Plant Growth Regulation **33:** 87–93, 2001.

Inhibition of methyl jasmonate-promoted senescence in rice leaves by a metal chelator, 2,2′-bipyridine

Wei-Ching Fang and Ching Huei Kao*
*Department of Agronomy, National Taiwan University, Taipei, Taiwan, Republic of China; *Author for correspondence (e-mail: kaoch@ccms.ntu.edu.tw; fax: 886-2-23620879)*

Received 25 January 2000; accepted in revised form 16 March 2000

Key words: 1,10-phenanthroline, 2,2′-bipyridine, 8-hydroxyquinoline, Lipid peroxidation, Methyl jasmonate, *Oryza sativa*, Senescence

Abstract

The possible mediatory role of transition metals in methyl jasmonate- (MJ-) induced senescence of rice leaves was investigated. Metal chelators (2,2′-bipyridine, 8-hydroxylquinoline and 1,10-phenanthroline) reduced MJ-promoted senescence of rice leaves. The reduction of MJ-promoted senescence by 2,2′-bipyridine(BP) is closely associated with the decrease in lipid peroxidation and increase in activity of superoxide dismutase (SOD). Our results suggest that iron or copper plays a major role in MJ-promoted senescence of detached rice leaves. BP-reduced senescence of detached rice leaves induced by MJ was reversed by adding Fe^{2+} or Cu^{2+}, but not by Mn^{2+} or Mg^{2+}. Reduction of MJ-promoted senescence of detached rice leaves by BP is most likely mediated through chelation of iron or copper and an increase in SOD activity.

Abbreviations: APOD – ascorbate peroxidase, BP – 2,2′-bipyridine, GR – glutathione reductase, HQ – 8-hydroxylquinoline, MDA – malondialdehyde, MJ – methyl jasmonate, PA – 1,10-phenanthroline, SOD – superoxide dismutase

Introduction

Jasmonates are naturally occurring growth regulators found in higher plants (Sandy et al. 1987) and have been shown to be powerful promoters of leaf senescence (Chou and Kao 1992; Hung and Kao 1996, 1997; Ueda and Kato 1981; Weidhase et al. 1987). Lipid peroxidation is considered to be an important mechanism of leaf senescence (Dhindsa et al. 1981; Kunnert and Ederer 1985; Strother 1988; Thompson et al. 1987). Dhindsa et al. (1982) reported that the inhibition of senescence of detached oat and *Rumex* leaves by plant hormones was mediated through modulation of free-radical-induced lipid peroxidation. Recently, we demonstrated that methyl jasmonate- (MJ-) promoted senescence of detached rice leaves was a consequence of free radical-induced lipid peroxidation (Hung and Kao 1998). Superoxide can serve as a source to generate more active hydroxyl radicals by Haber-Weiss and Fenton reactions (Naqui and Chance 1986; Smirnoff 1993; Strother 1988). Transition metals, such as iron and copper, are able to accelerate Haber-Weiss and Fenton reactions (Gutteridge et al. 1981). Paraquat, also known as methyl viologen, is a widely used herbicide in agriculture and has long been known to exert its phytotoxic effects by catalyzing the transfer of electrons from photosystem I of chloroplast membranes to molecular oxygen, producing oxygen radicals that cause lipid peroxidation and membrane damage (Calderbank 1968). It has been demonstrated that iron or copper ions are essential mediators in paraquat toxicity in bacterial cells (Kohen and Chevion 1986; Korbashi et al. 1986), in mammalian cells in tissue culture (Sandy et al. 1987), in mice (Kohen and Chevion 1985), in pea leaves (Zer et al. 1994), and in rice leaves (Chang and Kao 1977).

It has been proposed that an iron- or copper-containing system plays an essential part in the initiation of the process of senescence in oat and rice leaves (Cheng and Kao 1984; Tetley and Thimann 1975). It is not known whether iron or copper plays a mediatory role in jasmonate-promoted leaf senescence. For this reason we investigated the role of transition metals in MJ-promoted senescence in rice leaves.

Materials and methods

Rice (*Oryza sativa* cv. Taichung Native 1) was cultured as described previously (Lin et al. 1999). Briefly, seedlings were planted on stainless steel net floating on modified half-strength Johnson's nutrient solution (Johnson et al. 1957) in a 500-ml beaker. The nutrient solution (pH 4.5) was replaced every 3 days. Rice seedlings were grown in a greenhouse with natural day light at a day/night temperature of 30/25 °C and humidity of 95%. 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 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 m*M* 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) and for enzyme assays. Malondialdehyde (MDA) was extracted with 5% (w/v) trichloroacetic acid and determined according to Health and Packer (1968). MDA content is routinely used as an index of lipid peroxidation.

The H_2O_2 level was measured colorimetrically as described by Jana and Choudhuri (1981). H_2O_2 was extracted by homogenizing 50 mg leaf tissue with 3 ml of phosphate buffer (50 m*M*, pH 6.5). The homogenate was centrifuged at 6 000 g for 25 min. To determine H_2O_2 levels, 3 ml of extracted solution was mixed with 1 ml of 0.1% titanium sulphate in 20% (v/v) H_2SO_4 and the mixture was then centrifuged at 6 000 g for 15 min. The intensity of the yellow colour of the supernant was measured at 410 nm. The H_2O_2 level was calculated using the extinction coefficient $0.28\ \mu mol^{-1}\,cm^{-1}$.

Superoxide dismutase (SOD) was determined according to Paoletti et al. (1986). Ascorbate peroxidase (APOD) was determined according to Nakano and Asada (1981). The decrease in ascorbate concentration was followed as the decline in optical density at 290 nm and activity was calculated using the extinction coefficient ($2.8\ mM\,cm^{-1}$ at 290 nm) for ascorbate. Glutathione reductase (GR) was determined by the method of Foster and Hess (1980).

Chlorophyll, protein, H_2O_2 and MDA levels and enzyme activities were expressed per g fresh weight. Absolute levels of each measurement varied among experiments because of seasonal effects. However, the patterns of responses to MJ were reproducible. For all measurements, each treatment was repeated four times. All experiments described here were repeated at least three times. Similar results and identical trends were obtained each time. The data reported here are from a single experiment.

Results and discussion

The senescence of detached rice leaves in the dark was followed by measuring the decrease of chlorophyll and protein. Increasing concentration of MJ from 11.25 to 45 μM progressively promoted senescence of detached rice leaves and no further promotion was observed at a concentration of 67.5 μM (Hung and Kao 1997). Changes in the levels of chlorophyll and protein 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.

Figure 2 demonstrates that MJ treatment resulted in a marked increase in MDA level, indicating that MJ brings about lipid peroxidation. Free radical scavengers (reduced glutathione and sodium benzoate) have been shown to be effective in preventing MJ-promoted senescence in detached rice leaves (Hung and Kao 1998). These results suggested that free radicals and lipid peroxidation are major contributors to MJ-promoted senescence of detached rice leaves. H_2O_2 can be used in Fe- or Cu-catalyzed Haber-Weiss or Fenton reaction. Thus it is of great interest to know the effect of MJ on the level of H_2O_2 in detached rice leaves incubated in the dark. Similar to the effect of MJ on lipid peroxidation, MJ resulted in an increase in the level of H_2O_2 in detached rice leaves (Figure 2).

The present work is focused on the possible mediatory role of metal ions in rice leaves. We investigated this by using the metal chelators BP, HQ and

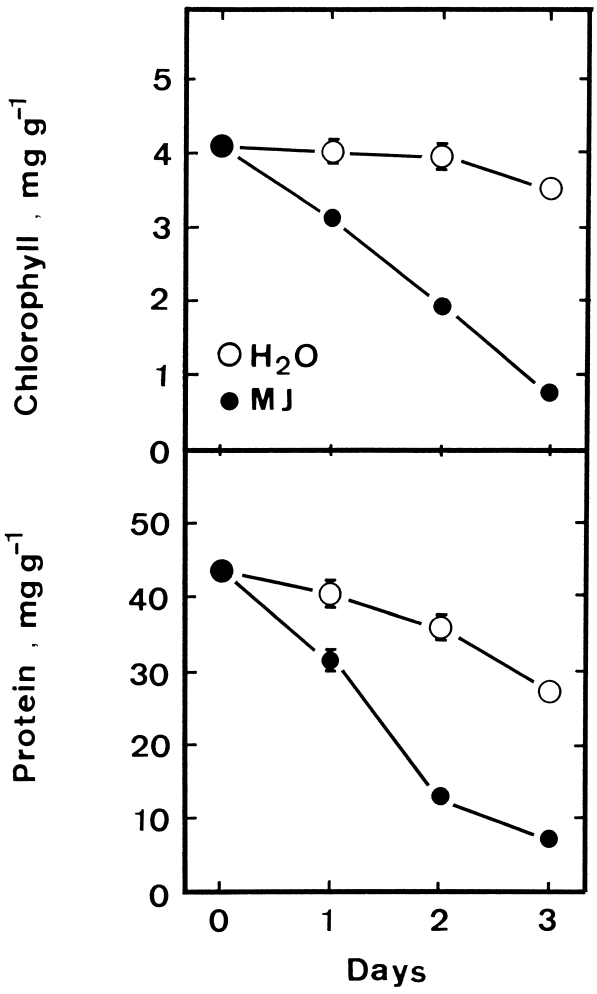

Figure 1. Changes in levels of chlorophyll and protein in detached rice leaves treated with MJ. Detached rice leaves were treated with either water or 45 μM MJ in the dark. Vertical bars represent standard errors (n = 4). Only those standard errors larger than the symbols are shown.

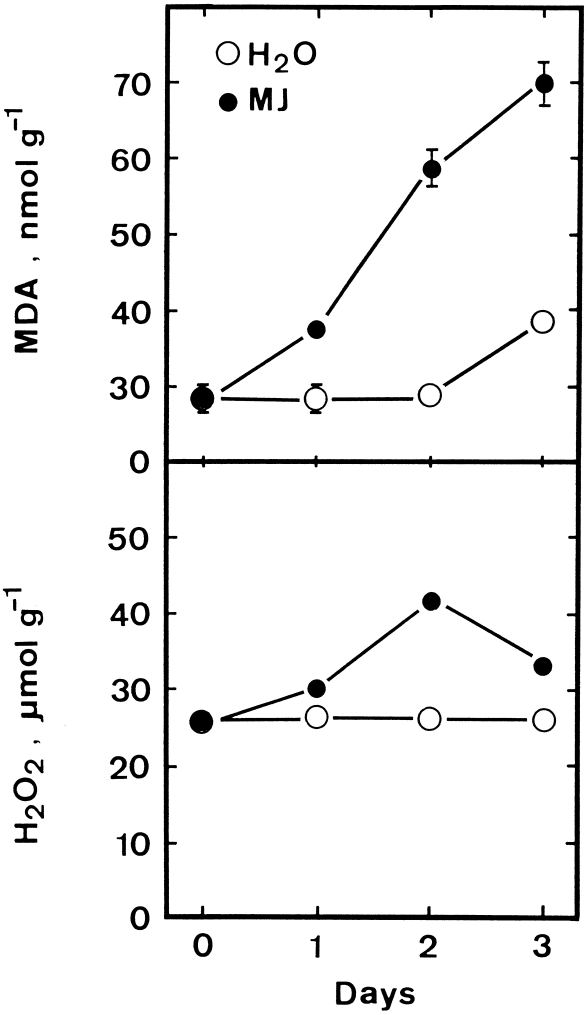

Figure 2. Changes in levels of MDA and H_2O_2 in detached rice leaves treated with MJ. Detached rice leaves were treated with either water or 45 μM MJ in the dark. Vertical bars represent standard errors (n = 4). Only those standard errors larger than the symbols are shown.

PA. All these chelators at the optimum concentration (1 m*M*) able to reduce MJ-promoted senescence of rice leaves (Figure 3). Since BP appears to be more effective in reducing MJ-promoted senescence of rice leaves, the effect of BP on MJ-promoted leaf senescence was studied in further detail.

Figure 4 shows the effect of pretreatment with BP on chlorophyll, protein and MDA levels of detached rice leaves treated with MJ. Pretreatment with BP caused a reduction of MJ-induced loss of chlorophyll and protein and a reduction in MJ-induced increase in MDA level.

The increase in lipid peroxidation seen in leaves treated with MJ alone (pretreatment with water) may be a reflection of the decrease in the activities of protective enzymes such as SOD, APOD and GR (Halliwell 1974). Treatment with MJ alone resulted in a decrease in SOD and GR activities, and an increase in APOD activity (Figure 5) Also shown in Figure 5 is the protection of SOD by BP against the MJ-induced loss of its activity. BP pretreatment had no effect on MJ-increased APOD activity and resulted in a further decrease in GR activity (Figure 5). These results indicate that BP-reduced MJ-induced leaf senescence is closely associated with the decrease in lipid peroxidation and increase in SOD activity in rice leaves.

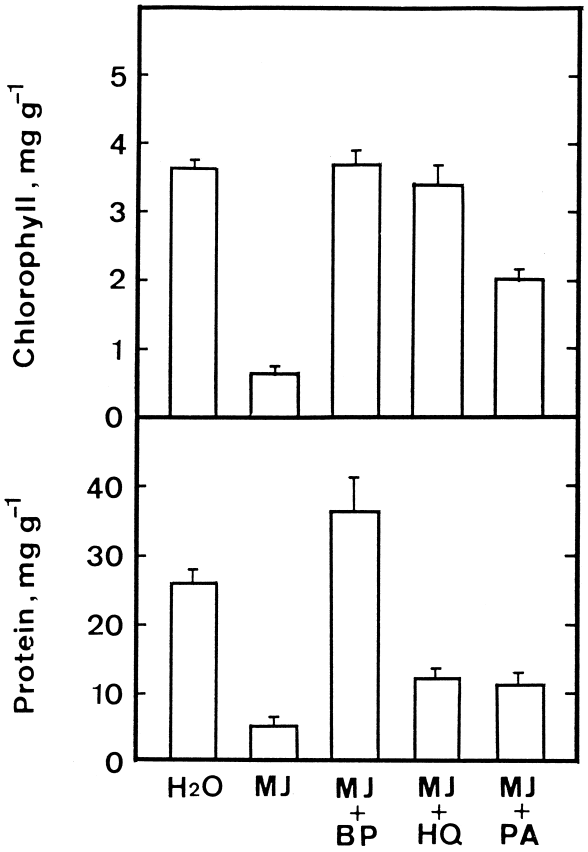

Figure 3. Effects of metal chelators on the levels of chlorophyll and protein in detached rice leaves treated with MJ. Detached rice leaves were treated with 1 m*M* metal chelators and 45 μM MJ for 3 days in the dark. Vertical bars represent standard errors (n = 4).

The decrease in SOD activity in leaves treated with MJ would result in an accumulation of superoxide radicals. The superoxide radicals can react with H_2O_2 through Fe- or Cu-catalyzed Haber-Weiss and Fenton reactions to produce hydroxyl radicals (Naqui and Chance 1986; Smirnoff 1993; Strother 1988). In the present work, H_2O_2 accumulates in MJ-treated detached rice leaves (Figure 2). It seems that MJ-induced lipid peroxidation in detached rice leaves is mediated through hydroxyl radicals.

The reversal of the reducing effect of BP on MJ-promoted leaf senescence was studied using Fe^{2+}, Cu^{2+}, Mn^{2+} and Mg^{2+} (Figure 6). The effect of BP could be reversed by Fe^{2+} and Cu^{2+}. However, Mn^{2+} and Mg^{2+} had no effect. These results indicate that BP can reduce MJ-promoted senescence by chelation of Fe^{2+} or Cu^{2+} and provide further evidence that iron or copper play a major role in MJ-induced senescence of leaves.

The activities of SOD, APOD and GR in detached rice leaves pretreated with either water or BP for 6 h in the dark (prior to MJ treatment) were determined, in an effort to explain the observed BP-reduced MJ-induced senescence. BP treatment results in an increase in SOD, but not in APOD and GR activities (Table 1).

Basically, BP can reduce senescence of rice leaves induced by MJ by several mechanisms including blockage of MJ-uptake, chelation of iron or copper and stimulation of protection enzymes activities. Since detached rice leaves are exposed to BP and MJ separately, the reducing effect of BP on MJ-induced senescence is unlikely caused by the blockage of MJ uptake by BP. The results of this study indicate that the effect of BP is mediated through chelation of iron or copper and an increase in SOD activity.

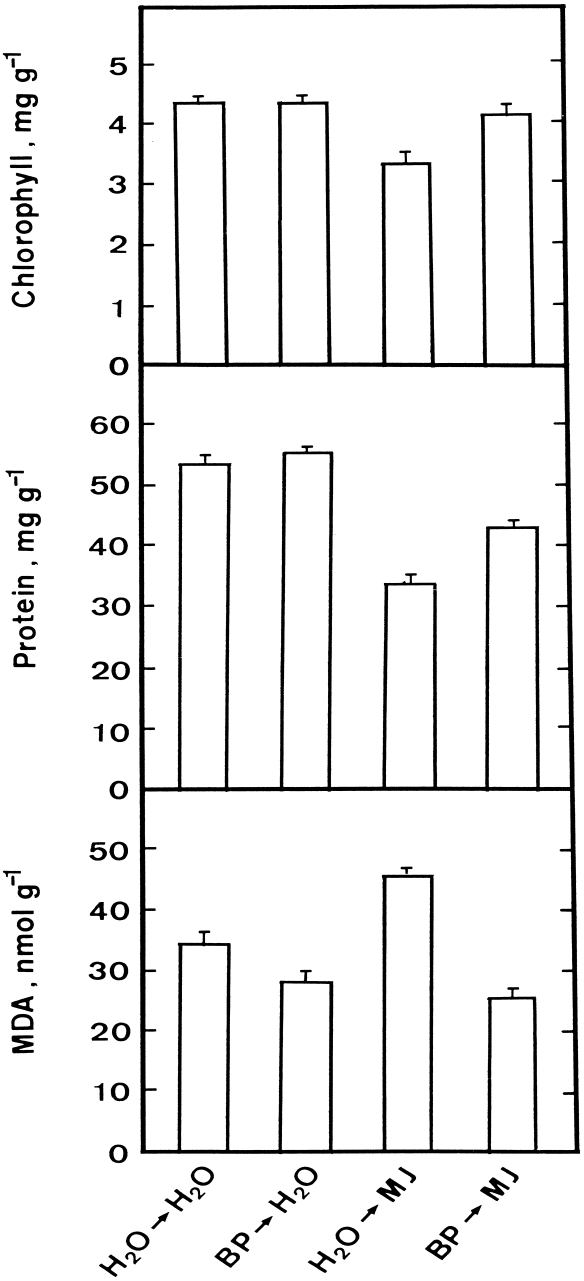

Figure 4. Effects of BP on levels of chlorophyll, protein and MDA in detached rice leaves. Detached rice leaves were pretreated with either water or 1 m*M* BP for 6 h in the dark and then treated with either water or 45 μM MJ for 24 h in the dark. Vertical bars represent standard errors (n = 4).

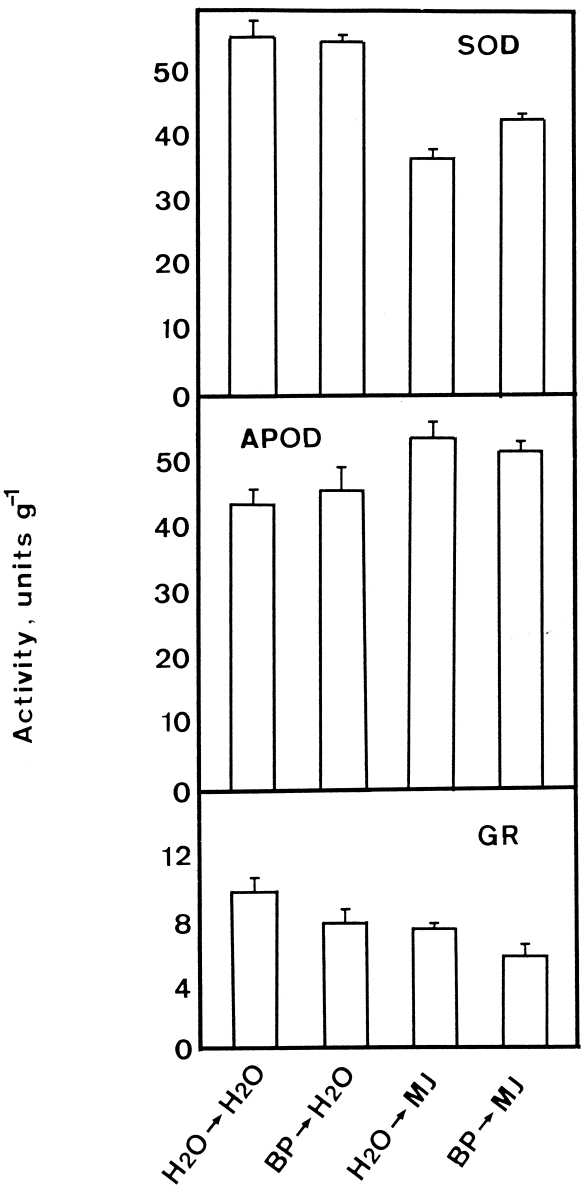

Figure 5. Effects of BP on activities of SOD, APOD and GR in detached rice leaves. Detached rice leaves were pretreated with either water or 1 m*M* BP for 6 h in the dark and then treated with either water or 45 μM MJ for 24 h in the dark. Vertical bars represent standard errors (n = 4).

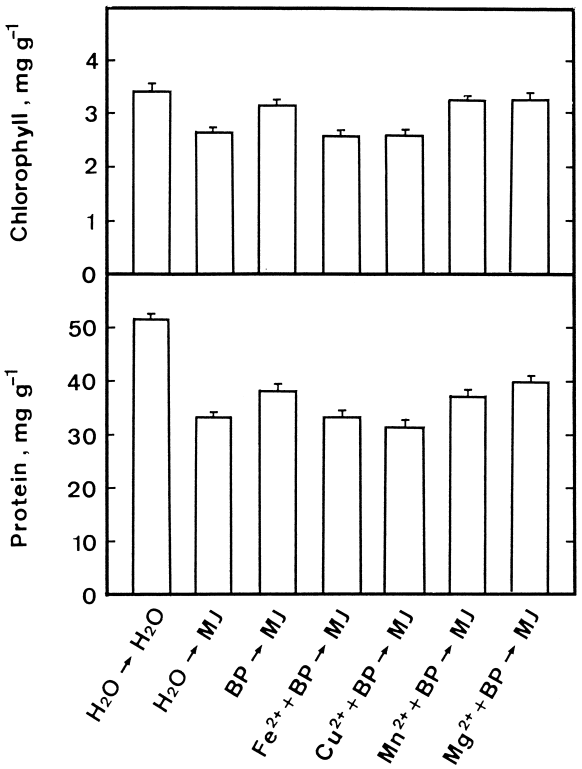

Figure 6. Reversal of BP-reduced senescence of detached rice leaves induced by MJ. Detached rice leaves were pretreated either water or 1 m*M* BP or 1 m*M* BP plus 10 m*M* metal (sulfate salt) for 6 h in the dark and then treated with either water or 45 μM MJ for 24 h in the dark. Vertical bars represent standard errors (n = 4).

Table 1. Effects of BP on activities of SOD, APOD, and GR in detached rice leaves
Detached rice leaves were treated with either water or 1 m*M* BP for 6 h in the dark.

Treatment	Enzyme activity, unit g^{-1}		
	SOD	APOD	GR
H_2O	60.2 ± 0.6	45.9 ± 0.1	7.3 ± 0.4
BP	64.1 ± 1.4	46.1 ± 1.5	6.8 ± 0.2

Acknowledgements

This work was supported by grant NSC 88-2321-B002-066 from the National Science Council of the Republic of China.

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