

An Increase in Ethylene Sensitivity Is Associated with Jasmonate-Promoted Senescence of Detached Rice Leaves

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Abstract. The role of ethylene in jasmonate-promoted senescence of detached rice leaves was investigated. Ethylene production in methyl jasmonate-treated leaf segments of rice was lower than in the control leaves. Treatment of leaf segments with silver nitrate or/and silver thiosulfate, inhibitors of ethylene action, inhibited methyl jasmonate-, jasmonic acid-, linolenic acid-, and abscisic acid-promoted senescence of detached leaves. We suggest that an increase in ethylene sensitivity, but not ethylene level, is the initial event triggering the enhanced senescence by jasmonates of detached rice leaves.

Key Words. Ethylene—Jasmonates—*Oryza sativa*—Senescence

Jasmonic acid (JA) and its methyl ester, methyl jasmonate (MJ), are naturally occurring growth regulators found in higher plants (Meyer et al. 1984, Sembdner and Parthier 1993). Jasmonates have been shown to be powerful promoters of leaf senescence (Chou and Kao 1992, Cuello et al. 1990, Sembdner and Parthier 1993, Ueda and Kato 1981, Weidhase et al. 1987). However, the mechanism whereby jasmonates promote leaf senescence remains unclear. Ethylene was found to play a significant role in promoting senescence of leaves (Mattoo and Aharoni 1988). Using cucumber cotyledons, Abeles et al. (1989) found that MJ was as effective as ethylene in inducing senescence. In addition to this observation, silver thiosulfate (STS) had no effect on the ability of MJ to promote senescence. Based on these results they suggested that the action of MJ was inde-

pendent of ethylene. Cuello et al. (1990) also reported that MJ and ethylene had different mechanisms in promoting the senescence of detached barley leaves. In this study the possible involvement of ethylene in the regulation of jasmonate-promoted senescence of detached rice leaves was investigated.

Materials and Methods

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

Chlorophyll was determined according to Wintermans and De Mott (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 17,600 ×g for 20 min, and the supernatants were used for determination of protein by the method of Bradford (1976).

For determination of ethylene, leaf segments were placed vertically in test tubes that were plugged with rubber stoppers and incubated in darkness at 27°C for 1 h. The ethylene in the gas phase of the plugged tubes was determined by analysis of a 1-mL sample withdrawn with a hypodermic syringe as described elsewhere (Kao and Yang 1983).

In experiments with Ag⁺ treatments, leaf segments were pretreated with 10 mg/mL silver nitrate for 30 min and then transferred to a Petri dish containing test solution. When STS was used as a source of Ag⁺, leaf segments were treated with STS and test solution. A stock solution of STS was prepared by mixing equal volumes of 0.01 M AgNO₃ and 0.04 M Na₂S₂O₃ (Liu et al. 1990).

All experiments were repeated at least twice. Data are presented as the results of a single experiment typical of the trends seen in the repeated experiments.

Results and Discussion

The senescence of detached rice leaves was followed by measuring the decrease of chlorophyll and protein. Fig. 1 shows the time courses of protein and chlorophyll levels of detached rice leaves floating on water or MJ (45 μM) in the dark. It is clear that the MJ significantly promotes

Abbreviations: JA, jasmonic acid; MJ, methyl jasmonate; STS, silver thiosulfate; ABA, abscisic acid.

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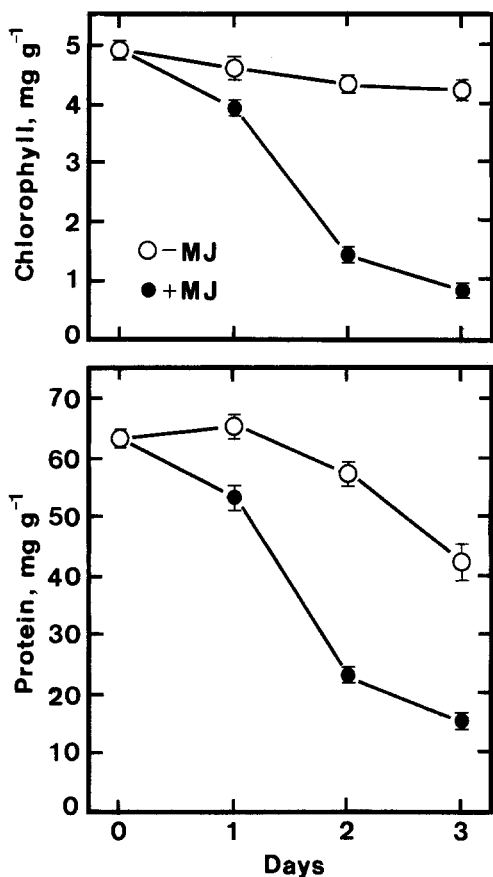


Fig. 1. Time course of chlorophyll and protein levels in detached rice leaves floating on water or MJ (45 μ M) in darkness. Bars indicate S.E. ($n = 4$).

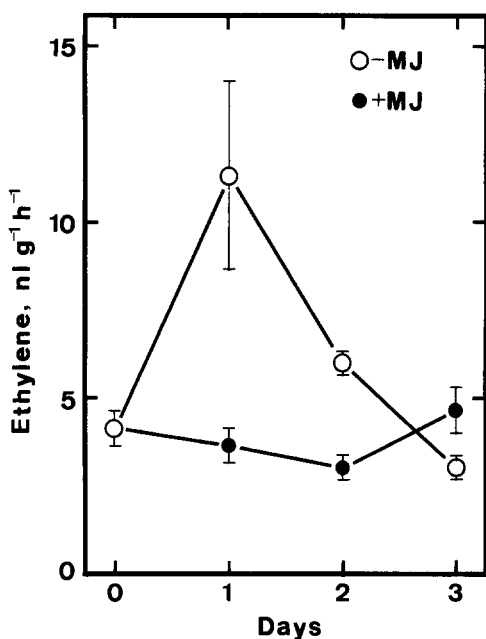


Fig. 2. Time course of ethylene production in detached rice leaves floating on water or MJ (45 μ M) in darkness. Bars indicate S.E. ($n = 4$).

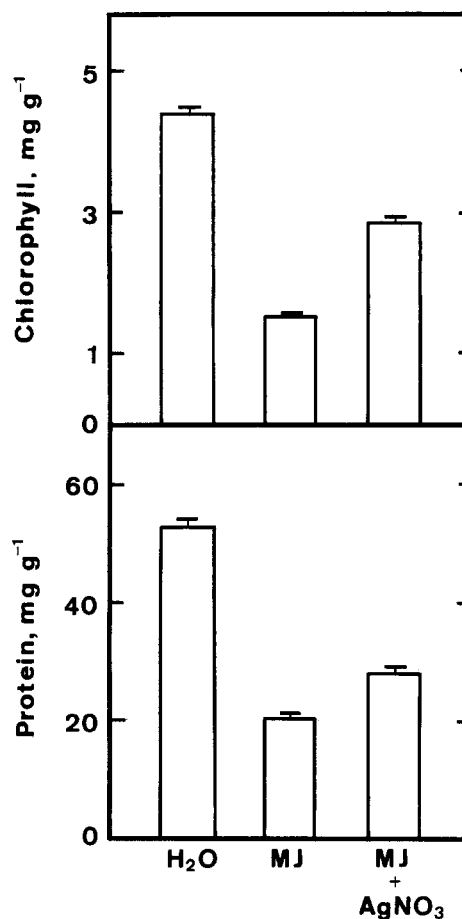


Fig. 3. Effect of silver nitrate pretreatment on the MJ-promoted senescence of detached rice leaves. Leaf segments were pretreated with silver nitrate (10 mg/mL) for 30 min in darkness and then transferred to distilled water or MJ (45 μ M) for 3 days. Bars indicate S.E. ($n = 4$).

the senescence of detached rice leaves. These results are in agreement with earlier reports of other investigators (Chou and Kao 1992, Cuello et al. 1990, Ueda and Kato 1981, Weidhase et al. 1987).

Ethylene production in water-treated leaf segments increased significantly during the first 24 h and decreased subsequently (Fig. 2). This result is consistent with our early finding that ethylene production precedes the senescence of detached rice leaves (Kao and Yang 1983), indicating that ethylene production participates in the regulation of rice leaf senescence. If MJ-promoted senescence of detached rice leaves is mediated through ethylene production, then ethylene production in MJ-treated leaf segments is expected to be higher than that in water-treated leaf segments. However, as indicated in Fig. 2, this does not seem to be the case.

If a change in ethylene production is excluded as an explanation for the MJ-promoted senescence of detached rice leaves, a change in sensitivity to ethylene is an alternate possibility. This possibility was tested by using

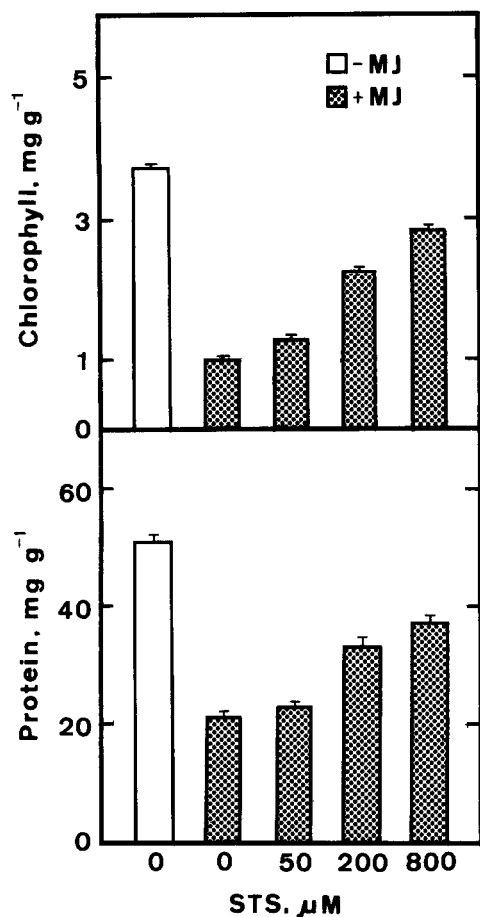


Fig. 4. Effect of STS on the MJ-promoted senescence of detached rice leaves. The concentration of MJ was 55 μM . Chlorophyll and protein levels were determined after 3 days in darkness. Bars indicate S.E. ($n = 4$).

the inhibitor of ethylene action, Ag^+ (Beyer 1976). Leaf segments were pretreated with silver nitrate for 30 min and then transferred to distilled water or MJ for 3 days in the dark. As indicated in Fig. 3, silver nitrate pretreatment resulted in an inhibition of MJ-promoted senescence of detached rice leaves. Toxic effects of silver nitrate have been reported (Lis et al. 1984, Liu et al. 1990). The advantages of using STS have been described by others and are related to lower free Ag^+ in STS solutions and a lower phytotoxicity of this compound (Liu et al. 1990). Thus, a change in ethylene sensitivity in MJ-treated leaf segments was tested further by using STS. STS was also found to be effective in inhibiting MJ-promoted senescence of detached rice leaves (Fig. 4).

JA was also observed to be effective, although to a lesser extent, in promoting the senescence of detached rice leaves (Fig. 5). Similar to MJ, the response of JA was also inhibited by STS (Fig. 5).

Linolenic acid (18:3) is known to be the precursor in the proposed biosynthetic pathway of JA (Sembdner and Parthier 1993) and has been shown to be a senescence-

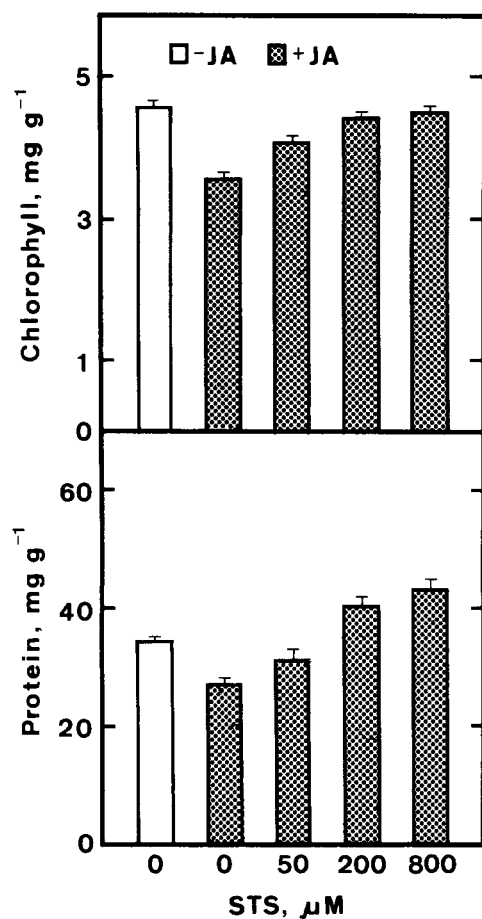


Fig. 5. Effect of STS on the JA-promoted senescence of detached rice leaves. The concentration of JA was 45 μM . Chlorophyll and protein levels were determined after 3 days in darkness. Bars indicate S.E. ($n = 4$).

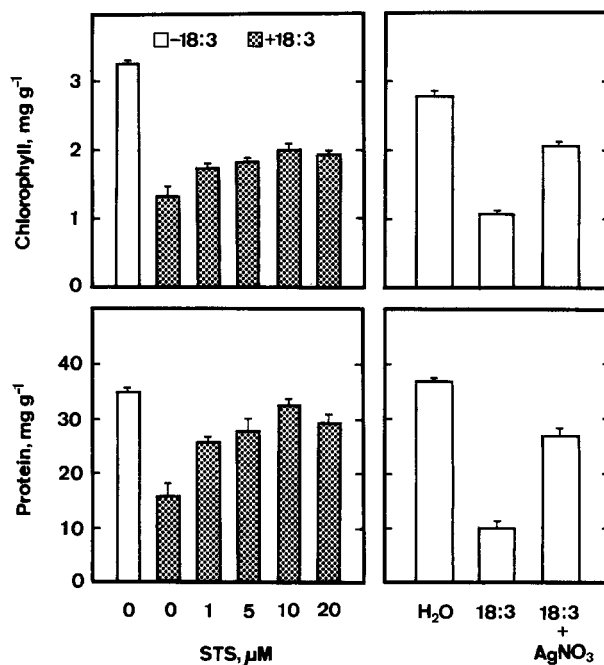


Fig. 6. Effect of STS and silver nitrate on linolenic acid-promoted senescence of detached rice leaves. Linolenic acid (18:3, 5 mM) was dissolved in 0.1% Tween 20. Water treatment included 0.1% Tween 20. For silver nitrate experiments, leaf segments were pretreated with silver nitrate (10 mg/mL) for 30 min in darkness. Chlorophyll and protein levels were determined after 3 days in darkness. Bars indicate S.E. ($n = 4$).

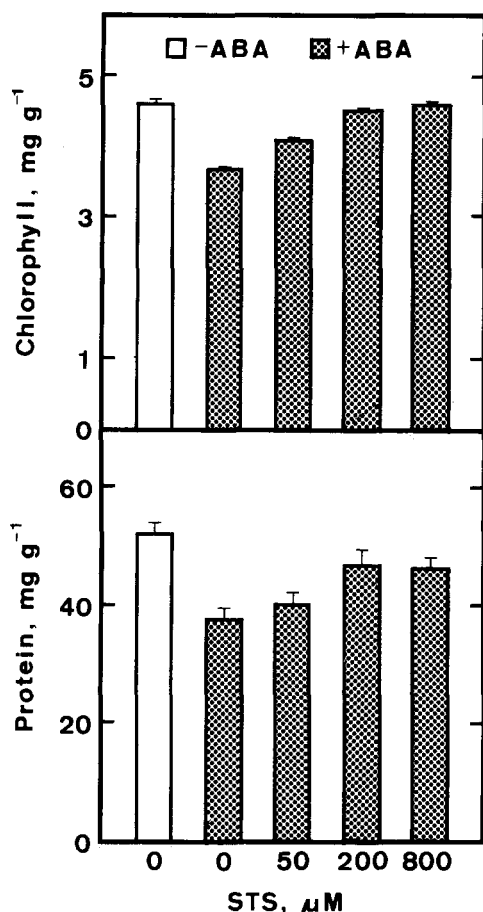


Fig. 7. Effect of STS on ABA-promoted senescence of detached rice leaves. The concentration of ABA was 45 μM . Chlorophyll and protein levels were determined after 3 days in darkness. Bars indicate S.E. ($n = 4$).

promoting substance (Ueda and Kato 1982). Our unpublished data also show that the promotive effect of linolenic acid on the senescence of detached rice leaves may be the result of an effect of JA. Thus, it is of great interest to know whether the promotive response of linolenic acid on the senescence of detached rice leaves is mediated through a change in ethylene sensitivity. As indicated in Fig. 6, linolenic acid indeed changes the sensitivity of ethylene, as judged by the effect of silver nitrate and STS.

In our earlier work we found that ABA promoted the senescence of detached rice leaves but inhibited ethylene production (Kao and Yang 1983). We proposed, but did not prove, that ABA increases tissue sensitivity to ethylene. Since jasmonates and ABA have both chemical and physiological similarity (Anderson et al. 1989), we investigated the effect of STS on ABA-promoted senescence of detached rice leaves. The results in Fig. 7 indicate that our early proposal appears to be correct.

Although it has been shown that the action of JAs was

independent of ethylene in other cases (Abeles et al. 1989, Cuello et al. 1990), our results strongly support the notion that ethylene plays a regulatory role in the JA-promoted senescence of detached rice leaves. It is ethylene sensitivity rather than ethylene level which JAs affect during the course of the senescence of detached rice leaves.

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