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

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(Manuscript received 1 September, 2010; revised 1 December, 2010; accepted 1 December, 2010)

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$_2$O$_2$) in maize leaves, while NO donor prevented UV-B induced increase in the contents of malondialdehyde (MDA) and H$_2$O$_2$. These results demonstrate that NO serves as antioxidant agent able to scavenge H$_2$O$_2$ 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$\omega$-nitro-L-arginine (LNNA), an inhibitor of nitric oxide synthase (NOS), significantly increased H$_2$O$_2$ 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.
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 \( \text{H}_2\text{O}_2 \). CAT is one of the main \( \text{H}_2\text{O}_2 \)-scavenging enzymes that dismutates \( \text{H}_2\text{O}_2 \) into water and \( \text{O}_2 \), and APX is a specific peroxidase that catalyzes the elimination of toxic product of \( \text{H}_2\text{O}_2 \) at the expense of oxidizing ascorbate to monodehydroascorbate (Yannarelli et al., 2005). Peroxidases are enzymes that catalyze the \( \text{H}_2\text{O}_2 \)-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 dormancy, 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