Promotive effect of jasmonates on the senescence of detached maize leaves

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Abstract

Promotion of senescence of detached maize leaves by jasmonates was investigated. Senescence of detached maize leaves was promoted by linolenic acid, the precursor of biosynthesis of jasmonic acid, and retarded by inhibitors of lipoxygenase, the first enzyme in the biosynthetic pathway of jasmonic acid. Results support a role of endogenous jasmonates in the regulation of senescence of detached maize leaves. Silver thiosulfate, an inhibitor of ethylene action, was found to inhibit methyl jasmonate, linolenic acid- and abscisic acid-promoted senescence of detached maize leaves. It seems that jasmonate-promoted senescence is mediated through an increase in ethylene sensitivity in detached maize leaves.

Abbreviations: ABA = abscisic acid; MJ = methyl jasmonate; STS = silver thiosulfate

Introduction

Methyl jasmonate (MJ) and free acid, jasmonic acid, have been found to occur throughout the plant kingdom [11]. Leaf senescence is the sum of various physiological processes that involve the loss of chlorophyll, proteins and nucleic acids, disassembly of membrane, and alterations in gene expression program and in enzyme activities [20]. It has been shown that exogenous jasmonates promoted leaf senescence [6, 16, 21]. However, it remains to be established whether endogenous jasmonates regulate leaf senescence.

Ethylene production by MJ in tomato fruits has been described [9]. Chou and Kao [7] demonstrated that MJ had no effect on ethylene production in detached rice leaves, but stimulated 1-aminocyclopropane-1-carboxylic acid-dependent ethylene production. Ethylene is also known to promote leaf senescence [2, 10, 13, 17]. Abeles *et al.* [1] reported that ethylene was not involved in MJ-promoted senescence of excised cucumber cotyledons. Cuello *et al.* [8] also demonstrated that MJ and ethylene promoted senescence of barley leaf senescence by different mechanisms.

In this study, we examined the role of endogenous jasmonates on senescence of detached maize leaves by using precursor and several inhibitors of lipoxygenase, the first enzyme in the proposed biosynthetic pathway of jasmonic acid. The possible involvement of ethylene in regulating MJ-promoted senescence of detached maize leaves was also investigated.

Materials and methods

Seedlings of maize (*Zea mays* cv. Taimung 1) were grown in vermiculite in a greenhouse with natural light at 30 °C day/25 °C night for 7 days, by which time the primary leaves were fully expanded. The apical 2.5-cm segments were excised from the primary leaves and were placed in Petri dishes containing 15 mL of distilled water or test solutions and incubated at 27 °C in the dark.

Chlorophyll was extracted and determined following the method of Wintermans and De Mots [25] after extraction in 96% ethanol. For protein determination, leaf segments were homogenized in 50 mM sodium phosphate buffer (pH 6.8). The extracts were centri-

Figure 1. Effect of methyl jasmonate on chlorophyll and protein levels in detached maize leaves. Chlorophyll and protein levels were determined after 4 days in darkness. Bars indicate SE ($n = 4$). Only those SE larger than symbol size are shown.

fused at 17,600 g for 20 min, and the supernatant liquids were used for determination of protein by the method of Bradford [5]. Chlorophyll and protein levels were expressed as mg g^{-1} fresh weight.

In experiments with Ag^+ , STS was used as a source. Leaf segments were treated with STS and test solution. A stock solution of STS was prepared by mixing equal volumes of 0.01 M AgNO_3 and 0.04 M $\text{Na}_2\text{S}_2\text{O}_3$ [14].

Results and discussion

The senescence of detached maize leaves in the dark was followed by measuring the decrease of chlorophyll and protein. Figure 1 shows the effect of MJ on the levels of chlorophyll and protein in detached maize

Figure 2. Effect of linoleic acid (18:2) and linolenic acid (18:3) on chlorophyll and protein levels in detached maize leaves. All treatments included 0.1% Tween 20. Chlorophyll and protein levels were determined after 3 days in darkness. The concentration of 18:2 and 18:3 was 5 mM . Bars indicate SE ($n = 4$).

leaves. MJ significantly promoted the senescence of detached maize leaves. MJ concentration at 11.25 μM is as effective as that at 45 μM in promoting senescence of detached maize leaves.

Oxidation of linolenic acid (18:3) by lipoxygenase is the first step in the biosynthesis pathway of jasmonic acid [24]. The availability of linoleic acid (18:2), linolenic acid and several inhibitors of lipoxygenase provided the opportunity to investigate whether endogenous jasmonates regulate senescence of detached maize leaves.

If endogenous jasmonates play a role in the regulation of senescence of detached maize leaves, linolenic acid, but not linoleic acid, is expected to promote senescence, whereas lipoxygenase inhibitors are expected to retard senescence. Figure 2 shows the effect of linoleic acid and linolenic acid on the levels of chlorophyll and protein in detached maize leaves. It is clear that linolenic acid, but not linoleic acid, signifi-

Figure 3. Effect of lipoygenase inhibitors on chlorophyll level in detached maize leaves. The pH of all solutions was maintained near neutrality with 0.2 mM potassium phosphate, pH 7.0. Chlorophyll level was determined after 4 days in darkness. Bars indicate SE ($n = 4$). Only those SE larger than symbol size are shown.

cantly promoted senescence of detached maize leaves. However, Ueda and Kato [22] demonstrated that both linoleic acid and linolenic acid promoted senescence of oat leaves. The effect of various compounds previously shown to inhibit lipoygenase [15, 19] on senescence of detached maize leaves in darkness is

presented in Figures 3 and 4. All five lipoygenase inhibitors at the appropriate concentration retarded senescence of maize leaves.

Although the inhibitors we used are diverse in their structures, there are similarities among them. We cannot rule out the possibility that the inhibitors acted

Figure 4. Effect of lipoxigenase inhibitors on protein level in detached maize leaves. The pH of all solutions was maintained near neutrality with 0.2 mM potassium phosphate, pH 7.0. Protein level was determined after 4 days in darkness. Bars indicate SE ($n = 4$). Only those SE larger than symbol size are shown.

by a mechanism other than the inhibition of lipoxigenase, although the variety of compounds tested suggests otherwise. Salicylhydroxamic acid and *n*-propyl gallate are chelators of divalent cations and they inhibit the alternative respiratory pathway [18]. It will be important to establish whether jasmonic

acid increases during senescence of detached maize leaves and whether the lipoxigenase inhibitors block the increase in jasmonic acid level.

The results that linolenic acid, precursor of jasmonic acid biosynthesis, promoted senescence and the lipoxigenase inhibitors retarded senescence

Figure 5. Effect of silver thiosulfate (STS) on methyl jasmonate (MJ)-promoted senescence of detached maize leaves. The concentration of MJ was 45 μM . Chlorophyll and protein levels were determined after 4 days in darkness. Bars indicate SE ($n = 4$).

support a role for endogenous jasmonic acid in regulating senescence of detached maize leaves.

Previously, we have shown that ethylene production in detached maize leaves was very low (less than 10 nL g⁻¹ 24 h⁻¹) and remained relatively constant during the course of senescence in darkness [12]. These results indicate that ethylene production does not play a role in regulating dark-induced senescence of detached maize leaves. If a change of ethylene level is excluded as an explanation for the senescence of detached maize leaves, a change in sensitivity to ethylene is an alternate possibility. This possibility was tested by using ethylene action inhibitor, Ag⁺ [4]. In the present investigation, STS was used as a source of Ag⁺. The advantage of using STS are related to a lower free Ag⁺ in STS solutions and a lower phytotoxicity of this compound [14, 23]. The effect of STS on MJ- and

Figure 6. Effect of silver thiosulfate (STS) on linolenic acid (18:3)-promoted senescence of detached maize leaves. The concentration of 18:3 was 5 mM. All treatments included 0.1% Tween 20. Chlorophyll and protein levels were determined after 4 days in darkness. Bars indicate SE ($n = 4$).

linolenic acid-promoted senescence of detached maize leaves was shown in Figures 5 and 6, respectively. STS was observed to inhibit MJ- and linolenic acid-promoted senescence of detached maize leaves. These results indicate that an increase in ethylene sensitivity is involved in regulating MJ- and linolenic acid-promoted senescence in detached maize leaves. Our results are in contrast to those of other workers, who showed that the action of MJ on senescence was independent of ethylene [1, 8]. Since STS is less effective in inhibiting linolenic acid-promoted senescence of maize leaves, other mechanisms that may be involved in regulating linolenic acid-induced senescence can not be excluded.

Figure 7. Effect of silver thiosulfate (STS) on abscisic acid (ABA)-promoted senescence of detached maize leaves. The concentration of ABA was $45 \mu\text{M}$. The pH of all test solutions was adjusted to 5.5 by 0.1 N HCl . Chlorophyll and protein levels were determined after 4 days in darkness. Bars indicate SE ($n = 4$).

Previously, we found that ABA promoted senescence of detached maize leaves but inhibited ethylene production [12]. We suggested, but did not prove, that ABA increased tissue sensitivity to ethylene [12]. Jasmonates and ABA have both chemical and physiological similarity [3]. Thus it is of great interest to know whether the promotive response of ABA on senescence of detached maize leaves is also mediated through a change in ethylene sensitivity. As indicated in Figure 7, ABA-promoted senescence is indeed mediated through a change in ethylene sensitivity.

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