Scientia Horticulturae 162 (2013) 1–10

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Nitric oxide mediates abscisic acid induced light-tolerance in leaves of tall fescue under high-light stress

Yuefei Xu [a], Juanjuan Fu [a], Xitong Chu [a], Yongfang Sun [a], He Zhou [b], Tianming Hu [a,*]

[a] Department of Grassland Science, College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi Province 712100, China
[b] College of Animal Science and Technology, China Agricultural University, Beijing 100193, China

ARTICLE INFO

Article history:
Received 25 March 2013
Received in revised form 1 July 2013
Accepted 30 July 2013

Keywords:
Abscisic acid
Antioxidant enzymes
High-light
Nitric oxide
Tall fescue

ABSTRACT

Abscisic acid (ABA) and nitric oxide (NO) are both extremely important signaling molecules involved in many physiological processes. In this study, the effect of ABA and NO on oxidative damage caused by high levels of light was investigated in leaves of two varieties of tall fescue (Arid3 and Houndog5). Pretreatment of these leaves with exogenous ABA, prior to exposure to high-light (HL) stress, resulted in reduced light-induced ion leakage and reduced content of malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and superoxide radical. The activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) increased in both varieties in the presence of ABA under HL stress. These responses could be reversed by pretreatment with fluridone (the ABA biosynthesis inhibitor). A pronounced increase in nitric oxide synthase (NOS)-like activity and NO release by exogenous ABA treatment was found in light-tolerant Arid3 plants after exposure to high light, while only a small increase was observed in more sensitive Houndog5. Furthermore, inhibition of NO accumulation by 2-(4-carboxy-2-phenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (PTIO) or N^{ω}-nitro-L-arginine (LNNA) blocked the protective effect of exogenous ABA, whereas inhibition of ABA by fluridone had no influence on the protective effect of NO. These results suggested that NO was involved in the ABA-induced activities of antioxidant enzymes, further protecting against injuries caused by high intensity light.

1. Introduction

Growth and development of plant is strongly influenced by the quality and amount of light they receive, but when the amount of absorbed light exceeds the amount required for photosynthesis, the excess light can be harmful because it causes the accumulation of reactive oxygen species (Asada, 2006), including singlet oxygen ($O_2{}^1$), hydrogen peroxide (H_2O_2), superoxide radical ($O_2{}^{\bullet -}$) and hydroxyl radical ($HO^{\bullet}$), which leads to lipid peroxidation, damages to the cell membrane, consequently inhibits the photosynthesis and respiration and plant growth. To avoid ROS-induced cellular injury, plants utilize various antioxidative enzymes as well as low molecular weight antioxidants (Mittler, 2002; Apel and Hirt, 2004). Recent studies indicate that high-light (HL) stress induced increased nitric oxide synthase (NOS, 1.14.13.39) activity leading to elevated nitric oxide (NO). This NO acts as a signaling molecule triggering enhanced activities of antioxidant enzymes, further protecting against injuries caused by high intensity light (Xu et al., 2010).

NO is an important inter- and intracellular signaling molecule involved in many plant physiological processes (Lamattina et al., 2003), and in regulating growth and developmental processes, such as seed germination, de-etiolation, cell senescence and programmed cell death (Beligni and Lamattina, 2000; Neill et al., 2003). Moreover, NO was found to mediate plant responses to abiotic stress caused by drought, salinity, UV-B radiation or heavy metals (Laspina et al., 2005; Zhao et al., 2008; Vital et al., 2008; Zhang et al., 2009; Corpas et al., 2011). Previous studies indicate that NO activates antioxidant defenses during heat and drought stresses, and that in these cases there may be a similar link between NO and abscisic acid (Song et al., 2008; Lu et al., 2009). Abscisic acid (ABA) is an important phytohormone that regulates plant adaptive responses to various environmental stresses and diverse physiological and development processes. The involvement of ABA in plant abiotic stress response has been recognized. Signal molecules, such as NO, H_2O_2 and Ca^{2+}, are involved in ABA-induced enhanced activities of antioxidant enzymes (Jiang and Zhang, 2003; Bright et al., 2006; Garcia-Mata and Lamattina, 2007; Tossi et al., 2012). ABA-induced SOD and CAT activities were mediated sequentially

Abbreviations: ABA, abscisic acid; APX, ascorbate peroxidase; CAT, catalase; GR, glutathione reductase; HL, high-light; LNNA, N^{ω}-nitro-L-arginine; NO, nitric oxide; NOS, nitric oxide synthase; PPFD, photosynthetic photon flux density; PTIO, 2-(4-carboxy-2-phenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide; ROS, reactive oxygen species; SNP, sodium nitroprusside; SOD, superoxide dismutase.

* Corresponding author. Tel.: +86 29 87091953; fax: +86 29 87092355.
E-mail addresses: hutianming@126.com, hutianming58@126.com (T. Hu).

0304-4238/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.scienta.2013.07.042

by H_2O_2 and NO under drought tolerance (Lu et al., 2009), or by NO under chilling temperature (Zhou et al., 2005). In addition, NO acts as an intermediate molecule mediating ABA-induced thermotolerance under heat stress (Song et al., 2008). Our earlier work has revealed that NO acts as a signal inducement of light-tolerant tall fescue by activating active oxygen scavenging enzymes (Xu et al., 2010). However, the induction mechanism of the antioxidant enzymes induced by NO and ABA in leaves of light-tolerant tall fescue has not been elucidated.

Tall fescue (*Festuca arundinacea*) is a widely used cold-season turf grass. It is often grown under fluctuating photosynthetic photon flux densities due to the impact of vegetation canopies, buildings or different weather patterns. In a preliminary experiment, two varieties of tall fescue, Arid3 and Houndog5, were found to exhibit distinct photo-acclimation behavior. Houndog5 was photobleached under HL stress while Arid3 was not affected, suggesting that Houndog5 is more susceptible to HL stress than Arid3. High-light stress leads to an enhanced generation of reactive oxygen species. Increased activities of antioxidant enzymes were reported under HL stress that mitigated oxidative damage (Burritt and Mackenzie, 2003). In our previous studies also demonstrated that HL stress induced increased NOS-like activity leading to elevated NO. This NO might act as a signaling molecule triggering enhanced activities of antioxidant enzymes, further protecting against injuries caused by high intensity light. The objective of this study was to elucidate (a) the role of ABA (applied exogenous ABA or inhibited endogenous ABA) in alleviating light-induced oxidative damage in leaves of two varieties of tall fescue (Arid3 and Houndog5), and (b) how ABA and NO interact and interrelate.

2. Materials and methods

2.1. Plant materials and treatments

Seeds of tall fescue [*F. arundinacea* (Schreb.) cvs. Arid3 and Houndog5] were obtained from Beijing Clover Seed & Turf CO., Ltd., China. Seeds were surface sterilized in 0.1% (w/v) sodium hypochlorite, rinsed several times in distilled water, and germinated on moistened filter paper at room temperature for 7 d. Seedlings were selected and placed into 5 L black plastic containers containing 4 L of nutrient solution. Each plastic container contained six plants. Seedlings cultured hydroponically in a continuously aerated nutrient solution containing 4 mM $Ca(NO_3)_2$, 4 mM KNO_3, 2 mM $MgSO_4$, 1 mM $NH_4H_2PO_4$, 46 μM H_3BO_3, 10 μM $MnSO_4$, 1.0 μM $ZnSO_4$, 0.95 μM $CuSO_4$, 0.05 μM H_2MoO_4 and 50 μM Fe-EDTA. The nutrient solution pH was adjusted close to 6.5 by adding H_2SO_4 or KOH. Nutrient solution was renewed once a week. The plants were grown in a plant incubator (Percival E-36L, Percival Scientific. Inc., USA) at a day/night temperature 25/20 °C, a relative humidity of 70%, a day/night regime of 14/10 h and a photosynthetic photon flux density (PPFD) at the height of the plants of 100 μmol m^{-2} s^{-1}. Lighting system is lit by (16) 17 W cool white fluorescent lamps and (2) 40 W incandescent lamps properly spaced for uniform light intensity.

Stress treatments were carried out after 21 d of pre-culture. The plants were treated under high light (500 μmol m^{-2} s^{-1} PPFD), at the same time, control plants were treated under low light (100 μmol m^{-2} s^{-1} PPFD). Sodium nitroprusside (SNP; Sigma, USA) was used as NO donor. The potassium salt of 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (PTIO; Sigma, USA) was used as NO scavenger. N^{ω}-nitro-L-arginine (LNNA; Sigma, USA) was used as NOS inhibitor. Fluridone was used as the ABA biosynthesis inhibitor (Hansen and Grossmann, 2000). To investigate the role of ABA in the NO induced light-tolerance. ABA at concentrations from 0 to 25 μM, 10 μM fluridone, 100 μM SNP, 150 μM LNNA or 200 μM PTIO were applied to tall fescue seedlings through the roots incubated in 4 L of nutrient solution (regenerated once a day) with HL treatments. The 21-d-old seedlings were incubated in the solutions for 7 d (Jiang et al., 2004, 2005) at a day/night temperature 25/20 °C, a relative humidity of 70%, a day/night regime of 14/10 h. After 7 d of treatments, plants were harvested and frozen in liquid N_2, and then stored at −80 °C for determinations of other physiological parameters.

2.2. Ion leakage assay

The fresh leaves (0.5 g) were harvested and cut into 20 mm pieces. They were washed in deionized water and placed in Petri dishes with 5 mL of deionized water at 25 °C for 2 h. After the incubation, the conductivity was measured ($C1$). Then, the samples were boiled for 20 min and conductivity was read again ($C2$). Relative ion leakage was expressed as a percentage of the total conductivity after boiling (relative ion leakage% = $C1/C2 \times 100$) (Song et al., 2008).

2.3. Determination of H_2O_2 and superoxide radical production

The content of H_2O_2 was measured according to Veljovic-Jovanovic et al. (2002). Leaves (0.5 g) were ground in liquid nitrogen and the powder was extracted in 2 mL 1 M $HClO_4$ in the presence of 5% polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 12,000 × *g* for 10 min, and the supernatant was neutralized with 5 M K_2CO_3 (pH 5.6) in the presence of 0.1 mL of 0.3 M phosphate buffer (pH 5.6). The solution was centrifuged at 12,000 × *g* for 1 min, and the sample was incubated for 10 min with 1 U ascorbate oxidase to oxidize ascorbate prior to assay. The reaction mixture contained 0.1 M phosphate buffer (pH 6.5), 3.3 mM 3-(dimethylamino) benzoic acid (DMAB), 0.07 mM 3-methyl-2-benzothiazoline hydrazone (MBTH), 0.3 U guaiacol peroxidase (POX) and 200 μL supernatant. Changes in absorbance at 590 nm were monitored at 25 °C.

The production rate of $O_2^{\bullet -}$ was determined by the modified method according to Elstner and Heupel (1976). Leaves (1.0 g) were homogenized in 3 mL of 50 mM potassium phosphate buffer (pH 7.8) and centrifuged at 12,000 × *g* for 20 min. The incubation mixture contained 1 mL of supernatant, 1 mL of 50 mM potassium phosphate buffer (pH 7.8) and 1 mL of 1 mM hydroxylaminoniumchloride and the mixture was incubated in 25 °C for 20 min. The mixture was subsequently incubated with 2 mL of 17 mM sulphanilic acid and 2 mL of 7 mM α-naphthyl amine at 25 °C for 20 min. The final solution was mixed with an equal volume of ethyl ether, and the absorbance of the pink phase was read at 530 nm. The production rate of $O_2^{\bullet -}$ was calculated based on a standard curve.

2.4. Analysis of lipid peroxidation

Membrane lipid peroxidation was estimated by the level of malondialdehyde (MDA) production with a slight modification of the thiobarbituric acid method of Buege and Aust (1978). Leaves (0.5 g) were homogenized with a mortar and pestle in 10% trichloroacetic acid, and then the homogenate was centrifuged at 4000 × *g* for 30 min. A 2 mL aliquot of supernatant was mixed with 2 mL of 10% trichloroacetic acid containing 0.5% thiobarbituric acid. The mixture was heated at 100 °C for 30 min. The absorbance of the supernatant was measured at 532 nm with a reading at 600 nm subtracted from it to account for non-specific turbidity.

2.5. Antioxidative enzyme activity assay

Leaves (1.0 g) were homogenized with a mortar and pestle at 4 °C in 5 mL 50 mM phosphate buffer (pH 7.8) containing