Nitric oxide acts as an antioxidant and delays methyl jasmonate-induced senescence of rice leaves

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Summary

In the present study, we evaluate the protective effect of nitric oxide (NO) against senescence of rice leaves promoted by methyl jasmonate (MJ). Senescence of rice leaves was determined by the decrease of protein content. MJ treatment resulted in (1) induction of leaf senescence, (2) increase in H₂O₂ and malondialdehyde (MDA) contents, (3) decrease in reduced form glutathione (GSH) and ascorbic acid (AsA) contents, and (4) increase in antioxidative enzyme activities (ascorbate peroxidase, glutathione reductase, peroxidase and catalase). All these MJ effects were reduced by free radical scavengers such as sodium benzoate and GSH. NO donors [N-*tert*-butyl- α -phenylnitron (PBN), sodium nitroprusside, 3-morpholinosydnonimine, and AsA+NaNO₂] were effective in reducing MJ-induced leaf senescence. PBN prevented MJ-induced increase in the contents of H₂O₂ and MDA, decrease in the contents of GSH and AsA, and increase in the activities of antioxidative enzymes. The protective effect of PBN on MJ-promoted senescence, MJ-increased H₂O₂ content and lipid peroxidation, MJ-decreased GSH and AsA, and MJ-increased antioxidative enzyme activities was reversed by 2-(4-carboxy-2-phenyl)-4,4,5,5-tetramethyl-imidazoline-1-oxyl-3-oxide, a NO-specific scavenger, suggesting that the protective effect of PBN is attributable to NO released. Reduction of MJ-induced senescence by NO in rice leaves is most likely mediated through its ability to scavenge active oxygen species including H₂O₂.

Key words: active oxygen species – lipid peroxidation – methyl jasmonate – nitric oxide – *Oryza sativa*

Abbreviations: AOS = active oxygen species. – APOD = ascorbate peroxidase. – AsA = ascorbic acid. – CAT = catalase. – c-PTIO = 2-(4-carboxy-2-phenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide. – FW = fresh weight. – GR = glutathione reductase. – GSH = reduced glutathione. – MDA = malondialdehyde. – MJ = methyl jasmonate. – NO = nitric oxide. – PBN = N-*tert*-butyl- α -phenylnitron. – POD = peroxidase. – SB = sodium benzoate. – SIN-1 = 3-morpholinosydnonimine. – SNP = sodium nitroprusside. – SOD = superoxide dismutase

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Introduction

Jasmonic acid and its methyl ester, methyl jasmonate (MJ), collectively named jasmonates, have been proposed as naturally occurring plant growth regulators because of their wide natural distribution (Meyer et al. 1991) and their effects on many physiological processes in plants (Sembdner and Parthier 1993, Creelman and Mullet 1997, Wasternack and Parthier 1997). Jasmonates have been shown to be powerful promoters of leaf senescence (Ueda and Kato 1981, Weidhase et al. 1987, Chou and Kao 1992, Tsai et al. 1996, Chen and Kao 1998, Hung and Kao 1998). Recent molecular studies have confirmed that jasmonic acid may play a role in leaf senescence (He et al. 2002).

Lipid peroxidation is considered to be an important mechanism of leaf senescence (Thompson et al. 1987, Strother 1988). Active oxygen species (AOS) can initiate lipid peroxidation (Kellogg and Fridovich 1975). It has been shown that MJ causes the generation of H_2O_2 (Orozco-Cárdenas and Ryan 1999, Orozco-Cárdenas et al. 2001) and lipid peroxidation expressed as malondialdehyde (MDA) production in plant cells (Hung and Kao 1998). Thus, MJ leads to oxidative stress in plant cells.

Nitric oxide (NO) is a bioactive free radical implicated in a number of physiological functions, including intra- and inter-cellular mediation of some animal responses (Anbar 1995, Lamattina et al. 2003). NO is involved in many physiological responses of plants: pathogen response (Delledonne et al. 1998, Klessig et al. 2000, Orozco-Cárdenas et al. 2001), programmed cell death (Pedroso et al. 2000, Beligni et al. 2002), growth (Leshem and Haramaty 1996), germination (Beligni and Lamattina 2000), root organogenesis (Pagnussat et al. 2002), phytoalexin production (Noritake et al. 1996), internal iron availability (Graziano et al. 2002), salt and heat tolerance (Uchida et al. 2002), cytokinin action (Scherer and Holk 2000), abscisic acid-dependent stomatal closure (Garcia-Mata and Lamattina 2002, Neill et al. 2002), and ethylene emission (Leshem 2000).

Several reports convincingly demonstrate that NO is able to counteract the toxicity of paraquat and diquat, which are known to generate AOS, in potato and rice leaves (Beligni and Lamattina 1999 a, b, 2002, Hung et al. 2002), and block H_2O_2 production induced by jasmonic acid in tomato leaves (Orozco-Cárdenas and Ryan 2002). Thus, a possible participation of NO in the antioxidant system in plants, as observed in animals (O'Donnell et al. 1997, d'Ischia et al. 2000), is suggested. In rice leaves, it has been shown that NO donors reduced paraquat toxicity through a decrease in lipid peroxidation (Hung et al. 2002). More recently, we showed that the promotion of rice leaf senescence caused by abscisic acid, which induces H_2O_2 production and lipid peroxidation, can be counteracted by NO donors (Hung and Kao 2003). Jasmonates have both chemical and physiological similarities to abscisic acid (Anderson et al. 1989). It is logical to speculate that NO is also effective in counteracting MJ-induced senescence of rice leaves.

Materials and Methods

Plant material and chemicals

Rice (*Oryza sativa* L., cv. Taichung Native 1) was sterilized with 2.5 % sodium hypochlorite for 15 min and washed extensively with distilled water. These seeds were then germinated in Petri dishes with wetted filter paper at 37 °C under dark condition. After 48 h incubation, uniformly germinated seeds were selected and cultivated in a 500 mL beaker containing half-strength Kimura B solution as described previously (Chu and Lee 1989). The hydroponically cultivated seedlings were grown for 12 days in a Phytotron with natural light 30 °C day (12 h)/25 °C night (12 h) and 90 % relative humidity. The apical 3 cm of the third leaf was used in all experiments. A group of ten segments was floated in a Petri dish containing 10 mL of test solution. Incubation was carried out at 27 °C in the dark.

Test solutions included MJ, NO donors, a NO scavenger [2-(4-carboxy-2-phenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide; c-PTIO], and free radical scavengers. PBN (N-tert-butyl- α -phenylnitron), 3-morpholino-sydonimine (SIN-1) and sodium nitroprusside (SNP) were used as NO donors. We also used a solution containing ascorbic acid (AsA) and $NaNO_2$ as another NO donor. Sodium benzoate (SB) and reduced glutathione (GSH) were used as free radical scavengers. All chemicals were purchased from Sigma Co. (St. Louis, MO, USA).

Determinations of protein, H_2O_2 , lipid peroxidation, GSH, and AsA

The senescence of detached rice leaves was followed by measuring the decrease of protein content. For protein extraction, leaf segments were homogenized in 50 mmol L⁻¹ sodium phosphate buffer (pH 6.8). The extracts were centrifuged at 17,600 g_n for 20 min, and the supernatants were used for determination of protein by the method of Bradford (1976) and enzyme activities. The H_2O_2 content was measured colorimetrically as described by Jana and Choudhuri (1981). H_2O_2 was extracted by homogenizing leaf tissue with phosphate buffer (50 mmol L⁻¹, pH 6.5) containing 1 mmol L⁻¹ hydroxylamine. The homogenate was centrifuged at 6,000 g_n for 25 min. To determine H_2O_2 content, the extracted solution was mixed with 0.1 % titanium chloride in 20 % (v/v) H_2SO_4 . The mixture was then centrifuged at 6,000 g_n for 25 min. The absorbance was measured at 410 nm. The H_2O_2 content was calculated using the extinction coefficient 0.28 μ mol⁻¹ cm⁻¹. MDA, routinely used as an indicator of lipid peroxidation, was extracted with 5 % (w/v) trichloroacetic acid and determined according to Heath and Packer (1968). GSH in 3 % sulfosalicylic acid extract and AsA in 5 % (w/v) trichloroacetic acid extract were determined as described by Smith (1985) and Laws et al. (1983), respectively.

Enzyme assays

Peroxidase (POD) activity was measured using a modification of the procedure of MacAdam et al. (1992). Activity was calculated using the extinction coefficient [26.6 (mmol L⁻¹)⁻¹ cm⁻¹ at 470 nm] for tetraguaia-

col. Catalase (CAT) activity was assayed by measuring the initial rate of disappearance of H_2O_2 (Kato and Shimizu 1987). The decrease in H_2O_2 was followed as the decline in absorbance at 240 nm, and activity was calculated using the extinction coefficient [$40 (\text{mmol L}^{-1})^{-1} \text{cm}^{-1}$ at 240 nm] for H_2O_2 (Kato and Shimizu 1987). 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 AsA concentration was followed as the decline in optical density at 290 nm and activity was calculated using the extinction coefficient [$2.8 (\text{mmol L}^{-1})^{-1} \text{cm}^{-1}$ at 290 nm] for AsA. Glutathione reductase (GR) was determined by the method of Foster and Hess (1980). One unit of activity for CAT, POD, SOD, APOD, and GR was defined as the amount of enzyme which degraded $1 \mu\text{mol}$ H_2O_2 per min, caused the formation of $1 \mu\text{mol}$ tetraguaiacol per min, inhibited by 50 % the rate of NADH oxidation observed in control, degraded $1 \mu\text{mol}$ of AsA per min, and decreased $1 A_{340}$ per min, respectively.

Experimental design

Protein, H_2O_2 , MDA, AsA, and GSH contents were expressed per g fresh weight (FW). Enzyme activities were expressed as unit mg^{-1} protein. In the present investigation, rice seedlings were grown for 12 days in a greenhouse, where natural light was provided. The growth of rice seedlings is very sensitive to light and varies with different light intensities. Experiments were carried out at different times of the year. Thus, absolute levels of each measurement varied among experiments because of seasonal effects. However, the patterns of response to MJ and/or NO donors were reproducible. All measurements described here were repeated three times. Similar results and identical trends were obtained each time. The data reported here are from a single experiment.

Results

The obvious character of leaf senescence is yellowing. Chlorophyll loss has been considered to be the principal criterion of senescence. The protein breakdown during leaf senescence has been realized from earlier studies. We have shown that protein breakdown precedes chlorophyll loss during rice leaf senescence (Kao 1980). Thus, senescence of rice leaves in the present investigation was followed by measuring the decrease of protein. The changes in protein and MDA contents in detached rice leaves treated with $45 \mu\text{mol/L}^{-1}$ MJ in the dark are shown in Figures 1A and 1B. The decrease in protein and increase in MDA were evident at day 1 after MJ treatment. Clearly, MJ is effective in promoting senescence of rice leaves. MJ treatment resulted in a marked increase in MDA, indicating that MJ brings about lipid peroxidation. Lipid peroxidation is caused by AOS (Kellogg and Fridovich 1975, Thompson et al. 1987). MJ treatment also caused an increase in H_2O_2 content (Fig. 1C). In the present investigation, when free radical scavengers such as SB and GSH, were used together with MJ, it was found that they partially inhibited the reduction in protein content (Fig. 2A) and the increase in MDA

Figure 1. Changes in the contents of protein (A), MDA (B), and H_2O_2 (C) in rice leaves treated with MJ. Detached rice leaves were treated with either water or $45 \mu\text{mol L}^{-1}$ MJ in the dark. Data are means ($\pm$ SE) of four replicates of a single typical experiment. Three series of independent experiments were carried out giving reproducible results.

(Fig. 2B) and H_2O_2 (Fig. 2C) contents caused by MJ. These results support the involvement of AOS as the chemical species inducing MJ-enhanced senescence in rice leaves.

Plant cells are equipped with several AOS detoxifying enzymes and antioxidants to protect them against oxidative damage. Antioxidative enzymes include SOD, APOD, GR, CAT, and POD (Foyer et al. 1997). AsA and GSH are two main water-soluble antioxidants (Foyer et al. 1997). The striking increase in lipid peroxidation seen in rice leaves treated with MJ may be a reflection of the change of the activities of antioxidative enzymes and the contents of antioxidants. As shown in Figures 3A, 3C, and 3D, MJ-treated rice leaves had higher activities of APOD, POD, and CAT than the controls at day 1 after dark incubation. Higher GR activity in MJ-treated rice leaves was observed only at 2 days after dark incubation (Fig. 3B). But MJ treatment had no effect on SOD activity in

Figure 2. Effect of free radical scavengers (SB and GSH) on protein (A), MDA (B), and H₂O₂ (C) contents in rice leaves treated with MJ. The concentrations of MJ, SB, and GSH were 45 μmol L⁻¹, 10 mmol L⁻¹, and 30 mmol L⁻¹, respectively. All measurements were determined 3 days after treatment in the dark. Data are means (± SE) of four replicates of a single typical experiment. Three series of independent experiments were carried out giving reproducible results.

rice leaves (data not shown). Treatment with MJ significantly decreased AsA and GSH contents compared with the control leaves; it occurred at day 1 after treatment (Figs. 4A and 4B). The increased activities of antioxidative enzymes and the decreased contents of AsA and GSH in response to MJ further suggest a strong induction of oxidative stress. As expected, treatment with free radical scavengers (SB and GSH) inhibited the increase in the activities of antioxidative enzymes (Figs. 5A, 5B, and 5C) and the decrease in the contents of AsA and GSH (Figs. 5D and 5E) in rice leaves caused by MJ.

We show that the promotion of senescence of detached rice leaves by MJ is linked to lipid peroxidation or oxidative stress. NO is known to counteract oxidative stress in plants (Beligni and Lamattina 1999 a, b, 2002, Beligni et al. 2002,

Table 1. Effect of NO donors on protein content in rice leaves treated with MJ.

Treatment	Protein (mg g ⁻¹ FW)
H ₂ O	50.1±1.1
MJ	38.1±0.21
MJ + PBN	47.2±0.91
MJ + SIN-1	43.7±0.11
MJ + SNP	42.9±0.73
MJ + AsA + NaNO ₂	41.8±0.18

The concentrations of MJ, PBN, SIN-1, SNP, AsA, and NaNO₂ were 45, 100, 100, 100, 100, and 200 μmol L⁻¹, respectively. Protein content was determined 1 day after treatment in the dark. Data are means (± SE) of four replicates of a single typical experiment. Three series of independent experiments were carried out giving reproducible results.

Cheng et al. 2002, Hung et al. 2002, Hung and Kao 2003). Thus, it is of great interest to know whether the protective role of NO is also active in MJ-promoted senescence of rice leaves. Consequently, detached rice leaves were treated with MJ in the presence or absence of NO donors, such as PBN, SIN-1, SNP, and a mixture of AsA and NaNO₂ in the dark. As indicated in Table 1, all NO donors were effective in inhibiting MJ-promoted senescence of rice leaves. We also observed that PBN is more effective in inhibiting MJ-promoted leaf senescence than SIN-1, SNP, and AsA plus NaNO₂ (Table 1).

PBN alone had no effect on protein content (Fig. 6). When applied together with MJ, PBN concentrations between 25 and 200 μmol L⁻¹ produced a clear protection against protein loss (Fig. 6). The optimal concentration of PBN in inhibiting MJ-promoted leaf senescence was observed to be 100 μmol L⁻¹ (Fig. 6).

To investigate whether the protective effect induced by PBN treatment was the result of the production of NO, 100 μmol L⁻¹ c-PTIO, a NO-specific scavenger, was applied along with 100 μmol L⁻¹ PBN. The effect of PBN on MJ-promoted protein loss (Fig. 7A) and increase in MDA and H₂O₂ contents (Figs. 7B and 7C) could be reversed by c-PTIO. We also observed that PBN counteracted MJ-induced increase of activities of antioxidative enzymes (CAT, POD and APOD) and c-PTIO reversed the effect of PBN-decreased enzyme activities (Figs. 8A, 8B, and 8C). Furthermore, the effect of PBN on MJ-decreased AsA and GSH contents could be reversed by c-PTIO (Figs. 8D and 8E). Clearly, the effect of NO donor PBN is attributable to NO released.

Discussion

Jasmonates have both chemical and physiological similarities to abscisic acid (Anderson et al. 1989). Since endogenous

Figure 3. Changes in the activities of APOD (A), GR (B), CAT (C), and POD (D) in rice leaves treated with MJ. Detached rice leaves were treated with either water or $45 \mu\text{mol L}^{-1}$ MJ in the dark. Data are means ($\pm$ SE) of four replicates of a single typical experiment. Three series of independent experiments were carried out giving reproducible results.

Figure 4. Changes in the contents of AsA (A) and GSH (B) in rice leaves treated with MJ. Detached rice leaves were treated with either water or $45 \mu\text{mol L}^{-1}$ MJ in the dark. Data are means ($\pm$ SE) of four replicates of a single typical experiment. Three series of independent experiments were carried out giving reproducible results.

abscisic acid content decreased in MJ-treated rice leaves (Wang and Kao 1999), it is unlikely that the effect of MJ on promoting senescence of rice is mediated through abscisic acid.

It has been shown that MJ can cause an increased production of H_2O_2 (Orozco-Cárdenas and Ryan 1999) and induce lipid peroxidation (Hung and Kao 1998). These suggest that MJ treatment causes oxidative stress in plants. Our results not only have shown that MJ increased the content of H_2O_2 (Fig. 1 C) and the activities of APOD (Fig. 3 A), GR (Fig. 3 B), CAT (Fig. 3 C), and POD (Fig. 3 D), but also demonstrate that MJ caused a decrease in AsA (Fig. 4 A) and GSH contents (Fig. 4 B). Meanwhile, protein loss (Fig. 1 A) and lipid peroxidation (Fig. 1 B) were observed in MJ-treated rice leaves. All these results suggest that MJ causes oxidative stress and that MJ-promoted senescence of rice leaves is mediated through oxidative stress. This suggestion is further supported by the observations that free radical scavengers (SB and GSH) inhibit MJ-induced senescence (Fig. 2 A), MJ-increased lipid peroxidation (Fig. 2 B) and H_2O_2 content (Fig. 2 C), MJ-increased antioxidative enzyme activities (Figs. 5 A, 5 B, and 5 C), and MJ-decreased antioxidants (Figs. 5 D and 5 E).

There is only limited information about the mechanism of MJ-induced H_2O_2 production. In several model systems investigated in plants, the accumulation of H_2O_2 appears to be mediated by the activation of a membrane-bound NADPH oxidase complex (Orozco-Cárdenas and Ryan 1999, Pei et al. 2000, Zhang et al. 2001, Jiang and Zhang 2002). Our unpublished observations indicate that diphenyleneiodonium chloride and imidazole, inhibitors of NADPH oxidase (Cross 1990, Pei et al. 2000), prevented MJ-induced H_2O_2 production, MJ-promoted senescence, MJ-increased antioxidative enzyme activities and MJ-decreased antioxidants in rice leaves. Similar results were obtained by using dimethylthiourea, a chemical trap for H_2O_2 (unpublished data). Here, we show that MJ

Figure 5. Effect of free radical scavengers on the activities of antioxidative enzymes [CAT (A), POD (B), and APOD (C)] and the contents of AsA (D) and GSH (E) in rice leaves treated with MJ. The concentrations of MJ, SB, and GSH were $45 \mu\text{mol L}^{-1}$, 10mmol L^{-1} , and 30mmol L^{-1} , respectively. All measurements were determined 3 days after treatment in the dark. Data are means ($\pm$ SE) of four replicates of a single typical experiment. Three series of independent experiments were carried out giving reproducible results.

caused the increase in H_2O_2 content in rice leaves (Fig. 1C). In a short term experiment, we also observed that the increase in H_2O_2 content caused by MJ preceded the de-

Figure 6. Effect of PBN concentrations on protein contents of rice leaves treated with MJ. Detached rice leaves were treated with either water or $45 \mu\text{mol L}^{-1}$ MJ for 1 day in the dark. Data are means ($\pm$ SE) of four replicates of a single typical experiment. Three series of independent experiments were carried out giving reproducible results.

crease in protein content and increase in MDA content (unpublished data). It appears that H_2O_2 is a key signaling molecule involved in MJ-induced senescence of rice leaves.

AsA is a major antioxidant in photosynthetic and non-photosynthetic tissues which reacts directly with AOS and is utilized as a substrate for APOD-catalysed H_2O_2 detoxification (Noctor and Foyer 1998). GSH is involved in AsA regeneration and functions also as a direct antioxidant of AOS (Noctor and Foyer 1998). Elevated AsA and GSH contents have been measured in plants subjected to abiotic stress (May et al. 1998, Kocsy et al. 2001, Khan et al. 2002, Ruiz and Blumwald 2002). However, there are other reports indicating that AsA and GSH levels decreased in plants in response to abiotic stress (Boo and Jung 1999, Hernandez et al. 2000, Shalta et al. 2001). In the present investigation, we observed that MJ treatment resulted in decreased AsA and GSH in rice leaves (Figs. 4A and 4B). We made the same observation for H_2O_2 (unpublished data). The decrease in AsA and GSH contents in rice leaves treated with MJ is believed to be the result of impaired AsA and GSH metabolism.

It has been suggested that NO is an antioxidant in plants and its action may be explained by its ability to scavenge AOS (Beligni and Lamattina 1999 a, b, 2002, Cheng et al.

Figure 7. Effect of PBN on protein (A), MDA (B), and H₂O₂ (C) contents in MJ-treated rice leaves in the presence or absence of c-PTIO. The concentrations of MJ, PBN, and c-PTIO were 45, 100, and 100 $\mu\text{mol L}^{-1}$, respectively. All measurements were determined 1 day after treatment in the dark. Data are means ($\pm$ SE) of four replicates of a single typical experiment. Three series of independent experiments were carried out giving reproducible results.

2002, Hung et al. 2002, Hung and Kao 2003). If NO acts as an antioxidant, NO may reduce AOS levels in MJ-treated rice leaves. Since H₂O₂ is involved in MJ-induced senescence of rice leaves (Fig. 1C and unpublished data) and NO reduces MJ-increased H₂O₂ content (Fig. 7C), it appears that NO indeed has the ability to scavenge AOS. Orozco-Cárdenas and Ryan (2002) also reported that NO blocked the H₂O₂ production that was induced by jasmonic acid.

In the present investigation, we found that NO reduced MJ-increased MDA content (Fig. 7B) and antioxidative enzyme activities (Figs. 8A, 8B, and 8C) in rice leaves. These results are in agreement with our previous work, in which we demonstrated that NO counteracted paraquat-increased MDA content and antioxidative enzyme activities (Hung et al. 2002). Because lipid peroxidation and the increase in antioxidative enzyme activities are the consequence of AOS overproduction (Kellogg and Fridovich 1975, Thompson et al. 1987) and since NO acts as an AOS scavenger, the reduction of MDA content and antioxidative enzyme activities could be a result of low levels of H₂O₂ in rice leaves treated with NO and MJ. The fact that NO counteracts MJ-decreased AsA and GSH (Figs. 8D and 8E) results in an increase in the capacity of NO to scavenge H₂O₂ in rice leaves treated with NO and MJ compared to rice leaves treated with MJ alone and might account in part for the decreased contents of H₂O₂ observed in rice leaves treated with NO and MJ (Fig. 7C).

CAT, APOD, and POD have been shown to be directly inhibited by NO (Ferrer and Barceló 1999, Clark et al. 2000). However, our results show that PBN treatment alone did not affect antioxidative enzyme activities in rice leaves (data not shown). Thus it is unlikely that the reduction of MJ-induced increase in antioxidative enzyme activities by NO is due to a direct NO-mediated inhibition of the enzymes.

In animals, NO displays cytotoxic activity but may also play a cytoprotective role in oxidative stress (Kroncke et al. 1997). In plants that the combination of NO with AOS is also described as either toxic or protective depending on the circumstances (Beligni and Lamattina 1999c, 2001). AOS can react with NO to form peroxynitrite (Kim and Han 2000, Martinez et al. 2000), which is often thought to have cytotoxic effects (Huie and Padmaja 1993, Alamillo and Garcia-Olmedo 2001, Beligni and Lamattina 2001). Peroxynitrite has been shown to react with H₂O₂ to yield nitrite ion and oxygen (Martinez et al. 2000). This reaction has been suggested to be the mechanism of NO cytoprotection in animals (Wink et al. 1993). Based on our results, it appears that this mechanism is operating in rice leaves as well.

Previously, we have shown that NO counteracts oxidative stress in rice leaves induced by paraquat, dehydration, polyethylene glycol, and abscisic acid (Cheng et al. 2002, Hung et al. 2002, Hung and Kao 2003). Herein, we report that NO donors act similarly as free radical scavengers (SB and GSH) in inhibiting MJ-promoted rice leaf senescence. These results strongly suggest that the antioxidant properties of NO are counteracting oxidative stress.

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Figure 8. Effect of PBN on the activities of antioxidative enzymes [CAT (A), POD (B), APOD (C)] and the contents of AsA (D) and GSH (E) in MJ-treated rice leaves in the presence or absence of c-PTIO. The concentrations of MJ, PBN, and c-PTIO were 45, 100, and 100 $\mu\text{mol L}^{-1}$, respectively. All measurements were determined 1 day after treatment in the dark. Data are means ($\pm$ SE) of four replicates of a single typical experiment. Three series of independent experiments were carried out giving reproducible results.

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