Nitric oxide counteracts the senescence of rice leaves induced by abscisic acid

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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 ABA. Senescence of rice leaves was determined by the decrease of protein content. ABA treatment resulted in (1) induction of leaf senescence, (2) increase in H_2O_2 and malondialdehyde (MDA) contents, (3) decrease in reduced form glutathione (GSH) and ascorbic acid (AsA) contents, and (4) increase in antioxidative enzyme activities (superoxide dismutase, ascorbate peroxidase, glutathione reductase, and catalase). All these ABA 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 + $NaNO_2$] were effective in reducing ABA-induced leaf senescence. PBN prevented ABA-induced increase in the contents of H_2O_2 and MDA, decrease in the contents of GSH and AsA, and increase in the activities of antioxidative enzymes. The protective effect of PBN on ABA-promoted senescence, ABA-increased H_2O_2 content and lipid peroxidation, ABA-decreased GSH and AsA, and ABA-increased antioxidative enzyme activities was reversed by 2-(4-carboxy-2-phenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide, a NO-specific scavenger, suggesting that the protective effect of PBN is attributable to NO released. Reduction of ABA-induced senescence by NO in rice leaves is most likely mediated through its ability to scavenge active oxygen species including H_2O_2 .

Key words: abscisic acid – active oxygen species – lipid peroxidation – 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. – ELISA = enzyme-linked immunosorbent assay. – FW = fresh weight. – GR = glutathione reductase. – GSH = reduced glutathione. – MDA = malondialdehyde. – 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

The plant hormone ABA is a sesquiterpenoid synthesized from xanthophylls (Creelman 1989, Taylor et al. 2000, Seo and Koshiba 2002) and appears to influence several physiological and developmental events (Creelman 1989, Kende and Zeevaart 1997). It has been suggested that ABA is one of the most effective plant hormones in terms of promoting leaf senescence (Nooden 1988). Applied ABA has been found to promote leaf senescence in a wide range of plant species (Nooden 1988, Creelman 1989). An increase in endogenous ABA has been shown to coincide with senescence of leaves (Gepstein and Thimann 1980, Yang 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 ABA causes generation of AOS including H_2O_2 (Guan et al. 2000, Pei et al. 2000, Jiang and Zhang 2001) and lipid peroxidation expressed as malondialdehyde (MDA) production in plant cells (Bueno et al. 1998). Thus, ABA leads to an oxidative stress in plant cells.

Nitric oxide (NO) is a free radical gas with well-characterized signaling roles in mammalian systems, acting as a second messenger during vasorelaxation, neurotransmission, immunity, and cytotoxicity (Furchgott 1995). It is now evident NO plays essential roles in animal physiology. In contrast to the large body of knowledge regarding NO in animal cells, the physiology and biochemistry of NO in plants is less well understood. There are an increasing number of reports indicating that NO could also participate in many physiological responses of plants: pathogen response (Delledonne et al. 1998, Klessig et al. 2000), programmed cell death (Pedroso et al. 2000, Beligni et al. 2002), growth (Leshem and Hara-maty 1996), germination (Beligni and Lamattina 2000), root organogenesis (Pagnussat et al. 2002), phytoalexin production (Noritake et al. 1996), cytokinin action (Scherer and Holk 2000), ABA-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, Hung et al. 2002). Recently, Beligni and Lamattina (2002) provided strong evidence that NO interferes with plant photo-oxidative stress by detoxifying AOS. Thus, a possible participation of NO in antioxidant system in plants, as it does 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 decrease in lipid peroxidation (Hung et al. 2002). More recently, we showed that the promotion of rice leaf senescence caused by polyethylene glycol and dehydration, which induce lipid peroxidation, can be counteracted by NO donors, but the senescence caused by sorbitol, which has no effect on lipid peroxi-

dation, cannot be counteracted by NO donors (Cheng et al. 2002). In the present investigation, we examined the effect of NO on ABA-promoted 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 ABA, NO donors, a NO scavenger (c-PTIO), and free radical scavengers. PBN, SIN-1 and SNP were used as NO donors. We also used a solution containing ascorbic acid and $NaNO_2$ as another NO donor. Sodium benzoate (SB) and 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, extracted solution was mixed with 0.1 % titanium sulphate 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 $\mu\text{mol}^{-1}\text{cm}^{-1}$. MDA, routinely used as an indicator of lipid peroxidation, was extracted with 5 % (w/v) trichloroacetic acid and determined according 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). 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 $1 \mu\text{mol H}_2\text{O}_2$ per min, caused the formation of $1 \mu\text{mol tetraguaiacol}$ per min, inhibited 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.

ABA assay

For extraction of abscisic acid (ABA), leaf samples were homogenized with a motor and pestle in extraction solution (80 % methanol containing 2 % glacial acetic acid). To remove plant pigments and other non-polar compounds which could interfere in the immunoassay, extracts were first passed through polyvinylpyrrolidone column and C_{18} cartridges. The eluates were concentrated to dryness by vacuum-evaporation and resuspended in Tris-buffered saline before enzyme-linked immunosorbent assay (ELISA). ABA was quantitated by ELISA (Walker-Simmons 1987). ABA immunoassay detection kit

(PGR-1) was purchased from Sigma Chemical Co. (St Louis MO, USA), is specific for (+)-ABA. By evaluating ^3H -ABA recovery, ABA loss was less than 3 % by the method described here.

Experimental design

Protein, H_2O_2 , MDA, AsA, GSH, and ABA 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 ABA 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 the principal criterion of senescence for the largest number of workers. The protein break-

Figure 1. Changes in the contents of ABA, protein, MDA, and H_2O_2 in rice leaves treated with ABA. Detached rice leaves were treated with either water or $45 \mu\text{mol L}^{-1}$ ABA 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.

Table 1. Effect of free radical scavengers (SB and GSH) on protein, MDA, and H_2O_2 contents in rice leaves treated with ABA.

Treatment	Protein (mg g^{-1} FW)	MDA (nmol g^{-1} FW)	H_2O_2 ($\mu\text{mol g}^{-1}$ FW)
H_2O	33.7 ± 0.32	33.6 ± 0.55	29.0 ± 1.3
ABA	22.1 ± 0.70	53.2 ± 1.2	38.6 ± 0.92
ABA + SB	27.7 ± 0.21	37.5 ± 2.6	30.0 ± 0.55
ABA + GSH	26.3 ± 1.1	35.6 ± 1.8	29.2 ± 0.68

The concentrations of ABA, SB, and GSH were $45 \mu\text{mol L}^{-1}$, 10mmol L^{-1} , and 30mmol L^{-1} , respectively. Protein, MDA, and H_2O_2 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.

down during leaf senescence has been realized from earliest 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 $45 \mu\text{mol/L}$ ABA in the dark are shown in Figure 1. The decrease in protein and increase in MDA was evident at 2 days after ABA treatment. Clearly, ABA is effective in promoting senescence of rice leaves. ABA treatment resulted in a marked increase in MDA, indicating that ABA brings about lipid peroxidation. Lipid peroxidation is caused by AOS (Kellogg and Fridovich 1975, Thompson et al. 1987). ABA treatment also caused an increase in H_2O_2 content (Fig. 1). The increase in H_2O_2 was evident at 1 day after ABA treatment, which preceded the decrease in protein and increase in MDA. In the present investigation, when free radical scavengers such as SB and GSH, were used together with ABA, it was found that they partially inhibited the reduction in protein content and the increase in MDA and H_2O_2 contents caused by ABA (Table 1). These results support the involvement of AOS as the chemical species inducing ABA-enhanced senescence in rice leaves.

Figure 1 also shows the changes in endogenous ABA content in detached rice leaves treated with $45 \mu\text{mol L}^{-1}$ ABA. As expected, ABA-treated leaves had higher endogenous ABA content than H_2O -treated leaves. In H_2O -treated rice leaves, endogenous ABA content increased at 2 days after dark incubation and protein loss occurred at one day after dark in-

Figure 2. Changes in the activities of SOD, APOD, GR, and CAT in rice leaves treated with ABA. Detached rice leaves were treated with either water or $45 \mu\text{mol L}^{-1}$ ABA 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 3. Changes in the contents of AsA and GSH in rice leaves treated with ABA. Detached rice leaves were treated with either water or $45 \mu\text{mol L}^{-1}$ ABA 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.

cubation, indicating that endogenous ABA does not play a primary role in the regulation of dark-induced senescence of rice leaves, although exogenous application of ABA promoted senescence of rice leaves (Fig. 1). Figure 1 also show that no changes in MDA and H_2O_2 contents were observed in control leaves. It appears that there is no direct relationship

between lipid peroxidation and dark induced senescence of 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 ABA may be a reflection of the change of the activities of antioxidative enzymes and the contents of antioxidants. As shown in Fig. 2, ABA-treated rice leaves had higher activities of SOD, APOD, GR, and CAT than the controls (Fig. 2). But ABA treatment had no effect on POD activity in rice leaves (data not shown). Treatment with ABA significantly decreased AsA and GSH contents compared with the control leaves, it occurred at 2 days after treatment (Fig. 3). The increased activities of antioxidative enzymes and the decreased contents of AsA and GSH in response to ABA are further suggesting of strong induction of oxidative stress. As expected, treatment with free radical scavengers (SB and GSH) inhibited the increase in the activities of antioxidative enzymes and the decrease in the contents of AsA and GSH in rice leaves caused by ABA (Table 2).

Here we show that the promotion of senescence of detached rice leaves by ABA 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, Cheng et al. 2002, Hung et al. 2002). Thus, it is of great interest to know whether the protective role of NO is also active in ABA-promoted senescence of rice leaves. Consequently, detached rice leaves were treated with ABA in the presence or absence of NO donors, such as PBN, SIN-1, SNP, and a mixture of AsA and NaNO_2 in the dark. As indicated in Table 3, all NO donors were effective in inhibiting ABA-promoted senescence of rice leaves.

PBN alone had no effect on protein content (Fig. 4). When applied together with ABA, PBN concentrations between 25 and $200 \mu\text{mol/L}$ produced a clear protection against protein loss (Fig. 4). The optimal concentration of PBN in inhibiting

Table 2. Effect of free radical scavengers on the activities of antioxidative enzymes (CAT, SOD, APOD, and GR) and the contents of AsA and GSH in rice leaves treated with ABA.

Treatment	CAT (units mg^{-1} protein)	SOD (units mg^{-1} protein)	APOD (units mg^{-1} protein)	GR (units mg^{-1} protein)	AsA ($\mu\text{mol g}^{-1}$ FW)	GSH (nmol g^{-1} FW)
H ₂ O	0.18 ± 0.02	1.2 ± 0.06	1.1 ± 0.04	0.17 ± 0.01	7.6 ± 0.27	2.2 ± 0.05
ABA	0.29 ± 0.04	2.8 ± 0.10	3.0 ± 0.12	0.33 ± 0.02	5.7 ± 0.18	1.0 ± 0.08
ABA + SB	0.22 ± 0.01	2.2 ± 0.07	1.9 ± 0.07	0.26 ± 0.02	6.8 ± 0.23	2.6 ± 0.05
ABA + GSH	0.21 ± 0.01	1.8 ± 0.10	1.8 ± 0.03	0.26 ± 0.01	9.1 ± 0.13	11.0 ± 0.69

The concentrations of ABA, SB, and GSH were $45 \mu\text{mol L}^{-1}$, 10 mmol L^{-1} , and 30 mmol L^{-1} , respectively. All measurements were determined 3 days after treatment in the dark. Data are means ($\pm$ SE) of four replicates of a single typical experiment. Three series of independent experiments were carried out giving reproducible results.

Table 3. Effect of NO donors on protein content in rice leaves treated with ABA.

Treatment	Protein (mg g ⁻¹ FW)
H ₂ O	40.2±1.1
ABA	28.7±0.51
ABA + PBN	31.9±0.64
ABA + SIN-1	30.5±1.1
ABA + SNP	31.8±1.2
ABA + AsA + NaNO ₂	33.8±1.9

The concentrations of ABA, PBN, SIN-1, SNP, AsA, and NaNO₂ were 45, 100, 100, 100, 100, and 200 μmol L⁻¹, respectively. Protein content was determined 2 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.

Figure 4. Effect of PBN concentrations on protein contents of rice leaves treated with ABA. Detached rice leaves were treated with either water or 45 μmol L⁻¹ ABA 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.**Table 4.** Effect of PBN on protein, MDA, and H₂O₂ contents in ABA-treated rice leaves in the presence or absence of c-PTIO.

Treatment	Protein (mg g ⁻¹ FW)	MDA (nmol g ⁻¹ FW)	H ₂ O ₂ (μmol g ⁻¹ FW)
H ₂ O	40.2±1.2	31.6±0.31	31.2±1.1
ABA	28.8±1.5	36.7±0.40	39.3±2.4
ABA + PBN	35.2±2.8	31.8±0.56	31.0±0.96
ABA + PBN + c-PTIO	27.0±0.56	38.1±0.72	38.7±2.2

The concentrations of ABA, PBN, and c-PTIO were 45, 100, and 100 μmol L⁻¹, respectively. Protein, MDA, and H₂O₂ contents were determined 2 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.

ABA-promoted leaf senescence was observed to be 100 μmol/L (Fig. 4).

To investigate whether the protective effect induced by PBN treatment was the result of the production of NO, 100 μmol/L c-PTIO, a NO-specific scavenger, was applied along with 100 μmol/L PBN. The effect of PBN on ABA-promoted protein loss and increase in MDA and H₂O₂ contents could be reversed by c-PTIO (Table 4). We also observed that PBN counteracted ABA-induced increase of activities of antioxidative enzymes (SOD, APOD, GR and CAT) and c-PTIO reversed the effect of PBN-decreased enzyme activities (Table 5). Furthermore, the effect of PBN on ABA-decreased AsA and GSH contents could be reversed by c-PTIO (Table 5). Clearly, the effect of NO donor PBN is attributable to NO released.

Discussion

It has been shown that ABA can cause an increased production of H₂O₂, induce the expression of some antioxidant genes and increase the activities of antioxidative enzymes such

Table 5. Effect of PBN on the activities of antioxidative enzymes and the contents of AsA and GSH in ABA-treated rice leaves in the presence or absence of c-PTIO.

Treatment	CAT (units mg ⁻¹ protein)	SOD (units mg ⁻¹ protein)	APOD (units mg ⁻¹ protein)	GR (units mg ⁻¹ protein)	AsA (μmol g ⁻¹ FW)	GSH (nmol g ⁻¹ FW)
H ₂ O	0.16±0.00	1.0±0.03	1.6±0.08	0.15±0.01	9.6±0.29	1.7 ±0.16
ABA	0.20±0.00	1.8±0.01	2.3±0.11	0.20±0.01	7.0±0.22	0.77±0.10
ABA + PBN	0.16±0.01	1.1±0.01	1.9±0.10	0.15±0.01	11.3±0.20	1.5 ±0.09
ABA + PBN + c-PTIO	0.22±0.01	1.8±0.01	2.3±0.17	0.20±0.01	9.0±0.36	1.1 ±0.09

The concentrations of ABA, PBN, and c-PTIO were 45, 100, and 100 μmol L⁻¹, respectively. Enzyme activities, AsA, and GSH were determined 2 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.

as SOD, APOD, GR, CAT, and POD in plants (Bueno et al. 1998, Gong et al. 1998, Guan and Scandalios 1998, Guan et al. 2000, Jiang and Zhang 2001, Zhang et al. 2001). These suggest that ABA treatment causes an oxidative stress in plants. Our results not only have shown that ABA increased the content of H_2O_2 (Fig. 1) and the activities of SOD, APOD, GR, and CAT (Fig. 2), but also demonstrate that caused a decrease in AsA and GSH contents. Meanwhile, protein loss and lipid peroxidation were observed in ABA-treated rice leaves (Fig. 1). All these results suggest that ABA causes an oxidative stress and that ABA-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 ABA-induced senescence, ABA-increased lipid peroxidation and H_2O_2 content, ABA-increased antioxidative enzyme activities, and ABA-decreased antioxidants (Tables 1 and 2).

There is only limited information about the mechanism of ABA-induced H_2O_2 production. In guard cells, ABA-induced H_2O_2 generation is regulated by plasma-membrane NADPH oxidase (Pei et al. 2000, Zhang et al. 2001). Recently, Jiang and Zhang (2002) also reported that plasma-membrane NADPH oxidase is involved in ABA- and water stress-induced antioxidant defense in leaves of maize seedlings. Our unpublished observations indicate that diphenyleneiodonium chloride and imidazole, inhibitors of NADPH oxidase (Cross 1990, Pei et al. 2000), prevented ABA-induced H_2O_2 production, ABA-promoted senescence, ABA-increased antioxidative enzyme activities and ABA-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 the increase in H_2O_2 content caused by ABA preceded the decrease in protein content and increase in MDA content (Fig. 1). It appears that H_2O_2 is a key signaling molecule involved in ABA-induced senescence of rice leaves.

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. 2002, Hung et al. 2002). If NO acts as an antioxidant, NO may reduce AOS levels in ABA-treated rice leaves. Since H_2O_2 is involved in ABA-induced senescence of rice leaves (Fig. 1 and unpublished data) and NO reduces ABA-increased H_2O_2 content (Table 4), it appears that NO indeed has the ability to scavenge AOS.

In the present investigation, we found that NO reduced ABA-increased MDA content and antioxidative enzyme activities (Tables 4 and 5) 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 NO acts as an AOS scavenger, therefore, the reduction of MDA content and antioxidative enzyme activities could be a result of

low levels of H_2O_2 in rice leaves treated with NO and ABA. The fact that NO counteracts ABA-decreased AsA and GSH (Table 5) would result in the capacity of NO to scavenge H_2O_2 in rice leaves treated with NO and ABA compared to rice leaves treated with ABA alone and might account in part for the lower contents of H_2O_2 observed in rice leaves treated with NO and ABA (Table 4).

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, the reduction of ABA-induced increase in antioxidative enzyme activities by NO is unlikely due to a direct NO-mediated inhibition of the enzymes.

In animals, NO on one side displays cytotoxic activity and on the other side may play a cytoprotective role in oxidative stress (Kroncke et al. 1997). AOS can react with NO to form peroxynitrite (Kim and Han 2000, Martinez et al. 2000). Peroxynitrite is often thought to have cytotoxic effect (Huie and Padmaja 1993, Alamillo and Garcia-Olmedo 2001, Beligni and Lamattina 2001). Peroxynitrite has been shown to react with H_2O_2 to yield nitrite ion and oxygen (Martinez et al. 2000). This reaction has been suggested to be the mechanism of NO cytoprotective actions in animals (Wink et al. 1993). Based on our results, it appears that this mechanism is operating in rice leaves.

In rice leaves, we have shown that NO counteracts oxidative stress induced by paraquat, dehydration, and polyethylene glycol (Cheng et al. 2002, Hung et al. 2002). Herein, we observed that NO donors act similarly as free radical scavengers (SB and GSH) in inhibiting ABA-promoted rice leaf senescence. All these results strongly suggest that antioxidant properties of NO are operating for counteracting oxidative stress in rice leaves.

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