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Involvement of lipid peroxidation in methyl jasmonate-promoted senescence in detached rice leaves

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Abstract

Lipid peroxidation in relation to MJ-promoted senescence of detached rice leaves was investigated. Lipid peroxidation seems to be involved in the regulation of MJ-promoted senescence of detached rice leaves. This conclusion was based on the observations that (a) methyl jasmonate, which was found to promote senescence, increases the level of lipid peroxidation, (b) linolenic and linoleic acid, precursors of the biosynthesis of jasmonic acid, promoted senescence and increased lipid peroxidation level, (c) benzyladenine, a synthetic cytokinin, counteracted MJ-promoted senescence and reduced the increase in lipid peroxidation level, (d) calcium chloride effectively reduced MJ-promoted senescence and at the same time reduced MJ-promoted lipid peroxidation. Free radical scavengers (reduced glutathione and sodium benzoate) and an iron chelator (2, 2′-bipyridine) prevented MJ-promoted senescence, suggesting that lipid peroxidation induced by MJ is mediated through free radicals.

Abbreviations: BA = benzyladenine; BP = 2, 2′-bipyridine; GSH = reduced glutathione; MDA = malondialdehyde; MJ = methyl jasmonate; SB = sodium benzoate

1. Introduction

Jasmonates are endogenous substances that have been identified in many plants [12]. Jasmonates were found to be powerful promoter of leaf senescence [2, 8, 9, 17, 19]. Lipid peroxidation is considered to be an important mechanism of leaf senescence [3, 11, 15, 16]. Dhindsa et al. [4] demonstrated that the inhibition of senescence of detached oat and *Rumex* leaves by plant hormones was mediated through modulation of free radical-induced lipid peroxidation. In this study, effects of MJ, and linoleic and linolenic acids, precursors of jasmonic acid biosynthesis [18], on degradation of chlorophyll and protein and lipid peroxidation were investigated. Furthermore, the effects of two free radical scavengers (glutathione, GSH, and sodium benzoate, SB) [14, 21] and one metal chelator (2,2′-bipyridine, BP) on degradation of chlorophyll and protein were also examined.

2. Materials and methods

Rice (*Oryza sativa* cv. Taichung Native 1) was cultured as previously described [10]. 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 Mots [20]. After extraction in 96% (v/v) ethanol. For protein extraction, leaf segments were homogenized in 25 mM sodium phosphate buffer (pH 7.5). 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 [1]. Protein and chlorophyll levels were expressed as mg g^{-1} fresh weight. MDA was extracted with 5% (w/v) trichloroacetic acid and determined according to Heath and Packer [7]. MDA level was expressed as nmol g^{-1}

fresh weight and is routinely used as an index of lipid peroxidation.

For the experiments of the effect of linoleic and linolenic acids (5 mM) on chlorophyll, protein and MDA levels in detached rice leaves, all treatments included 0.1% Tween 20. Chlorophyll, protein and MDA levels were determined after 4 days in darkness. For those experiments with treatments that included GSH (30 mM) or SB (10 mM) together with MJ (45 μM), chlorophyll, protein and MDA levels were determined after 3 days in darkness. As to the effect of BP (1 mM) on chlorophyll and protein levels in detached rice leaves treated with MJ (45 μM), chlorophyll, protein and MDA levels were determined after 3 days in darkness.

MJ [(–)–MJ] was a product of Serva and other chemicals were purchased from Sigma Chemical Co.

3. Results and discussion

The senescence of detached rice leaves in the dark was followed by measuring the decrease of chlorophyll and protein. Changes in the levels of chlorophyll, protein, and MDA in detached rice leaves treated with 45 μM MJ are shown in Figure 1. It is clear that the promotion of chlorophyll loss and protein degradation by MJ was evident one day after MJ treatment. MDA level in MJ-treated detached rice leaves was observed to be higher than that in water-treated controls throughout the entire duration of incubation. This shows that MJ-promoted senescence of detached rice leaves is linked to lipid peroxidation.

It has been shown that cytokinins, known to delay senescence, counteract jasmonic acid effect with respect to chlorophyll degradation in detached barley leaves [19]. In detached rice leaves, we observed that BA, a synthetic cytokinin, counteracted MJ-promoted senescence (Figure 2). Figure 2 also shows that BA reduced the increase in MDA level in detached rice leaves treated with MJ.

Previously, we have shown that calcium interacts with jasmonates in the regulation of senescence of detached rice leaves [2]. In the present study, we found that treatment with calcium chloride effectively reduced MJ-promoted senescence and at the same time reduced MJ-increased lipid peroxidation (Figure 3).

It has been shown that oxidation of linolenic acid by lipoxygenase is the first step in the biosynthesis pathway of jasmonic acid [18]. Recently, we demonstrated that linoleic acid (18:2) and linolenic acid (18:3) promoted senescence of detached rice leaves [9]. Here, we reported that linoleic and linolenic acid -promoted

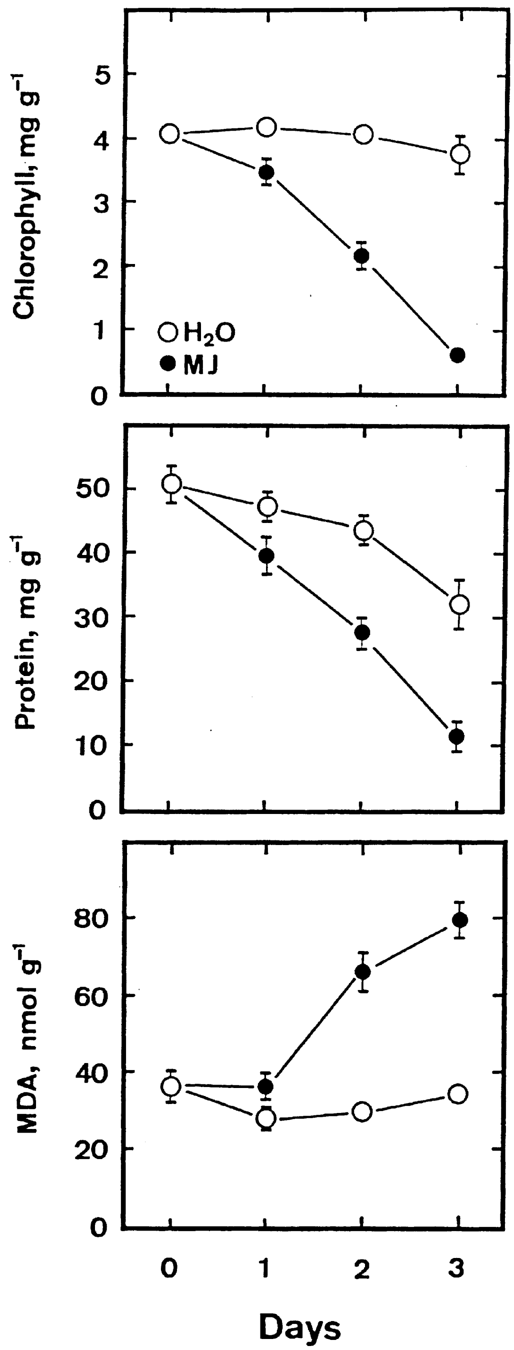

Figure 1. Changes in chlorophyll, protein, and MDA levels in detached rice leaves treated with MJ. The concentration of MJ was 45 μM. Bars indicate SE (n = 4).

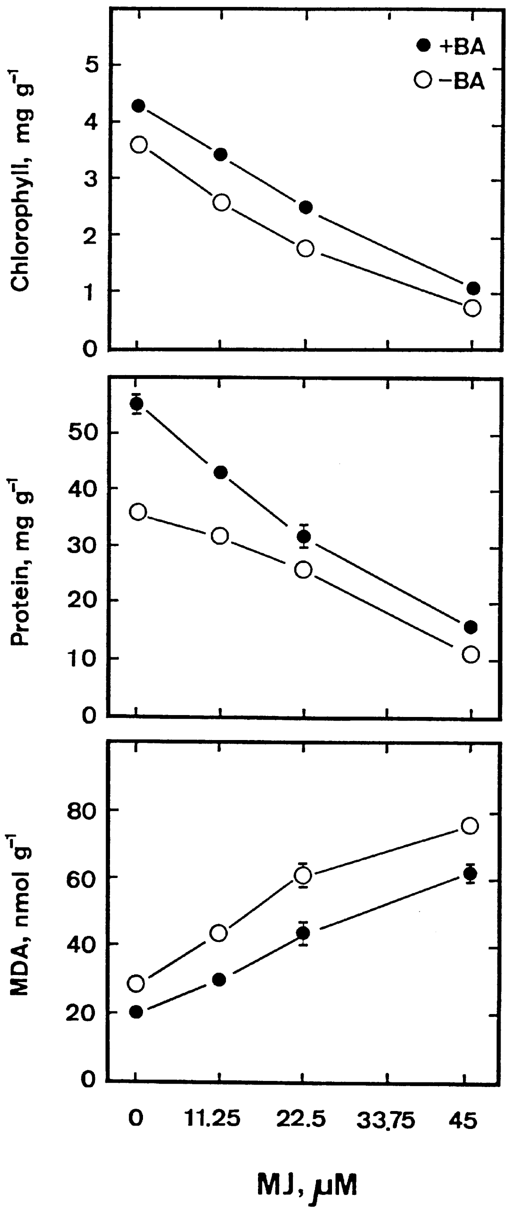

Figure 2. Effect of BA on MJ-promoted senesence and MJ-increased MDA level in detached rice leaves. Chlorophyll, protein, and MDA levels were determined after 3 days in darkness. Bars indicate SE (n = 4). Only those SE larger than symbol size are shown.

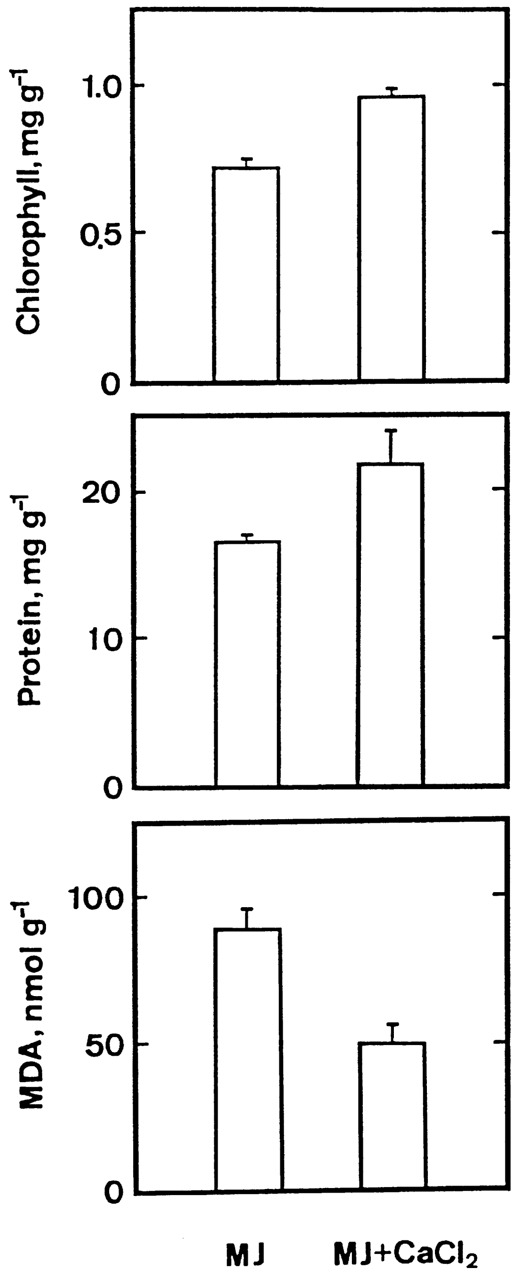

Figure 3. Effect of calcium chloride on chlorophyll, protein and MDA levels in detached rice leaves treated with MJ. Chlorophyll, protein and MDA were determined after 3 days in darkness. The concentration of MJ and calcium chloride were 45 μM and 10 mM, respectively. Bars indicate SE (n = 4).

senescence is linked to an increase in MDA level (Figure 4).

It is generally considered that lipid peroxidation is induced by free radicals [5]. If it is the case in the jasmonate-induced lipid peroxidation, then we would expect a reduction of MJ-increased MDA level and a corresponding inhibition of MJ-promoted senescence in detached rice leaves by the addition of free radical scavengers such as GSH and SB [14, 21]. This is essentially what we see in Table 1.

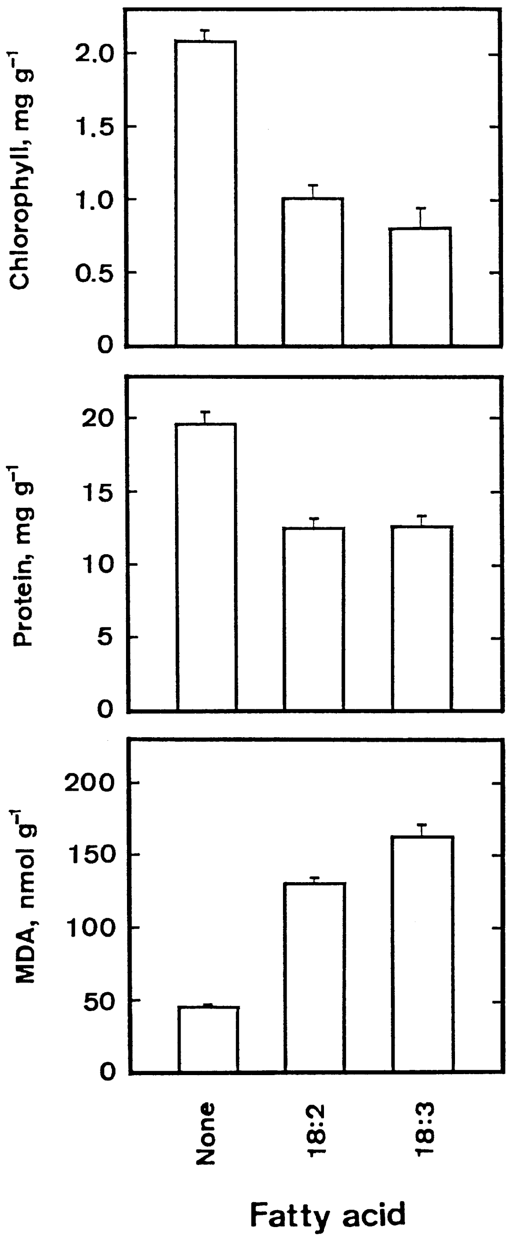

Figure 4. Effect of linoleic acid (18:2) and linolenic acid (18:3) on chlorophyll, protein and MDA Levels in detached rice leaves. All treatments included 0.1% Tween 20. Chlorophyll, protein and MDA levels were determined after 4 days in darkness. The concentration of 18:2 and 18:3 was 5 mM. Bars indicate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

Superoxide can serve as a source to generate more active hydroxyl radicals by Haber-Weiss and Fenton reaction [13, 15]. Transition metals, such as iron and copper, are able to accelerate Haber-Weiss and Fenton reaction [6]. We do not know whether MJ-promoted senescence of detached rice leaves requires metal ions. We investigated this by using BP, a metal chelator. BP was found to be able to inhibit MJ-promoted senescence of detached rice leaves (Table 1).

Table 1. Effects of free radical scavengers and BP on chlorophyll, protein and MDA levels in detached rice leaves treated with MJ. Chlorophyll, protein and MDA levels were determined after 3 days in darkness. The concentrations of MJ, GSH, SB, and BP were 45 μM, 30 mM, 10 mM and 1 mM, respectively. The data represent mean values ± SE (n = 4)

Treatment	Protein ($mg\ g^{-1}$)	Chlorophyll ($mg\ g^{-1}$)	MDA ($nmol\ g^{-1}$)
H_2O	45.34 ± 0.83	3.71 ± 0.14	24.33 ± 3.08
MJ	13.06 ± 0.83	0.74 ± 0.08	84.21 ± 1.32
MJ + GSH	18.48 ± 0.24	1.68 ± 0.05	34.83 ± 2.90
MJ + SB	21.47 ± 0.79	1.76 ± 0.14	25.14 ± 0.91
H_2O	43.67 ± 0.22	4.32 ± 0.03	
MJ	18.91 ± 0.60	0.70 ± 0.02	
MJ + BP	56.85 ± 2.07	4.40 ± 0.02	

In conclusion, the result of the present investigation suggest that MJ-promoted senescence is a consequence of free radical-induced lipid peroxidation.

Acknowledgements

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