll loss was induced by the increased amount of ethylene (Makhlouf et al., 1989; Tian et al., 1994; Kasai et al., 1996). Moreover, by treatment with 2,5-norbornadiene and 1-methylcyclopropene (MCP), inhibitors of ethylene action strongly suppressed the progress of senescence, indicating that endogenous ethylene is involved in the aging process (Kasai et al., 1996; Ku and Wills, 1999).

Ethylene is synthesized via methionine-ACC pathway in higher plants (Adams and Yang, 1979; Yang and Hoffman, 1984) which is catalyzed by ACC synthase and ACC oxidase, regulators of ethylene biosynthesis (Yang and Hoffman, 1984; Abeles et al., 1992; McKeon et al., 1995).

Other phytohormones affect ethylene production and senescence of broccoli florets, e.g., cytokinin application retarded the ACC-stimulated senescence of broccoli and extended its storage life (Fuller et al., 1977; Clarke et al., 1994). Jasmonates (jasmonic acid and MJ) exist widely in plants (Ueda and Kato, 1980; Yamane et al., 1981; Vick and Zimmerman, 1984) and exert a wide range of hormonal roles (Sembdner and Parthier, 1993; Creelman and Mullet, 1997), such as; a) tuberization (Koda, 1992), b) promotion of leaf senescence (Ueda and Kato, 1981), c) inhibition of sprouting (Wang, 1998), d) defense responses against damages by pathogens (Thomma et al., 1989) and wounding (Niki et al., 1998; Watanabe and Sakai, 1998), e) promotion of fruit ripening (Fan et al., 1998a; 1998b), f) promotion of ethylene biosynthesis (Saniewski et al., 1987; Chou and Kao, 1992; Fan et al., 1997) and g) nicotine biosynthesis (Imanishi et al., 1998). Jasmonic acid and MJ are synthesized from linolenic acid through 13-hydroperoxylinolenic acid (HPLA) and 12, 13-epoxylinolenic acid (Sembdner and Parthier, 1993). DIECA inhibits jasmonate biosynthesis by converting HPLA to 13-hydroxylinolenic acid (Farmer et al., 1994; Menke et al., 1999).

In this study, we found that application of MJ to broccoli florets accelerates senescence by promoting ethylene production. The role of MJ in this postharvest regulation of senescence during storage of broccoli florets based on ethylene biosynthesis and action in broccoli is discussed.

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*Corresponding author: e-mail: abhhyou@agr.shizuoka.ac.jp

Materials and Methods

Plant materials and treatment of methyl jasmonate and diethyldithiocarbamate

Broccoli heads (*Brassica oleracea* L. cv Italica), obtained from a local market in Shizuoka, were dissected into curds (segments averaging 17.1 g) and immersed in a solution of 1 mM MJ (Wako Pure Chemical Ind.) containing 1% ethanol or 10 mM DIECA (Sigma Chemical Co.) in 0.1% Tween 20 while being gently stirred for 1 hr at room temperature. The curds were removed from the solution, blotted lightly with paper towels and held at 20 ℃ under continuous light (5.2 μmol·sec^{-1}·m^{-2}) and a high humidity. Control broccoli curds were treated with 1% ethanol or 0.1% Tween 20, and stored under the same conditions as the treated ones. Periodically during the incubation, florets were excised from the curds and analyzed.

Chlorophyll determination

A gram of broccoli florets was homogenized with 20 ml of 96% ethanol with a mortar and pestle, and the homogenate centrifuged at 3,000 X g for 15 min. Chlorophyll content in the supernatant was determined by spectrophotometry, based on the absorbance at 665 nm and 649 nm and equations described by Wintermans and De Mots (1965).

Assay of ethylene production

Broccoli florets (1 g) were placed in an Erlenmeyer flask (63 ml), which was sealed with a rubber serum cap for 1 hr at 20 ℃. One-ml gas sample was withdrawn from the atmosphere in the flask and injected into a gas chromatograph (Hitachi 163) equipped with an activated alumina column at 70 ℃ and a flame ionization detector, and the ethylene concentration was recorded.

Extraction and assay of ACC synthase

Broccoli florets were homogenized with 5 times their volume of 100 mM N-(2-hydroxyethyl) piperazine-N'-(3-propanesulfonic acid) (EPPS) buffer, pH 8.5, containing 10 mM 2-mercaptoethanol (2-ME), and 10 μM pyridoxal phosphate (PLP) at 2 ℃ with a mortar and pestle. The homogenate was centrifuged at 14,000 X g for 20 min at 4 ℃, and the supernatant was gel-filtrated through PD-10 (Sephadex G-25, Amersham Pharmacia Biotech) column with 10 mM EPPS buffer, pH 8.5, containing 10 mM 2-ME and 10 μM PLP. The filtrate was assayed for ACC synthase activity in a reaction mixture that contained 50 mM EPPS buffer, pH 8.5, and 50 μM S-adenosylmethionine in a total of 1 ml. After incubation at 30 ℃ for 30 min, the reaction was stopped by adding 0.1 ml 40 mM $HgCl_2$. ACC formed in this reaction was assayed by the method of Lizada and Yang (1979). ACC synthase activity was expressed as nmol ACC formed per hr per g fresh weight of florets.

Extraction and assay of ACC

Florets (0.5 g) were homogenized in 10 ml of 80% ethanol. The homogenate was centrifuged at 3,000 X g for 15 min, and the supernatant concentrated to dryness in vacuo at 45 ℃. The residue was taken up in 5 ml of water, and an aliquot was assayed for ACC by the method of Lizada and Yang (1979).

Extraction and assay of ACC oxidase

A gram of florets was homogenized in 10 ml of 100mM Tris-HCl buffer, pH 7.2, containing 10% (w/v) glycerol, 10 mM sodium ascorbate, and 5 mM dithiothreitol at 2 ℃. The homogenate was centrifuged at 14,000 X g for 20 min at 4 ℃. The supernatant was assayed for ACC oxidase activity in a medium comprised of 100 mM Tris-HCl buffer, pH 7.2, 30% glycerol, 1 mM ACC, 10 mM sodium ascorbate, 50 μM $FeSO_4$, and 10 mM $NaHCO_3$ for a total of 2 ml including the enzyme. The enzyme reaction was performed in a sealed test tube with an atmospheric volume of 14.1 ml; after 1 hr the amount of ethylene produced was assayed by gas chromatography and the ACC oxidase activity expressed as nl ethylene produced per hr per g fresh weight of florets.

All data are representative of at least two separate experiments. Each sampling or assay was performed in triplicate, and the results are expressed as mean ± S.D.

Results

Stimulation of broccoli senescence by methyl jasmonate

Senescence of broccoli florets progressed rapidly at 20 ℃ after harvest, accompanied by the yellowing of sepals. The rate of ethylene production by florets significantly increased to a maximum and declined thereafter. Treatment of florets with 1 mM MJ significantly promoted ethylene production, attaining a higher peak much faster than in the control (Fig. 1). The progress of senescence as reflected by chlorophyll loss was also enhanced by the exogenous treatment (Fig. 2).

Changes in ACC synthase and ACC oxidase activities and ACC levels in broccoli florets during senescence by treatment with methyl jasmonate

ACC synthase in broccoli florets was induced by treatment with 1 mM MJ; its activity increased and reached a maximum 2 days after incubation, followed by a sharp decline (Fig. 3). The activity in control samples remained almost constant throughout the experimental period.

ACC oxidase activity in florets increased during senescence, attaining a broad peak from which it declined sharply (Fig. 4). Treatment of the curds with MJ hastened ACC oxidase activity faster than that of the control.

ACC contents in florets remained low during the early

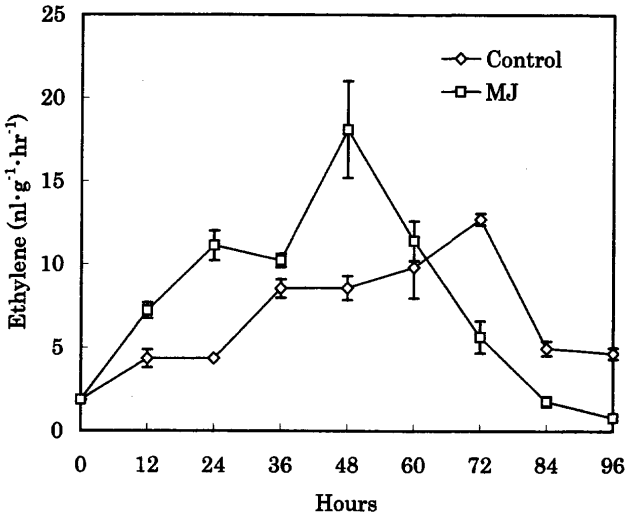

Fig. 1. Time course of ethylene production by broccoli florets by treatment with 1 mM MJ during storage at 20 ℃. (◇), control; (□), 1 mM MJ. Data are the mean of three replicates. Bars represent S. D.s when larger than symbols.

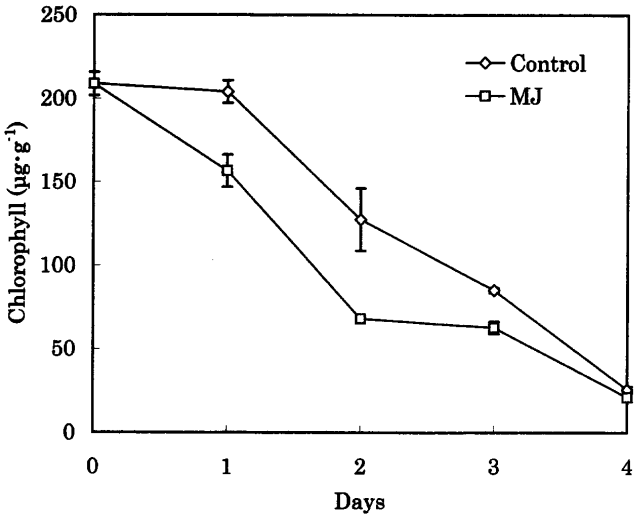

Fig. 2. Time course of chlorophyll degradation in MJ-treated and untreated broccoli florets. Chlorophyll content represents a total of chlorophyll 'a' and 'b'. (◇), control; (□), 1 mM MJ. Data are the mean of three replicates. Bars represent S. D.s when larger than symbols.

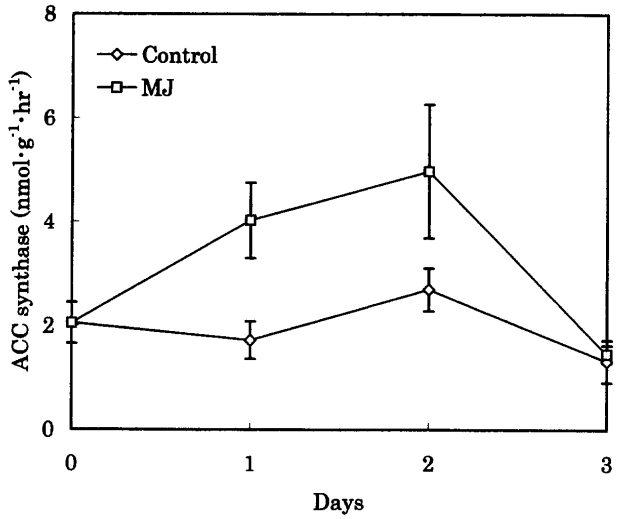

Fig. 3. Fluctuations in ACC synthase activity during senescence of MJ-treated and control broccoli florets. (◇), control; (□), 1 mM MJ. Data are the mean of three replicates. Bars represent S. D.s and are contained within the symbols when not shown.

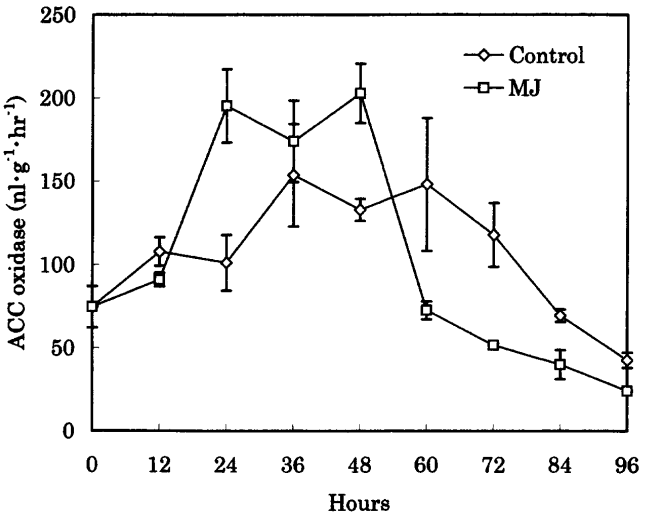

Fig. 4. Fluctuations in ACC oxidase activity of broccoli florets treated with 1 mM MJ vs. the control. (◇), control; (□), 1 mM MJ. Data are the mean of three replicates. Bars represent S. D.s and are contained within the symbols when not shown.

stage of senescence, but gradually increased with time (Fig. 5). After treatment with MJ the curds accumulated significantly more ACC than did the control.

Effect of diethyldithiocarbamate on ethylene production by broccoli florets

Ethylene production by broccoli florets treated with 10 mM DIECA was reduced and delayed in reaching a peak (Fig. 6). This inhibition of ethylene evolution is attributed to the suppression of jasmonate biosynthesis by DIECA. The slowing of ACC oxidase activity correlates with the reduction and delay of ethylene production.

Discussion

Broccoli florets senesce rapidly after harvest at ambient temperatures as evident by the yellowing of sepals. During the course of senescence ethylene production reached a maximum and then declined thereafter (Fig. 1). The trend and role of ethylene have been reported (Lieberman and Hardenburg, 1954; Makhlouf et al., 1989; Tian et al., 1994; Kasai et al., 1996). Ku and Wills (1999) demonstrated that MCP, an inhibitor of ethylene action delayed senescence, indicating that ethylene was closely associated with the aging process

In this study, exogenous application of MJ to broccoli florets significantly hastened senescence by accelerating

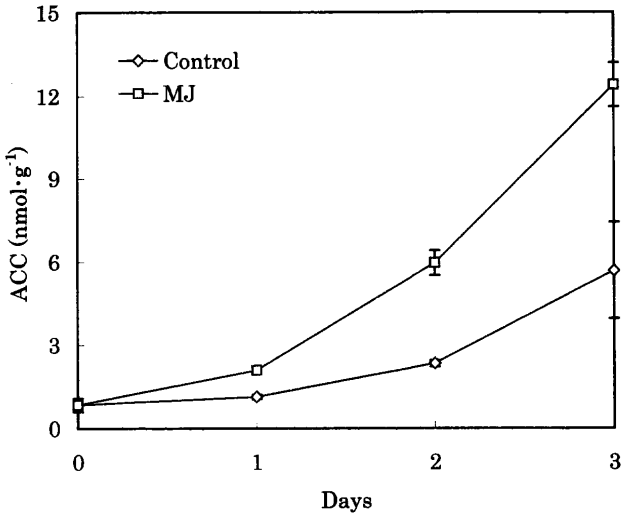

Fig. 5. Increase in ACC content by treatment of broccoli curds with 1 mM MJ. (◇), control; (□), MJ. Data are the mean of three replicates. Bars represent S. D.s and are included in the symbols when not shown.

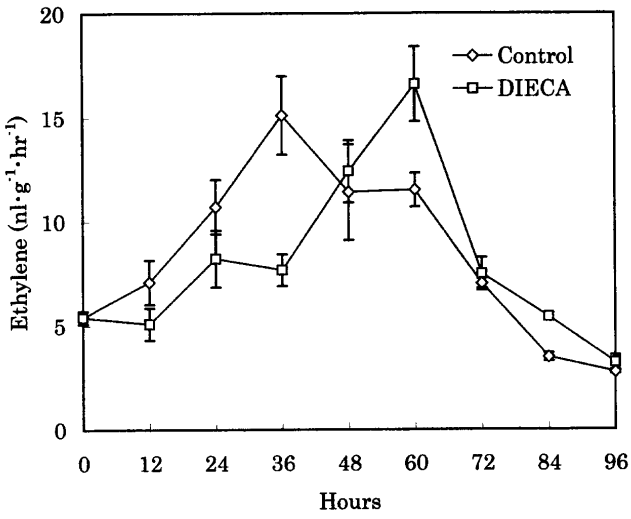

Fig. 6. Effect of treating broccoli curds with 10 mM DIECA on ethylene production during storage at 20 ℃. (◇), control; (□), 10 mM DIECA. Data are the mean of three replicates with bars representing S. D.s when larger than the symbols.

the rate of ethylene production in the florets (Fig. 1, 2), while increasing ACC oxidase activity in the florets (Fig. 4). The enhanced rate of ethylene production is attributed to the rapid rise in ACC synthase activity, leading to ACC formation (Fig. 3, 5). Treatment with 0.01, 0.1, and 1 mM MJ revealed that 1 mM was most effective although 0.1 mM MJ still had a stimulating effect.

Saniewski et al. (1987) found that MJ stimulated both ethylene production and in vivo ACC oxidase activity in either immature or mature green tomatoes, whereas Chou and Kao (1992) reported that MJ enhanced ACC-dependent ethylene production in detached rice leaves, mediated by ACC oxidase. In excised mesocarp tissue of *Cucurbita maxima*, Watanabe and Sakai (1998) found that MJ stimulated gene expression of ACC synthase induced by wounding. In apples ACC synthase activity was also promoted by MJ (Fan et al., 1998a). These results indicate that MJ accelerates ethylene production by stimulating gene expression and synthesis of ACC synthase and ACC oxidase.

During the senescence of broccoli florets ethylene synthesis is induced, but whether this response is the results of endogenous jasmonate formation and action is unknown. In this trial, we found that DIECA, supposedly a jasmonate biosynthesis blocker (Farmer et al., 1994; Menke et al., 1999), reduced ethylene production (Fig. 6), indicating that endogenous jasmonate is involved in the senescence process.

Niki et al. (1998) proposed a model for cross-signalling in the wound-induced transduction pathway between jasmonate and salicylic acid (SA). When the effect of 1 mM SA on rates of senescence and ethylene production in broccoli florets were sought during senescence, both chlorophyll breakdown and ethylene production were retarded (data not shown). Furthermore this treatment antagonized the effect of 1 mM MJ on stimulation of chlorophyll breakdown. Hence, we assume that the endogenous jasmonate is associated with the progress of senescence. But, because salicylate inhibits ACC oxidase activity (Leslie and Romani, 1988) it is possible that salicylate regulates ethylene biosynthesis by reducing ACC oxidase activity. Therefore, the primary role of ethylene is to interact somehow jasmonate and salicylate in regulating postharvest senescence of broccoli florets.

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ブロッコリー小花の老化に及ぼすジャスモン酸メチルの影響

渡辺和志・加茂知子・西川芙美恵・兵藤　宏

静岡大学農学部　422-8529　静岡市大谷 836

摘　要

ブロッコリー (*Brassica oleracea* L. cv Italica) の小花は収穫後室温で急速に老化した.

ブロッコリーの 20℃貯蔵における老化の過程で小花のエチレン生成量は明確に増加し，最大値に達した後減少した. エチレン生成量の増加はがく片の黄化 (クロロフィルの減少) の急速な進行と関連していた. 小花の 1-アミノシクロプロパン-1-カルボン酸 (ACC) 酸化酵素活性は急速に増加し，ピークに達した後急激に低下した. ACC 酸化酵素活性の増減はエチレン生成のそれとほぼ平行であった. 1 mM ジャスモン酸メチル (MJ) 処理により小花のエチレン生成と ACC 酸化酵素活性は対照区と比較して促進され，ピークは早く，そして高い値を示した. クロロフィルの分解も MJ により促進された. 1 mM MJ 処理により ACC 合成酵素活性は対照区と比較して高まり，ACC レベルの顕著な増加に関連していた. またエチレン生成量の増大はジャスモン酸生合成の阻害剤であると考えられている 10 mM ジエチルジチオカルバミン酸 (DIECA) 処理により抑制され，遅れた. これらの結果はジャスモン酸がブロッコリー小花においてエチレン生成を促進することによって老化の促進に関わっていることを示唆している.