

# Senescence of rice leaves XXXV. Promotive effects of jasmonates

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**Abstract.** Promotion of the senescence of detached rice leaves by jasmonates was investigated. The senescence of detached rice leaves was promoted by linolenic acid, the precursor of the biosynthesis of jasmonic acid, and retarded by inhibitors of lipoxygenase, the first enzyme in the biosynthetic pathway of jasmonic acid. Linolenic acid-promoted senescence was found to be inhibited by lipoxygenase inhibitors. Among the four major lipoxygenase pathway metabolites studied, jasmonic acid and methyl jasmonate were effective in promoting the senescence of detached rice leaves while traumatic acid and *trans*-2-hexenal had no or only marginal effect on it. The results support a role for endogenous jasmonates in regulating the senescence of detached rice leaves.

**Keywords:** Jasmonic acid; Leaf senescence; Methyl jasmonate; *Oryza sativa* L.

## Introduction

Jasmonates are endogenous substances that have been identified in many plants (Meyer et al., 1984). It has been shown that exogenous jasmonates promote leaf senescence (Chou and Kao, 1992; Parthier, 1991; Ueda et al., 1981; Weidhase et al., 1987). However, it remains to be established whether endogenous jasmonates regulate leaf senescence. In the present investigation, we examined the role of endogenous jasmonates in detached rice leaf senescence by using precursors, several inhibitors of lipoxygenase, the first enzyme in the proposed biosynthetic pathway of jasmonic acid (JA), and major lipoxygenase pathway metabolites including JA, methyl jasmonate (MJ), traumatic acid, and *trans*-2-hexenal. Both JA and MJ are derived from linolenic acid (18:3) via the lipoxygenase pathway (Vick and Zimmerman, 1984). *Trans*-2-hexenal and traumatic acid may also be formed from octadecanoid fatty acid linolenic acid by sequential enzyme steps involving lipoxygenase (Siedow, 1991; Vick and Zimmerman, 1984; Zimmerman and Coudron, 1979). The availability of linolenic acid, JA, MJ, *trans*-2-hexenal, traumatic acid, and several inhibitors of lipoxygenase provides the opportunity to investigate whether endogenous jasmonates regulate the senescence of detached rice leaves.

## Materials and Methods

Rice (*Oryza sativa* cv. Taichung Native 1) was cultured as previously described (Kao, 1980). The apical 3-cm segments excised from the third leaves of 12-day-old seedlings were used. A group of 10 segments, weighing about 45 mg, were floated in a Petri dish containing 10 mL of test solution. Incubation was carried out at 27°C in darkness.

Chlorophyll was determined according to Wintermans and De Mots (1965) after extraction in 96% (v/v) ethanol. For protein extraction, leaf segments were homogenized in 50 mM sodium phosphate buffer (pH 6.8). The extracts were centrifuged at 17,000 g for 20 min, and the supernatants were used for determination of protein by the method of Bradford (1976). Protein and chlorophyll levels were expressed as mg g<sup>-1</sup> fresh weight.

All experiments were run at least twice. Data are presented as the results of a single experiment typical of the trend seen in the repeated experiments. MJ [(-)-MJ] was a product of Serva. JA[(±)-JA] and other chemicals were purchased from Sigma Chemical Co.

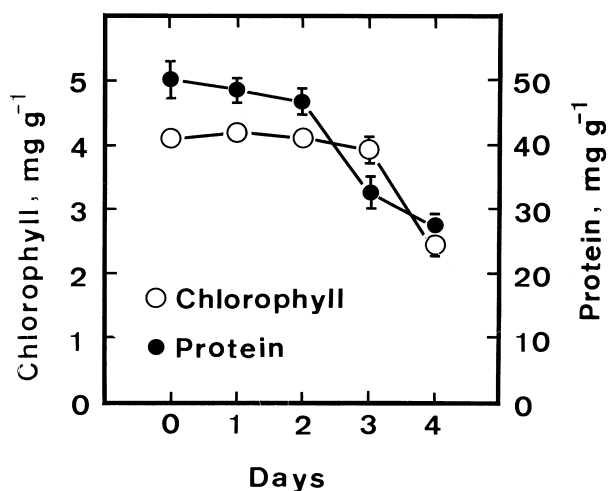
## Results and Discussion

The senescence of detached rice leaves in the dark was followed by measuring the decrease of chlorophyll and protein. Figure 1 shows the time courses of chlorophyll and protein levels of detached rice leaves. Chlorophyll levels remained unchanged for the first 3 days and subsequently declined. A decrease in protein was evident 3 days after leaf detachment.

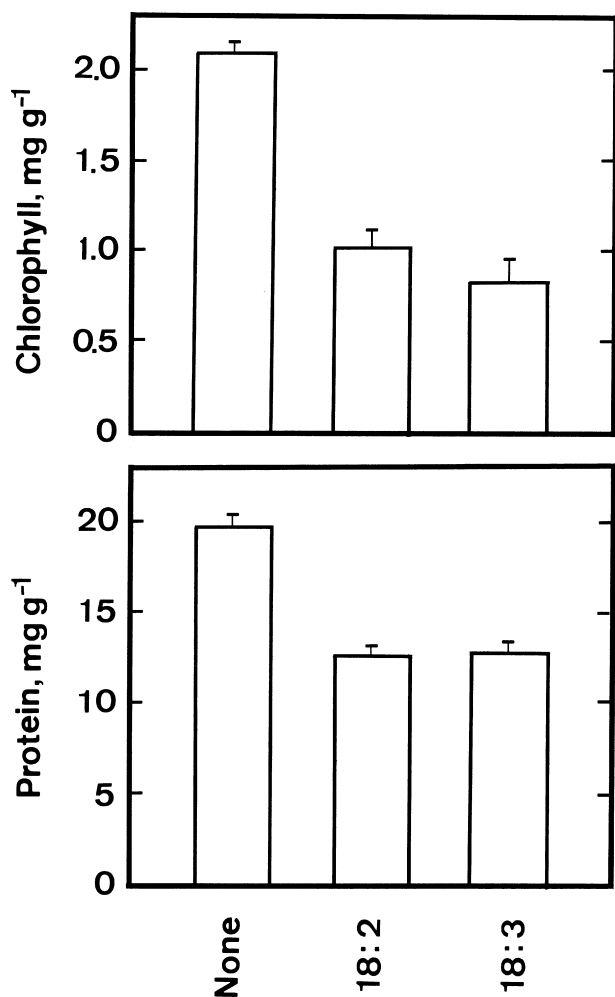
Oxidation of linolenic acid (18:3) by lipoxygenase is the first step in the biosynthesis pathway of JA (Vick and Zimmerman, 1984). The availability of linoleic acid, linolenic acid, and several inhibitors of lipoxygenase provided the opportunity to investigate whether endogenous jasmonates regulate the senescence of detached rice leaves. To examine the possible involvement of endogenous JA in regulating senescence of detached rice leaves, we first examined the effects of linoleic acid (18:2), linolenic acid (18:3) and lipoxygenase inhibitors on the senescence of detached rice leaves.

Figure 2 shows the effects of linoleic acid and linolenic acid on the levels of chlorophyll and protein in detached

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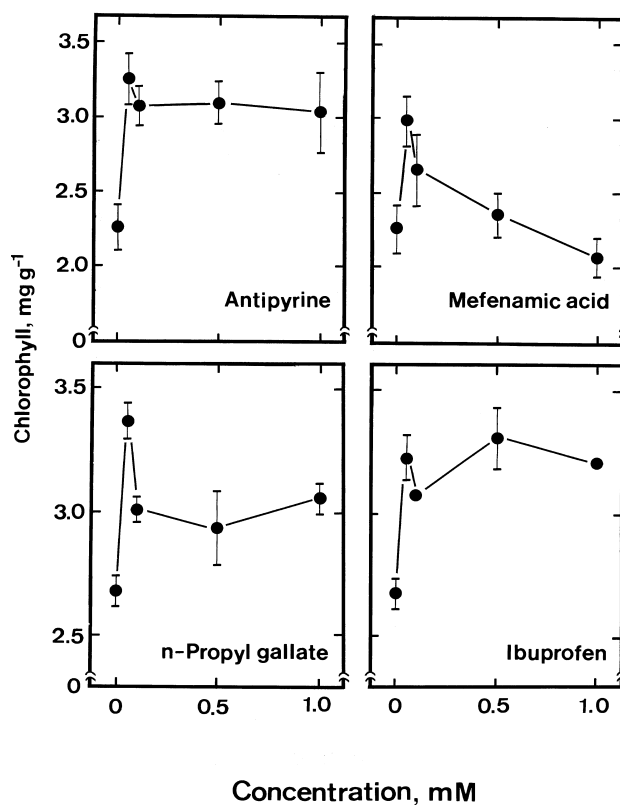
**Figure 1.** Chlorophyll and protein levels in detached rice leaves during dark-induced senescence. Bars indicate S. E. (n=4).



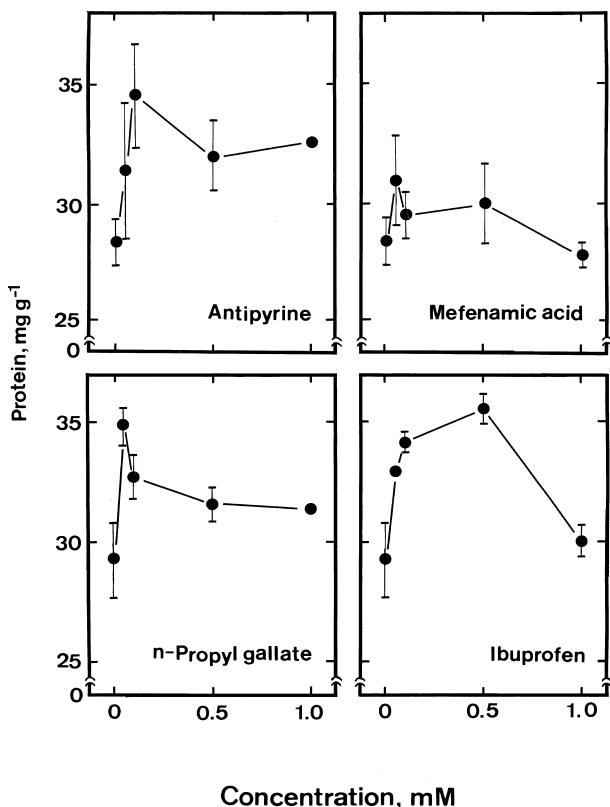
**Figure 2.** Effect of linoleic acid (18:2) and linolenic acid (18:3) on chlorophyll and protein levels in detached rice leaves. All treatments included 0.1% Tween 20. Chlorophyll and protein levels were determined after 4 days in darkness. The concentration of 18:2 and 18:3 was 5 mM. Bars indicate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

rice leaves. Clearly, both linoleic and linolenic acid were effective in promoting the senescence of detached rice leaves. The promotive effect of linoleic acid is not unexpected, since linoleic acid can be converted to linolenic acid and is also a primary endogenous substrate for lipoxygenase (Hildebrand, 1989; Thomas, 1986). Ueda and Kato (1982) also demonstrated that both linoleic acid and linolenic acid promoted senescence in oat leaves.

If endogenous jasmonates play a role in regulating the senescence of detached rice leaves, lipoxygenase inhibitors are expected to retard senescence. The effects of various compounds previously shown to inhibit lipoxygenase (Park and Polacco, 1989; Sircar et al., 1983) on senescence of detached rice leaves in darkness are presented in Figures 3 and 4. All four lipoxygenase inhibitors at 0.05 mM retarded the senescence of rice leaves. Although the inhibitors used are diverse in their structure, they have some similarities (Staswick et al., 1991). We can not rule out the possibility that the inhibitors acted by a mechanism other than the inhibition of lipoxygenase, although the variety of compounds tested suggests otherwise. Lipoxygenase inhibitors were also observed to inhibit linolenic acid-promoted senescence in detached rice leaves (Figure 5). These results suggest that linolenic acid-promoted senescence in detached rice leaves is mediated through lipoxygenase.



**Figure 3.** Effect of lipoxygenase inhibitors on chlorophyll level in detached rice leaves. The pH of all solutions was maintained near neutrality with 0.2 mM potassium phosphate, pH 7.0. Chlorophyll levels were determined after 4 days in darkness. Bars indicate S. E. (n=4).



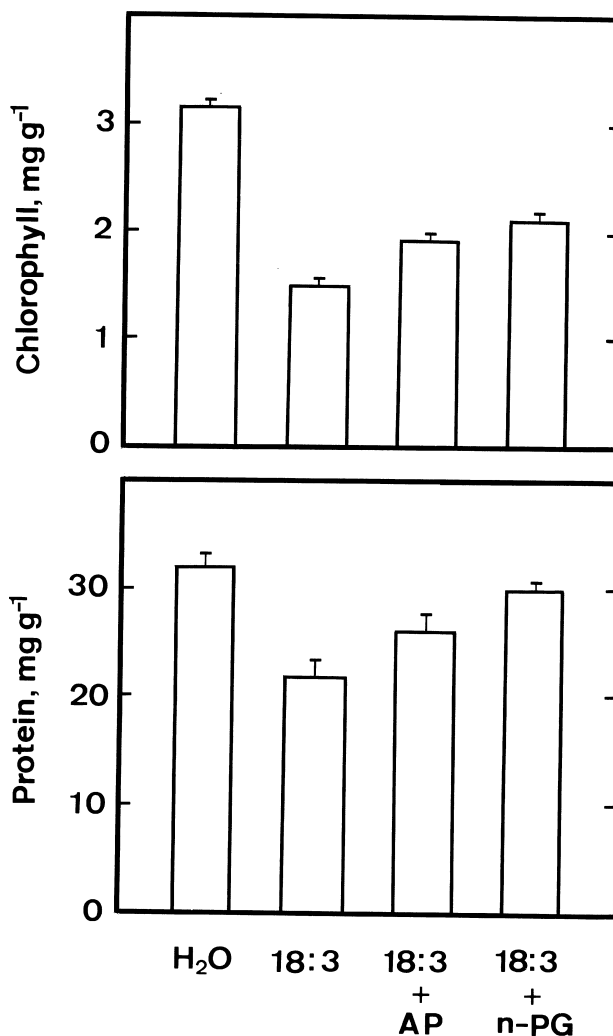
**Figure 4.** Effect of lipoxygenase inhibitors on protein level in detached rice leaves. The pH of all solutions was maintained near neutrality with 0.2 mM potassium phosphate, pH 7.0. Protein levels were determined after 4 days in darkness. Bars indicate S. E. (n=4).

After exploring the effects of lipoxygenase substrates and inhibitors on the senescence of detached rice leaves. We further examined the effects of major lipoxygenase pathway metabolites on this process.

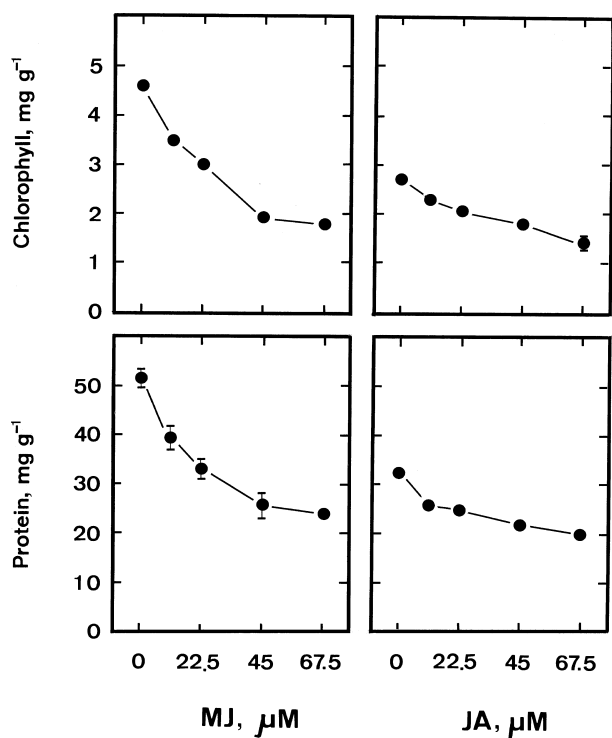
Lipoxygenase pathway metabolites tested in this study include MJ, JA, traumatic acid, and *trans*-2-hexenal. Figure 6 shows the effect of MJ and JA on the levels of chlorophyll and protein in detached rice leaves. MJ markedly promoted the senescence of detached rice leaves (Figure 6). Increasing MJ concentration from 11.25 to 45 μM progressively enhanced it. No further enhancement was observed at a concentration of 67.5 μM. As indicated in Figure 6, JA was also observed to be effective, though to a lesser extent, in promoting the senescence of detached rice leaves. Traumatic acid and *trans*-2-hexenal are C<sub>12</sub> and C<sub>6</sub> products of lipoxygenase pathway, respectively (Siedow, 1991; Zimmerman and Coudron, 1979). However, the treatment of detached rice leaves with traumatic acid did not have any effect on their senescence (Table 1). A slight promotive effect on protein degradation of detached rice leaves by *trans*-2-hexenal was observed at a concentration of 45 μM (Table 1). These results indicate jasmonates are the major metabolites influencing the senescence of detached rice leaves. This conclusion is further supported by the results that lipoxygenase inhibitors did not affect MJ- and JA-promoted senescence (data not shown).

**Table 1.** Effect of traumatic acid and *trans*-2-hexenal on the senescence of detached rice leaves. Chlorophyll and protein levels were determined after 3 days in darkness. Traumatic acid was dissolved in 10% (v/v) ethanol and 0.1% (v/v) Triton X-100.

Treatment	Protein, mg g <sup>-1</sup>	Chlorophyll, mg g <sup>-1</sup>
Traumatic acid		
0	51.3±2.1	4.1±0.1
45 μM	52.2±0.8	4.1±0.1
90 μM	50.3±1.1	4.2±0.1
<i>trans</i> -2-Hexenal		
0	39.2±0.9	4.0±0.1
22.5 μM	37.6±1.9	4.0±0.1
45 μM	31.7 ±0.2	3.6±0.1



**Figure 5.** Effect of antipyrine and n-propyl gallate in the linolenic acid-promoted senescence of detached rice leaves. All treatments included 0.1% Tween 20. Chlorophyll levels were determined after 4 days in darkness. The concentrations of linolenic acid (18:3), antipyrine (AP) and n-propyl gallate (n-PG) were 5, 0.5 and 0.05 mM, respectively. Bars indicate S. E. (n=4).



**Figure 6.** Effect of jasmonates on chlorophyll and protein levels in detached rice leaves. Chlorophyll and protein levels in MJ- and JA-treated detached rice leaves were determined after 2 and 3 days in darkness, respectively. Bars indicate S. E. (n=4). Only those S. E. larger than the symbol are shown.

All the results described above seem to support a role for endogenous jasmonates in the regulation of the senescence of detached rice leaves. However, it will be important to establish whether JA levels increase during the senescence and whether the lipoxygenase inhibitors block the increase in JA level.

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