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Protective Effect of Nitric Oxide against Oxidative Stress under UV-B Radiation in Maize Leaves

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Abstract

The effect of nitric oxide (NO) on antioxidant system and protective mechanism against oxidative stress under UV-B radiation was investigated in leaves of maize (*Zea mays* L.) seedlings during 3 days growth period. UV-B irradiation caused a decrease of leaf biomass including leaf length, width and weight during growth. Application of NO donor, sodium nitroprusside (SNP), significantly alleviated UV-B stress induced growth suppression. NO donor permitted the survival of more green leaf tissue preventing chlorophyll content reduction and of higher quantum yield for photosystem Ⅱ than in non-treated controls under UV-B stress, suggesting that NO has protective effect on chloroplast membrane in maize leaves. Flavonoids and anthocyanin, UV-B absorbing compounds, were significantly accumulated in the maize leaves upon UV-B exposure. Moreover, the increase of these compounds was intensified in the NO treated seedlings. UV-B treatment resulted in lipid peroxidation and induced accumulation of hydrogen peroxide (H_2O_2) in maize leaves, while NO donor prevented UV-B induced increase in the contents of malondialdehyde (MDA) and H_2O_2. These results demonstrate that NO serves as antioxidant agent able to scavenge H_2O_2 to protect plant cells from oxidative damage. The activities of two antioxidant enzymes that scavenge reactive oxygen species, catalase (CAT) and ascorbate peroxidase (APX) in maize leaves in the presence of NO donor under UV-B stress were higher than those under UV-B stress alone. Application of 2-(4-carboxyphenyl)-4, 4, 5, 5-tetramethylimidazoline-1-oxyl-3- oxide (PTIO), a specific NO scavenger, to the maize leaves arrested NO donor mediated protective effect on leaf growth, photosynthetic pigment and free radical scavenging activity. However, PTIO had little effect on maize leaves under UV-B stress compared with that of UV-B stress alone. N^{ω}-nitro-L-arginine (LNNA), an inhibitor of nitric oxide synthase (NOS), significantly increased H_2O_2 and MDA accumulation and decreased antioxidant enzyme activities in maize leaves under UV-B stress. This demonstrates that NOS inhibitor LNNA has opposite effects on oxidative resistance. From these results it is suggested that NO might act as a signal in activating active oxygen scavenging system that protects plants from oxidative stress induced by UV-B radiation and thus confer UV-B tolerance.

Key Words : Antioxidant enzymes, Nitric oxide, Oxidative stress, UV-B radiation, *Zea mays*

1. Introduction

Significant reductions in the stratospheric ozone layer led to an increase in solar ultraviolet-B (UV-B : 280-320 nm) radiation reaching the earth's surface (Mackerness, 2000). Numerous studies have demonstrated several detrimental effects of UV-B on plant development, morphology and physiology, including biomass reduction, decreased protein synthesis inhibition of photosynthetic activity and growth, photooxidation of pigment and DNA damage (Greenberg et al., 1997; An et al., 2005). In order to prevent these harmful effects of UV-B radiation, plants have developed several defense mechanisms

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including repair of inflicted damage and screening of the internal tissues against the radiation. Among these defense mechanisms are UV-absorbing molecules such as flavonoid derivatives and their biosynthetic machineries, reactive oxygen scavenging compounds and enzymes, pathogenesis-related defense proteins and DNA repair mechanisms (Brosche and Strid, 2003). The secondary metabolites, mainly flavonoid and related phenolic compounds, have the capacity to not only shield the tissue by UV absorption but also to scavenge the reactive oxygen species (ROS) generated (Harborne and Williams, 2000).

High doses of UV-B light produce oxidative stress, increasing ROS generation such as singlet oxygen, superoxide anion, hydrogen peroxide and hydroxyl radicals (Mackerness et al., 2001). ROS severely affect the normal structure and functioning of plant organelles, including damage to proteins, lipids and DNA, and affecting the cell integrity, morphology and physiology of plants (Frohnmeyer and Staiger, 2003). Plant have evolved complex mechanisms involving antioxidant system to scavenger these ROS and thereby protect cellular membranes, pigments and organelles. Nevertheless, recent studies confirm that ROS are signaling molecules that modulate various plant response to abiotic and biotic stresses (Apel and Hirt, 2004).

An efficient antioxidant defense system is present in plants to counteract oxidative stress. Catalase (CAT), ascorbate peroxidase (APX) and a variety of general peroxidases catalyze the breakdown of H_2O_2. CAT is one of the main H_2O_2-scavenging enzymes that dismutates H_2O_2 into water and O_2, and APX is a specific peroxidase that catalyzes the elimination of toxic product of H_2O_2 at the expense of oxidizing ascorbate to monodehydroascorbate (Yannarelli et al., 2005). Peroxidases are enzymes that catalyze the H_2O_2-dependent oxidation of a wide variety of substances, mainly phenolics. These antioxidant enzymes play cooperative roles to protect cellular membranes, pigment and organelles, and minimize tissue injury (Mittler, 2002).

Nitric oxide (NO), a highly reactive free radical molecule, is endogenously formed in many biological system. NO is an important secondary messenger in animal cells and accumulating evidence suggests that it is important in plant cells as well (Wendehenne et al., 2001). NO has been proposed to be a functional metabolite as an intra- and intercellular signaling molecule involved in growth, development and defense responses. In recent years, NO was reported to play important roles in diverse physiological responses in plants, including stimulation of seed germination, reduction of seed domancy, regulation of plant maturation and senescence, regulation of stomatal closure, induction of apoptosis /programmed cell death, and suppression of floral transition (Neill et al., 2003 ; Zhang et al., 2003b ; Qiao and Fan, 2008). NO is itself a reactive nitrogen species and its effects on different types of cells have proved to be either cytoprotective or cytotoxic depending on its concentration and on the status of the environments (Beligni and Lamattina, 2001). NO is evidently highly versatile in its physiological effects.

NO was suggested to be a signal molecule mediating responses to abiotic and biotic stresses, such as drought, salinity, heat, UV-B radiation and disease infection (Song et al., 2006 ; Qiao and Fan, 2008). Application of exogenous NO can mediate various physiological processes to abiotic stresses, thus enhance plant tolerance to specific stresses. The protective effect of NO against abiotic stresses is closely related to the NO-mediated reduction of ROS in plants (Zhang et al., 2003b). Abiotic stresses including UV-B radiation alter NO production and NOS is extensively activated by UV-B radiation (An et al., 2005). NO can be enzymatically synthesized from nitrite by nitrate reductase, nitrite reductase and NO synthase (NOS) activity (Wilson et al., 2008). If

UV-B radiation is received by NOS contributed to NO production, the elevated level of NO could rapidly cross biological membranes and trigger various different processes in a short period of time (Mackerness et al., 2001). NO formed through the increased NOS activity in responding to UV-B can be an early signaling molecule, and thus NOS is perhaps one of the photoreceptors for UV-B-induced growth inhibition.

Recent studies have provided evidence to further explain that NO might act as the main signal of growth and development in response to UV-B stress (Tossi et al., 2009 ; Zhang et al., 2009). Nevertheless, further investigations would be needed to further elucidate the relationship between NO and oxidative stress under UV-B, and the role of NO for inducing adaptive responses. Therefore, the present study was conducted to determine whether NO can induce tolerance against UV-B induced oxidative stress in maize (*Zea mays* L.) seedlings. Anatomical and physiological changes in UV-B- and NO-treated seedlings were monitored to characterize the patterns of damage induced in this crop species and to investigate possible mechanisms of NO induced protection.

2. Materials and Methods

2.1. Plant material and growth conditions

Seeds of maize (*Zea may* L.) were surface sterilized with 0.5% sodium hypochlorite for 20 min and rinsed thoroughly with distilled water. The seeds were then sown in pots containing a mixture of vermiculite and perlite (1:2, v/v) in a controlled environmental growth chamber with a 14 h/10 h photoperiod under 160 μmol photons $m^{-2}s^{-1}$ at 25℃, and watered daily until the second leaf was completely developed. Then half of the selected uniform seedlings were transferred to a another chamber supplemented with UV-B radiation. At the same time, the other half of the uniform seedlings were allowed to grow for a further 3 days for a group of controls without UV-B radiation. Over the course of growing period, the plants were treated with UV-B and chemicals alone or in combination. Test solutions included NO donor sodium nitroprusside (SNP), NO scavenger 2-(4-carboxyphenyl)-4, 4, 5, 5-tetramethyl-imidazoline-1-oxy-3-oxide (PTIO) and NOS inhibitor N^{ω}-nitro-L-arginine (LNNA). For treatments, 0.1 mM SNP, 0.1 mM PTIO or 0.2 mM LNNA were sprayed on the leaf surfaces of maize plants at 6-h intervals for 3 days, while only water was applied to the control plants. Leaf samples were taken from uniform plants in different parts of the pots at designed times. Fresh tissues were directly used for growth analysis or immediately frozen in liquid N_2 and stored at -70℃ for further analysis.

2.2. Ultraviolet-B treatment

UV-B radiation was provided with fluorescent UV-B lamps (VL-6, Viber lourmat, France) during the 14 h light periods, suspended 40 cm above the plant seedlings. Spectral irradiance was measured using a UV spectroradiometer (Li-1800, Lycosa, USA) and UV-B fluence rate at the sample surface was measured to be 6 $Wm^{-2}s^{-1}$.

2.3. Growth measurements

Leaf length, width and biomass were measured once a day during the growth of seedlings after the treatments. Leaf fresh weight was determined by using electronic balance. Leaf dry weight was measured following over drying at 80℃ for 72 h and reweighing.

2.4. Determination of photosynthetic pigments

Fully expanded leaves from the plants were frozon, then transferred to N,N- dimethylformamide and stored in the dark at 4℃ until they were analyzed. The chlorophyll and carotenoid contents were measured spectrophotometrically using specific

absorption coefficients of 470, 647 and 664 nm. The concentrations of chl a, chl b and carotenoid were calculated according to Inskeep and Bloom (1985).

2.5. Determination of flavonoids and anthocyanin contents

Flavonoids and anthocyanin were measured according to the method of Mirecki and Teramura (1984) with some modifications. Excised leaves (5 g) were homogenized with mortar and pestle at 4℃ in 5 ml of extraction buffer (99 : 1 methanol-HCl, v/v). The homogenate was centrifuged at 2,000 g for 10 min at 4℃, and the supernatant was used for assays. Flavonoids and anthocyanin contents were estimated by measuring to their absorbances at 300 and 530 nm, respectively.

2.6. Chlorophyll fluorescence measurement

Chlorophyll fluorescence induction parameters of leaves were measured at room temperature using a pulse-amplitude modulated fluorometer (PAM 2100, Walz, Germany) at the adaxial leaf surface of fresh plant material. Before each measurement, plants were dark-adapted for 10 min, and then fluorescence data was collected during a 5-min continuous illumination using the LED of the PAM as the actinic light source (63 μmol $m^{-2}s^{-1}$). Saturating pulses were given every 20 s and photochmical quantum yield of PS Ⅱ was calculated (Schreiber et al., 1986). The maximal photochemical efficiency of PS Ⅱ photochemistry was evaluated as $Fv/Fm=(Fm-Fo)/Fm$ where *Fo* and *Fm* represent the fluorescence levels under irradiation before and after a saturating pulse (3000 μmol $m^{-2}s^{-1}$), respectively.

2.7. Determination of lipid peroxidation

Lipid peroxidation was measured in terms of malondialdehyde (MDA) content following the method of Zhao et al. (1994). Fresh leaves (0.5 g) were homogenized in 10 ml of 5% trichloroacetic acid and the homogenate was then centrifuged at 4,000 g for 10 min. The supernatant (2 ml) was mixed with 2 ml of 10% trichloroacetic acid containing 0.5% thiobarbituric acid, and then boiled at 100℃ for 20 min. After centrifugation at 12,000 g for 10 min, the absorbance of the supernatant was measured at 532 and 620 nm using a spectrophotometer. The amount of MDA was calculated using an extinction coefficient of 155 $mM^{-1}cm^{-1}$.

2.8. Determination of H_2O_2 content

The contents of H_2O_2 were determined by peroxide-coupled assay according to Veljovic-Jovanovic et al. (2002). Excised leaves (1 g) was ground with a mortar and pestle in liquid nitrogen, and the powder was extracted in 2 ml of 1 M $HClO_4$ in the presence of insoluble polyvinylpyrrolidone (5%, w/v). The homogenate was centrifuged at 12,000 g for 10 min at 4℃, and then the supernatant was neutralized to pH 7.5 with 5 M K_2CO_3 in the presence of 0.1 ml of 0.3 M phosphate buffer (pH 5.6). The solution was centrifuged at 12,000 g at 4℃ for 1 min, and the sample was incubated for 10 min with 1 U of ascorbate oxidase to oxidize ascorbate prior to assay. The reaction mixture consisted of 0.1 M phosphate buffer (pH 6.5), 3.3 mM 3-(dimethylamino) benzoic acid, 0.07 mM 3-methyl-2-benzothiazolinone hydrazone, and 0.3 U of peroxidase. This reaction was initiated by the addition of 0.2 ml of sample. The absorbance was recorded at 590 nm and compared with increases elicited by standard samples of hydrogen peroxide.

2.9. Determination of antioxidant enzyme activity

Fresh leaves (0.5 g) was homogenized in 10 ml of 100 mM potassium phosphate buffer (pH 7.5) containing 1 mM ascorbate and 0.5 mM EDTA in pre-cooled mortar and pestle. The homogenate was centrifuged at 22,000 g for 30 min at 4℃, and the supernatant was mixed as a crude extract for assays of enzyme activity.

CAT activity was assayed as described by Durner

and Klessing (1996). The activity was determined by monitoring the consumption of H_2O_2 (extinction coefficient 39.4 $mM^{-1}cm^{-1}$) at 240 nm for 2 min. APX activity was determined according to the Nakano and Asada (1981). The activity was monitored the rate of ascorbate oxidation at 290 nm, and calculated using an extinction coefficient of 2.8 $mM^{-1}cm^{-1}$ by following the decrease in absorbance of ascorbate for 2 min at 290 nm.

2.10. Statistical analysis

Each experiment was performed at least three times. Values represent the mean ±SD for four plants with three replicates per treatment. Statistical analysis was performed using the Student's t-test.

3. Results and Discussion

3.1. Effect of NO on leaf biomass under UV-B radiation

To specify the role of NO under UV-B stress, SNP as NO donor, LNNA as NOS inhibitor and PTIO as NO scavenger were used in the experiment. Leaf length and width were significantly decreased under UV-B radiation, while increased after the application of SNP at 36 h of treatment (Fig. 1.). The combined UV-B + SNP, UV-B + LNNA and UV-B + PTIO treatments enhanced to above the level of UV-B treatment alone, compromising the inhibitory effect of UV-B alone.

Fresh- and dry-matter accumulations were remarkably suppressed by enhanced UV-B radiation (Fig. 2). The combination of UV-B with NOS inhibitor relieved the inhibitions of the corresponding treatment (LNNA) in the leaf biomass. As compared to the control, LNNA and PTIO affected leaf biomass to a lesser extent, while SNP increased these parameters to a larger extent in the NO treated seedlings.

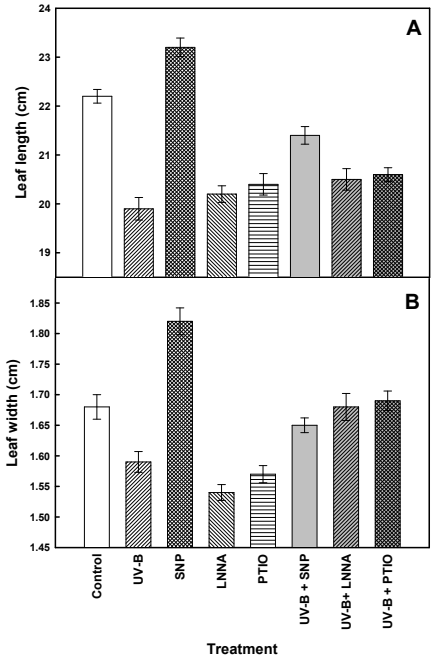

Fig. 1. Effect of NO on leaf length (A) and width (B) of maize seedlings in the presence or absence of UV-B radiation.

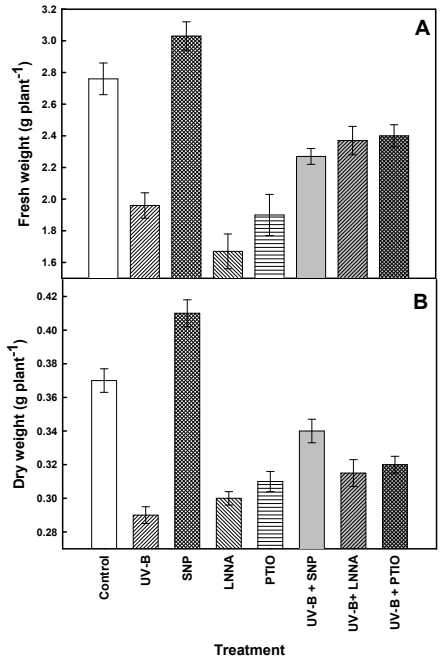

Fig. 2. Effect of NO on fresh weight (A) and dry weight (B) of maize leaves in the presence or absence of UV-B radiation.

Several mechanisms for tolerate to UV-B have been proposed, including leaf thickening, reduced leaf expansion, increased synthesis of epicuticular wax and enhanced synthesis of UV-B absorbing pigments. Exogenous NO was able to enhance leaf growth of maize seedlings, which was significantly inhibited by the decrease of leaf expansion and biomass after LNNA treatment. The application of SNP, NO donor, can induce rapid growth, exhibiting thicker and greener leaves than those of the non-treated controls. The increased size accounts for the significantly increased fresh mass of the leaves.

However, UV-B + LNNA treatment significantly relieved the growth arrest as compared to UV-B + SNP and UV-B treatment alone. It is probably that UV-B + SNP caused the leaves to release more 'over-produced NO' than UV-B + LNNA treatment, indicating that exogenous SNP application under UV-B was partially converted into 'over-produced NO' (An et al., 2005). Meanwhile, inhibition of UV-B induced leaf growth by LNNA is only partial, whereas recovery of the biomass is almost complete. The results strongly suggest that NO under UV-B stress might function as a signaling molecule of UV-B inhibiting growth, mainly by being responsible for the stress signaling transduction rather than acting as antistress molecule in the leaves during growth and development. It has been also proved that the effect of NO is in concentration-dependent manner. In rapidly growing foliage, NO treatment proved to have dual behavior. High concentration of SNP (2 mM) inhibited wheat and pea growth, while low micromolar NO concentrations produced an increase in the rate of leaf expansion (Tu et al., 2003).

Exogenous NO plays an important role in leaf cell enlargement without UV-B radiation, and NOS act as a catalyzer, which contributes to the generation of NO to induce leaf growth (Leshem and Haramaty, 1996). Nevertheless, the promotion of exogenous NO to leaf expansion was inconsistent with previous report that exogenous NO inhibited the mesocotyl elongation in maize seedlings at the same concentration and situation (Zhang et al., 2003a). Another light response stimulated by NO was the inhibition of hypocotyl and internode elongation (Beligni and Lamattina, 2000). This was a dose-dependent response and was arrested by addition of an NO scavenger, PTIO. This is possibly the result of diverse sensitivity of each organ to the exogenous NO. From the above findings, it has been implicated that NO as a stimulator molecule in plant photomorphogenesis.

3.2. Effect of NO on photosynthetic responses under UV−B irradiation

As shown in Table 1, treatment with SNP prevented completely or alleviated the decrease of chlorophyll and carotenoid in maize leaves induced by UV-B stress. However, there was no pronounced difference in photosynthetic pigments between UV-B treatment alone and UV-B + LNNA or UV-B + PTIO. From these findings, it is proved that the UV-B induced chlorophyll loss was reversed by NO donor and NO has protective effects on chloroplast membrane. Recent reports have demonstrated that NO protects chlorophyll levels in stressed leaves and NO participates in bring about partial greening in etioated seedlings by synergistic effect of NO and stress (Beligni and Lamattina, 2000).

Table 1. Effect of NO on chlorophyll a, chlorophyll b and carotenoid contents in maize leaves under UV-B radiation

Treatment	Chlorophyll content (mg g^{-1}FW)		Carotenoid content (mg g^{-1}FW)
	Chl a	Chl b	
Control	2.62 ± 0.05	0.37 ± 0.08	0.43 ± 0.07
UV-B	1.81 ± 0.03	0.20 ± 0.10	0.30 ± 0.05
UV-B + SNP	2.27 ± 0.10	0.32 ± 0.07	0.38 ± 0.08
UV-B + LNNA	1.90 ± 0.07	0.26 ± 0.09	0.33 ± 0.05
UV-B + PTIO	1.99 ± 0.06	0.27 ± 0.08	0.34 ± 0.09

The ratio of chlorophyll fluorescence (*Fv/Fm*) is a good indicator of photosynthetic efficiency in PS Ⅱ activity and a decline of this ratio suggests photoinhibitory damage. Without UV-B stress, PS Ⅱ activity of maize leaves treated with SNP was about the same as in the controls (Fig. 3). UV-B stress caused a drastic reduction in PS Ⅱ activity, indicating that PS Ⅱ is UV-B sensitive. None of the *Fv/Fm* ratio measured for the NO treated maize leaves were significantly decreased by the UV-B treatment. *Fv/Fm* steadily declined following UV-B treatment, however, *Fv/Fm* ratios of UV-B treated seedlings declined a lesser extent with application of NO, indicating that these seedlings underwent some photoinhibition, although that *Fv/Fm* ratios were slightly lower than those of the controls at all times after NO treatment. These results demonstrate that the reduction in PS Ⅱ activity was largely prevented by treatment with SNP and chloroplast membranes were protected to a greater extent from damage by NO treatment. It was shown that SNP pretreatment reduced salt and heat stress induced damage in rice seedlings and prevented the impairment of PS Ⅱ (Uchida et al., 2002).

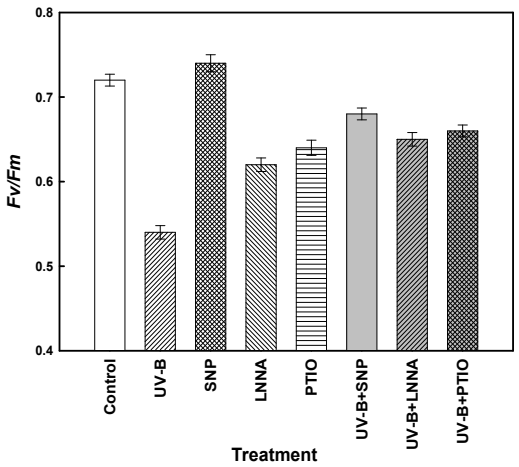

Fig. 3. Effect of NO on chlorophyll fluorescence in maize leaves in the presence or absence of UV-B radiation.

Tu et al. (2003) found that 0.1 mM SNP delayed the senescence of wheat leaves by inhibition of the degradation of chlorophyll and soluble proteins, especially Rubisco, while 0.5 mM would accelerate the process. NO can provoke both beneficial and harmful effects, which depends on the concentration and location of NO in plant cells. From these results, it is proved that NO has profound effect on the growth and photosynthetic metabolism.

3.3. Effect of NO on the antioxidant system under UV-B radiation

UV-B absorbing compounds such as flavonoids and anthocyanin are thought to protect plant from damaging effects of UV-B radiation because they absorb strongly in the UV-B range. The contents of both compounds were increased in a time-dependent manner under UV-B radiation, peaking at 36 h of exposure (Fig. 4). Accumulations were greater in the

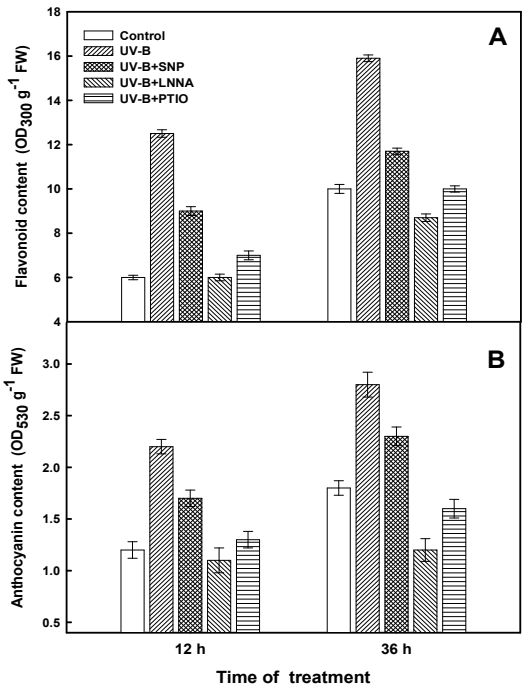

Fig. 4. Effect of NO on flavonoid (A) and anthocyanin (B) contents in maize leaves under UV-B radiation.

UV-B stress, being 159% and 156% over the control for flavonoids and anthocyanin, respectively, in the maize leaves. In the presence of radiation, SNP intensified the product of flavonoids and anthocynin by 17% and 13%, respectively, over the control after 36 h of radiation. However, their production was inhibited by LNNA with reduction of 13% in flavonoids and 33% in anthocyanin contents. Production of these compounds was enhanced by SNP but inhibited by LNNA, demonstrating that NO can enhance flavonoids and anthocyanin accumulation. Comparatively, there was no difference between PTIO treatment and the control.

Repair and acclimation responses are readily induced in response to UV exposure in many plant species (Frohmeyer and Staiger, 2003). Acclimation responses include the accumulation of UV-screening flavonoid and increased oxygen radical scavenging activity (Warren et al., 2002). Tolerance to UV-B irradiation has also been implicated ; treatment of *Arabidopsis thaliana* with PTIO, a scavenger of NO, prevented the up-regulation of the gene encoding chalcone synthase, a key enzyme in the flavonoid pathway that is believed to be important in conferring UV-B protection (Mackerness et al., 2001).

The oxidative stress is generated under UV-B exposure through the formation of ROS. Hydrogen peroxide(H_2O_2), as a main kind of ROS, accumulates under UV-B stress. Under UV-B radiation, H_2O_2 content in maize leaves increased to 172% of the control while it decreased to 113% in the presence of SNP (Fig. 5). Treatment with LNNA led to the reverse, stimulating more H_2O_2 production, i.e., to 165% of the control. PTIO blocked the effect of SNP on the H_2O_2 accumulation in leaves, while PTIO itself had no influence on H_2O_2 content under UV-B stress. PTIO in combination with SNP induced evident increase in H_2O_2 contents compared with that of UV-B treatment alone. These results indicate that NO might be involved in H_2O_2 production showing inhibition of H_2O_2 accumulation under UV-B exposure.

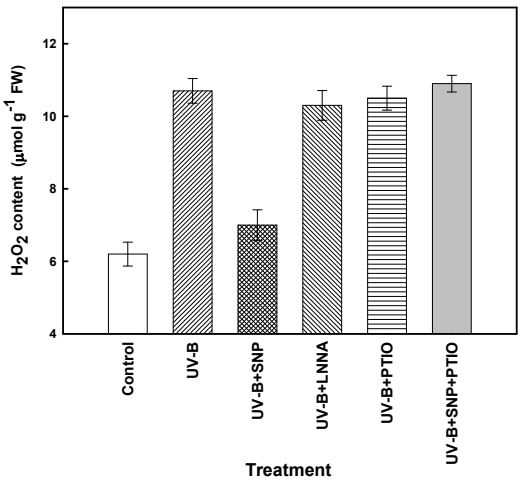

Fig. 5. Effect of NO on H_2O_2 content in the maize leaves under UV-B radiation.

The content of malondialdehyde (MDA) is an indicator of lipid peroxidation and oxidative damage to membrane due to free radicals. UV-B exposure dramatically potentiated MDA as a consequence of irradiation (Fig. 6). MDA content was 143% higher than the control after 36 h of irradiation. SNP treatment showed a distinct decrease in MDA contents by 34%, while LNNA treatment led to MDA value that was enhanced 148% higher over the control.

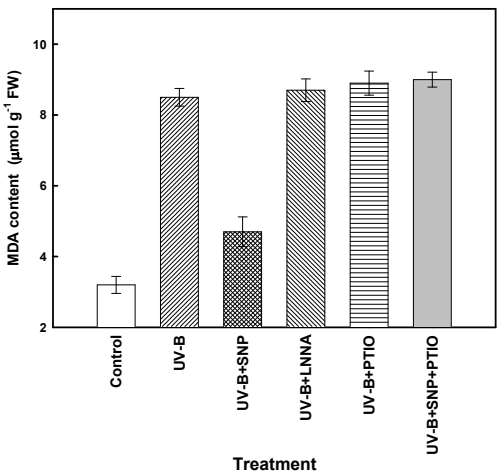

Fig. 6. Effect of NO on MDA content in the maize leaves under UV-B radiation.

The effect of SNP on UV-B induced stress and decrease in MDA contents could be recovered by PTIO. In the presence of PTIO or in combination with SNP, the MDA content was close to the level of UV-B treatment alone. These results demonstrate that NO severely inhibits the stimulation of MDA under UV-B exposure, suggesting that NO confers a certain degree of protection to maize leaves from deleterious effects of UV-B irradiation. The striking increase in lipid peroxidation and H_2O_2 seen in maize leaves treated with UV-B radiation may be a reflection of change in the specific activities of antioxidant enzymes and the contents of antioxidants.

The protective effect of NO may also be related to its ability to react with some ROS making NO act as a chain breaker and show its proposed antioxidant properties. Moreover, it has been reported that NO can react with lipid alcoxyl and peroxyl radicals, leading to the expectation that NO could stop the propagation of radical-mediated lipid oxidation in a direct fashion (Lamott et al., 2004), which is well agreement with our result in the decrease of MDA content. Our evidence adds the concept that H_2O_2 and NO can be important signal molecules for abiotic stress tolerance, although characterization of NO radicals as signal molecule in plants has been limited so far.

UV-B stress induce oxidative injury and alter the activities of antioxidant enzymes. CAT and APX are two key antioxidant enzymes that remove H_2O_2 in plants. Their activities showed a similar pattern with increasing initially then decreasing over time under UV-B exposure (Figs. 7 and 8). Maximum values for CAT in the maize leaves were 32% of the control after 12 h, while the values for APX peaked at 12 h measuring 33% of the control. SNP strongly induced CAT and APX activities, peaking at 46% and 59% higher than the control, respectively. In contrast, treatment with LNNA resulted in remarkable decrease in the activities of CAT and APX compared with those under UV-B stress alone. The promotive effect of NO donor on the antioxidant enzymes was reversed in the presence of NO scavenger, PTIO. The combination of UV-B with either PTIO or LNNA had lower CAT and APX activities than UV-B treatment alone. With PTIO in combination with SNP treatment, the activities of CAT and APX declined makedly compared with those under UV-B stress alone.

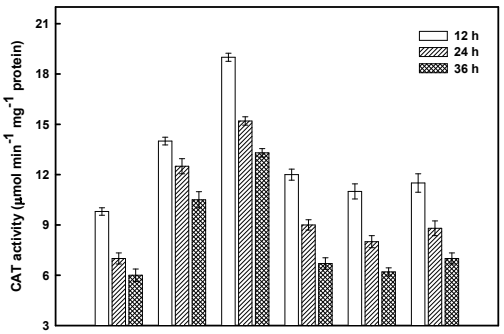

Fig. 7. Time course of changes in CAT activity in maize leaves under UV-B radiation.

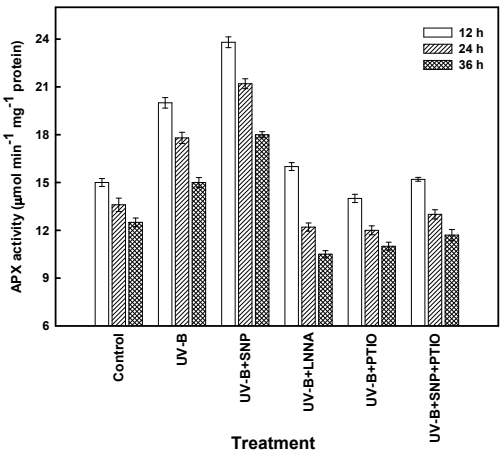

Fig. 8. Time course of changes in APX activity in maize leaves under UV-B radiation.

There are reports that NO counteracts oxidative stress under various types of environmental stress (Huang et al., 2002 ; Hsu and Kao, 2004). Thus, it is

of great interests to know whether the protective role of NO is also active in UV-B stress in maize leaves. NO donor was effective in reducing UV-B stress and UV-B-induced lipid peroxidation, suggesting that it is reasonable to infer NO resistance to active oxygen induced by irradiation.

Because lipid peroxidation and the increase in the specific activities of antioxidant enzymes are consequence of ROS overproduction and NO acts as an ROS scavenger, therefore, the reduction of the content of MDA and the specific activities of antioxidant enzymes could be a result of low levels of ROS including H_2O_2 in maize leaves treated with UV-B and NO (An et al., 2005). NO alleviates the harmfulness of ROS and reacts with other target molecules, and regulates the expression of stress responsive genes under various stress conditions (Qiao and Fan, 2008). We also observed that SNP counteracted UV-B induced increase in the specific activities of antioxidant enzymes (CAT and APX) and PTIO reversed the effect of SNP-increased specific activities. Clearly, the effect of NO donor SNP is attributable to NO released. The stimulating effect of NO donor were suppressed by the NO scavenger, indicating that NOS may play an important role in NO-mediated UV-B-induced antioxidant enzyme activity (Tossi et al., 2009). UV-B might first induce NO synthesis and the UV-B-induced NO then stimulates the antioxidant enzymes. The inducible effect of NO on the activity of antioxidant enzymes was observed in many other plant species under abiotic stresses such as drought, salinity, heat, herbicide and heavy metals (Song et al., 2006 ; Qiao and Fan, 2008). The present study also provide evidence that NO dependence on antioxidant activity serves as a signaling component in the induction of protective responses and is associated with UV-B tolerance in maize seedlings. However, it was reported that CAT and APX have been shown to be inhibited by NO in tobacco (Clark et al., 2000) and SNP treatment alone did not affect the specific activities of antioxidant enzymes in rice leaves (Hsu and Kao, 2004). Those lines of evidence implicate that the response to some stressors may be specific to certain species.

In *Arabidopsis* plants, endogenous NO was found to mediate UV-B induction of chalcone synthase (CHS) gene expression, which is important in conferring UV-B protection (Mackerness et al., 2001). Moreover, some antioxidant genes including CAT, APX were also found to be induced by NO in *Arabidopsis* suspension cells (Huang et al., 2002). In our work, application of exogenous NO remarkably elevated activities of CAT and APX and alleviated oxidative stress induced by UV-B. Thus, it is highly possible that the protective effect of NO may be mediated by increased level of expression of genes that encode active oxygen scavenging substances under UV-B stress. In conclusion, NO can induce the antioxidant system to prevent the accumulation of active oxygen and protect plants from oxidative damage under UV-B stress. Thus, it is suggested that NO is involved in the UV-B signaling pathway and may serve as a second messenger to mediate the adaptive response to UV-B stress.

4. Conclusions

In this work, we demostrated that the application of exogenous NO completely eliminated oxidative stress in maize leaves imposed by UV-B stress. Application of SNP as NO donor upon UV-B exposure prevented the reduction of leaf growth such as leaf length, width and weight and reduced the accumulation of flavonoids and anthocyanin, which are UV-absorbing compounds, induced by UV-B stress. The UV-B induced chlorophyll contents reduction was prevented by NO donor SNP. The *Fv/Fm* ratio, a good indicator of photosynthetic efficiency, was significantly declined by UV-B

exposure, however, the reduction in PS Ⅱ activity was largely prevented by NO donor. The promotion of H_2O_2 production and lipid peroxidation caused by UV-B in maize leaves could be counteracted by NO donor, showing declined levels of H_2O_2 and MDA. SNP also counteracted UV-B induced increase in the specific activities of antioxidant enzymes CAT and APX and eliminated H_2O_2 overproduction, thus conferring UV-B resistance. However, the protective effect of NO donor on the antioxidant system under UV-B stress was suppressed in the presence of NO scavenger, PTIO and the NOS inhibitor, LNNA. These results demonstrate that NO treatment mediates tolerance to elevated levels of UV-B radiation and precludes oxidative damage in maize leaves. Therefore, NO might function as a second messenger and an antioxidant of UV-B radiation during developmental growth of the leaves.

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