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Hydrogen sulfide regulates photosynthesis of tall fescue under low-light stress

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Abstract

Hydrogen sulfide (H_2S) is regarded as gaseous signaling molecule in plant response to various biotic and abiotic stresses. In this study, we investigated potential role of H_2S in modulating photosynthesis in low light (LL) stress-induced tall fescue (*Festuca arundinacea* Schreb.) seedlings. Results demonstrated that LL significantly reduced the plant height, leaf width, tiller number, dry mass, turf quality, chlorophyll content, net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, transpiration rate, maximal quantum yield of PSII photochemistry, photochemical efficiency of PSII, photochemical quenching, electron transport rate, and enzymatic activity of ribulose-1,5-bisphosphate carboxylase. Then, these parameters were found to be considerably alleviated by H_2S donor application. Moreover, exogenous application of NaHS decreased the concentration of malondialdehyde, increased activities of peroxidase, superoxide dismutase, ascorbate peroxidase, total soluble sugar, soluble protein, and endogenous concentration of H_2S. In addition, these responses could be reversed by treatment with hypotaurine (H_2S scavenger) and aminooxy acetic acid (H_2S biosynthesis inhibitor). These results suggested that H_2S was possibly involved in the regulation of photosynthesis to strengthen LL tolerance by maintaining a high level of photochemical efficiency and improving antioxidant enzyme activities in tall fescue.

Additional key words: ascorbate peroxidase; carotenoids; gas exchange; lipid peroxidation; superoxide dismutase.

Introduction

Light is an essential resource for photoautotrophic higher plants including turfgrass for survival, growth, site distribution, and development (Huang *et al.* 2011). In a natural environment, plants are often subjected to erratic variations with time due to light intensities and fluctuations regulated by sun, angle of clouds, and leaf movement (Hirth *et al*. 2013). Currently, it has been accepted that shade is one of the most common environmental limiting factors for aesthetic value and service life of turf. It is evaluated that as much as 25% of turfgrass in America (Emmons 2008) and approximately 50% of that in China (Yang *et al*. 2014) is subjected to varying degrees of shade.

During developmental changes at whole-plant or leaf level, LL stress was found to have an impact on morphological parameters, physiological characteristics, and photosynthetic efficiency of plants (Li *et al*. 2017). Previous studies have reported morphological changes of plants in response to LL stress, such as thinner leaves (Fu *et al.* 2014) and lower biomass (Gustafsson and Boström 2013). Another study reported that LL could decrease the biosynthesis of photosynthetic pigments (Zhang *et al.* 2018) and reduce the chlorophyll (Chl) fluorescence parameters (Chen *et al*. 2016). Under LL stress conditions, enzymes components of Calvin-Benson cycle, particularly Rubisco, exhibited an apparent tendency of diminishing activities leading to lower CO_2 assimilation rate, eventually resulting in prominent depression of plant growth (Yang *et al.* 2018). Furthermore, LL irradiance contributed to the overproduction of ROS by increasing the electron flow to O_2 during photosynthetic and respiratory processes, which resulted in membrane lipid peroxidation and protein denaturation (Li *et al.* 2017). To alleviate the negative effect of excessive ROS, antioxidant enzyme activities were enhanced in plants to deal with different oxidative stresses (Candan and Tarhan 2003).

H_2S is an endogenous messenger molecule, which

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Abbreviations: AOA – aminooxy acetic acid; APX – ascorbate peroxidase; Car – carotenoids; Chl – chlorophyll; C_i – intercellular CO_2 concentration; E – transpiration rate; EDTA – ethylene diamine tetraacetic acid; ETR – electron transport rate; F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_m' – maximal fluorescence yield of the light-adapted state; F_s – steady-state fluorescence yield; F_v/F_m – maximal quantum yield of PSII photochemistry; g_s – stomatal conductance; H_2S – hydrogen sulfide; HT – hypotaurine; LL – low light; MDA – malondialdehyde; P_N – net photosynthetic rate; POD – peroxidase; q_P – photochemical quenching coefficient; ROS – reactive oxygen species; RuBP – ribulose-1,5-bisphosphate; SOD – superoxide dismutase; Φ_{PSII} – effective quantum yield of PSII photochemistry.
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modulates various important physiological processes in plants (Zhang *et al*. 2008). Numerous studies have reported the effects of H_2S in plants. H_2S not only promoted root organogenesis (Zhang *et al*. 2009) and seed germination (Li *et al.* 2012) but also delayed senescence (Hu *et al*. 2015) in plants. Besides its function on growth regulation, H_2S also acts as a protective agent against multiple abiotic stresses induced by drought (Jin *et al*. 2013), heat (Li *et al*. 2012), salt (Wang *et al*. 2012), and heavy metals (Ali *et al*. 2014). In addition, H_2S could improve the photosynthesis in *Spinacia oleracea* seedlings through increased chloroplast biogenesis, photosynthetic enzyme expression, and thiol redox modification (Chen *et al*. 2011). In another study, H_2S was found to improve the photosynthesis in *Dendrobium officinale* under high light through the stimulation of antioxidant enzyme activities (Fan *et al.* 2014). However, the underlying mechanism of H_2S regulation of photosynthesis in tall fescue leaves induced by LL stress has not yet been illustrated.

Tall fescue (*Festuca arundinacea* Schreb.), a widespread perennial cold season forage or turfgrass, is known to survive various environmental stresses. It is usually cultivated under fluctuating light density regimes due to the detrimental effects of vegetation canopies, bushes, buildings or different weather patterns (Xu *et al*. 2013). In our previous study, it was reported that tall fescue suffered adverse effects on antioxidant defense system at different growth stages under LL stress, which were alleviated by application of sodium nitroprusside (nitric oxide donor) (Fu *et al.* 2014). However, little information is available with regards to H_2S mediated plant photosynthesis under LL stress, though previous study has shown that LL stress could cause diminution of photosynthetic characteristics (Zhong *et al.* 2014). In the present study, we hypothesized that H_2S is involved in photosynthesis of LL stress-induced tall fescue by maintaining a high photosynthetic rate and regulating antioxidant system. Therefore, the purpose of this study was to explore the role of H_2S in modulating carboxylation efficiency, Chl content, and PSII activity for better tolerance of tall fescue plants in LL stress conditions. This study could help us further understand the role of H_2S in improving LL tolerance of tall fescue.

Materials and methods

Plant materials and growth conditions: Tall fescue (*Festuca arundinacea* Schreb. cv. Arid3) seeds were obtained from *Beijing Clover Seed & Turf Co*., China. The seeds were surface sterilized using 0.1% (w/v) sodium hypochlorite, rinsed several times with distilled water, and subjected to germination on moistened filter paper at room temperature for 7 d. After 7-d period, three healthy and morphologically uniform sized seedlings were selected and transferred into black plastic pots (9-cm diameter × 15-cm depth) containing sterile quartz sand autoclaved at 180°C for 60 min. Seedlings were watered every day and fertilized once every 3 d with Hoagland's 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 approximately to 6.5 using H_2SO_4 or KOH. The plants were grown in a plant incubator at a day/night temperature of 25/20°C, relative humidity of 60/50%, 16-h photoperiod, and PPFD of 200 μmol m^{-2} s^{-1} placed at plant height. Irradiance system was provided by *20000K-20B* fluorescent lamps (*Nanjing Huaqiang Electronics Co.*, *Ltd.*, China) and appropriately spaced for uniform light intensity.

Treatment and experimental design: Stress treatments were carried out after 28 d of preculture. PPFD used for control and low light stress groups were at 200 and 40 μmol m^{-2} s^{-1}, respectively. To apply exogenous H_2S to tall fescue seedlings, NaHS (*Sigma*) was used as H_2S donor (Fan *et al.* 2014). For further treatment, seedlings were divided into two groups. In the first group, seedlings were supplied with different concentrations of NaHS (100, 300, 500; 1,000; and 1,500 μM) for 7 d to determine optimal concentration. In the second group, 28-d-old plants were separated into five groups exposed to different treatments for 7 d:

Treatment	
distilled water	control, CK
distilled water under low light	LL
500 μM NaHS under low light	LL+H_2S
300 μM HT (H_2S scavenger) under low light	LL+HT
300 μM AOA (H_2S biosynthesis inhibitor) under low light	LL+AOA

After the treatments, expanded leaves of plants were randomly sampled and immediately frozen in liquid nitrogen, and stored at –80°C for physiological and biochemical analysis. Fresh leaves were used for gas exchange and Chl fluorescence experiments.

Growth characteristics: Plant heights were measured with a ruler, while a vernier caliper was used to measure leaf widths, and total tiller number per seedling was individually counted. Three seedlings from each treatment were taken after removing the quartz sand and transferred to an oven at 80°C for 72 h. Dry mass was determined from each treatment until a constant mass was obtained. Turf quality was used to evaluate overall health and vigor of turfgrass based on color, density, and uniformity. It was given a score in 1–9 scale, where 1 denotes the worst and 9 indicates the best in all evaluation criteria (Zhang *et al*. 2017).

Photosynthetic pigment content: Chl and Car production in fresh leaves was determined using the method of Lichtenthaler (1987) with slight modifications. Leaves weighing approximately 0.1 g were used to extract Chl and Car by soaking in a blank solvent containing 10 mL of 80% acetone and 5 mL of 95% ethyl alcohol for 24 h in dark. Absorbance of the extracts was measured at 440, 663, and 645 nm spectrophotometrically (*LV-1800*, *MAPADA Co., Ltd*., Shanghai, China). Chl *a*, Chl *b*, Chl (*a*+*b*), and Car concentrations were calculated using the equations and

extinction coefficients provided by Lichtenthaler (1987). The Chl *a*/*b* ratio was computed by dividing the concentration of Chl *a* by that of Chl *b*.

Gas-exchange parameters: Net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and transpiration rate (E) were measured using a portable photosynthesis system (*Li-6400*, *LICOR*, *Inc.*, Lincoln, NE) from 8:30 to 11:30 h. To achieve full photosynthetic induction, all tall fescue seedlings were illuminated with 800 μmol m^{-2} s^{-1} of saturated PPFD for 30 min prior to measurement (Mittler *et al*. 2001). P_N, g_s, C_i, and E were monitored on the third fully expanded leaves from the top. During the process, ambient air temperature, relative humidity, and CO_2 concentration were maintained at 25°C, 60%, and 400 μmol mol^{-1}, respectively.

Chl fluorescence on the same leaf was measured using a portable pulse-amplitude-modulated fluorometer (*PAM 2500*, *Walz*, Effeltrich, Germany) with *PamWin* software as described by Oliveira and Peñuelas (2004). The third completely expanded leaves from the top were kept in dark using a leaf clip for at least 20 min of adaptation before measurement. F_0 was monitored using a weak modulated light irradiation which was low enough to not induce fluorescence at room temperature, while F_m was determined by 0.8-s saturation pulse flash at PPFD of 4,000 μmol m^{-2} s^{-1}. The leaf was illuminated with constant actinic light to determine F_s, after which a second saturation pulse was applied to determine F_m' in light-adapted state. Then actinic light was removed, and far-red light was turned on for several seconds to record the minimum fluorescence of light-adapted leaves (F_0'). Afterwards, $F_v/F_m = [(F_m - F_0)/F_m]$, $\Phi_{PSII} = [(F_m' - F_s)/F_m']$, ETR = ($\Phi_{PSII}$ × PPFD × 0.5), and $q_P = [\Delta F'/(F_m' - F_0')]$ were computed, respectively. For ETR and q_P calculation, 0.5 is a partition coefficient of energy between PSI and PSII. Leaf Chl fluorescence was determined simultaneously with leaf gas exchange on the same area of leaf at the day of gas-exchange measurement (Lu *et al*. 2003).

Total soluble sugar production: Total soluble sugar concentration was measured according to the method described by Xu *et al*. (2013). Fresh leaves (0.5 g) were grinded with a pestle in an icy mortar, and mixed with 5 mL of distilled water, immediately followed by incubating in boiling water for 30 min. After centrifugation at 4,000 × *g* for 5 min and a removal of supernatant, pellet was resuspended and reextracted twice. Three supernatants were transferred to 25-mL volumetric flask and distilled water was added to make up the volume. Afterwards, 1 mL of sample extract was added to 3 mL of anthrone reagent and mixed well. After heating the sample at 100°C for 10 min and cooling down, soluble sugar content was measured at the absorbance of 620 nm (*LV-1800*, *MAPADA Co., Ltd*., Shanghai, China).

Soluble protein content: Total soluble protein contents in leaves of stressed or non-stressed seedlings were determined using modified method of Bradford (1976). Leaves (0.3 g) were crushed in phosphate buffer (pH 7.0). The homogenate was centrifuged at 12,000 × *g* for 10 min. All steps were performed at 4°C. One mL of sample extract was added to 5 mL of 0.01% (w/v) *Coomassie Brilliant Blue G-250* containing 4.7% (w/v) ethanol and 8.5% (w/v) phosphoric acid, and mixed well. After incubation at room temperature for 2 min, absorbance was recorded at 595 nm (*LV-1800*, *MAPADA Co., Ltd*., Shanghai, China). Concentration of protein was calculated by standard curve using different concentrations of bovine serum albumin (BSA) and expressed as mg g^{-1}(FM).

Analysis of Rubisco enzyme activity*:* Rubisco activity was determined according to the method of Lilley and Walker (1974) with slight modifications. Samples (0.3 g) were crushed with a pestle in an ice-cold mortar (4°C) using a small amount of quartz sand and 1.5 mL of extraction buffer containing 40 mM Tris-HCl (pH 7.6), 10 mM $MgCl_2$, 0.25 mM EDTA, and 5.0 mM glutathione. Afterwards, the homogenate was centrifuged at 12,000 × *g* for 20 min at 4°C and supernatant was collected for the measurement of Rubisco activity. Sample extract (50 μL) was added to 900 μL of reaction volume consisting of 50 mM Tris-HCl (pH 7.8), 12 mM $MgCl_2$, 0.4 mM EDTA, 5 mM ATP, 5 mM NADH, 5 units of glyceraldehyde-3-phosphate dehydrogenase, 5 units of 3-phosphoglyceric phosphokinase, 17.5 units of creatine phosphokinase, and the reaction was initiated by adding 50 μL of 10 mM RuBP. Absorbance was measured at 340 nm (*LV-1800*, *MAPADA Co., Ltd*., Shanghai, China) every 10 s for 3 min corresponding to the oxidation of NADH. Extraction buffer was used to replace RuBP as a blank. The decrease of absorbance with the blank and the differences were used to determine the enzyme activity. according to the formulas: Rubisco activity [nmol(NADH) min^{-1} mg^{-1}(protein)] = $\Delta A/\Delta t \times N \times 109/(\varepsilon \times d \times C_{pr})$, where $\Delta A/\Delta t$ represents the change of optical density at 340 nm per min,N denotes the ratio of the extraction volume to the reaction volume, ε means the molar extinction coefficient of NADH at 340 nm, d is the optical distance of the cuvette, and C_{pr} represents the concentration of soluble protein in extraction solution.

Determination of lipid peroxidation: Membrane lipid peroxidation was determined by malondialdehyde (MDA) content using trichloroacetic acid method of Buege and Aust (1978). Fresh leaves (0.5 g) were grinded with pestle in a prechilled mortar containing a quartz sand and homogenized in 10 mL of 10% (w/v) trichloroacetic acid, immediately followed by centrifugation at 12,000 × *g* for 10 min and supernatant was collected as sample extract. Two mL of supernatant and 2 mL of mixture containing 10% trichloroacetic acid and 0.5% thiobarbituric acid were mixed well, and the solution was incubated in a boiling water bath for 15 min. Samples were refrigerated immediately and centrifuged again at 12,000 × *g* at 4°C for 10 min. The absorption of supernatant was recorded at 450, 532, and 600 nm using a spectrophotometer (*LV-1800*, *MAPADA Co., Ltd*., Shanghai, China).

Quantification of antioxidant enzymatic activity: Approximately 1.0 g of fresh leaves was finely homogenized with a mortar and pestle on ice in 5 mL of a solution containing 50 mM phosphate buffer (pH 7.8), 1 mM EDTA and 2% (w/v) polyvinylpolypyrrolidone. The homogenate was centrifuged at 12,000 × *g* at 4°C for 20 min and supernatant was collected for measuring enzyme activities (Zhou *et al*. 2005). The spectrophotometer *LV-1800* (*MAPADA Co., Ltd*., Shanghai, China) was used for following measurements.

Peroxidase (POD, EC 1.11.1.7) activity was analyzed as described by Upadhyaya *et al.* (1985) based on its capability to convert guaiacol to tetraguaiacol. Each POD reaction system was composed of 2.5 mL of 50 mM potassium phosphate buffer (pH 6.1), 1.0 mL of 1% (v/v) H_2O_2, and 1.0 mL of 1% (v/v) guaiacol followed by the addition of 10 mL of enzyme extract to initiate the reaction. Changes of absorption were determined at 420 nm within 3 min after the beginning of the reaction in 1-min interval. One unit of POD activity was defined as 1 μmol(H_2O_2 decomposed) mL^{-1} min^{-1}.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was measured following the photochemical nitroblue tetrazolium (NBT) method as described by Beauchamp and Fridovich (1971). Three mL of reaction mixture consisted of 13 μM methionine, 63 μM NBT, 1.3 μM riboflavin, 50 mM phosphate buffer (pH 7.8), and enzyme extract. One unit of SOD was defined as the amount of enzyme per fresh mass sample inhibiting the photochemical reduction of NBT by 50%. Absorbance at 560 nm of 1-cm cuvette was monitored. One unit of SOD was defined as the amount of enzyme per protein inhibiting the photochemical reduction of NBT by 50%.

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was measured by monitoring the rate of ascorbic acid oxidation according to the method of Nakano and Asada (1981). Three mL of APX assay mixture consisted of 100 mM phosphate (pH 7.0), 0.1 mM EDTA-Na_2, 0.3 mM ascorbic acid, 0.06 mM H_2O_2, and 100 μL of enzyme extract. The oxidation of ascorbate was observed by the decrease in absorption at 290 nm after adding enzyme extract. One unit of APX forms 1 μM of ascorbate acid oxidized per min under assay conditions.

Endogenous H_2S concentration was determined by the conversion of methylene blue from dimethyl-*p*-phenylenediamine in H_2SO_4 according to the method described by Chen *et al*. (2011) with some slight modifications. Leaves (0.5 g) of experimental plants were grinded on ice and mixed in 5 mL of 50 mM phosphate buffer solution (pH 6.8) including 0.1 M EDTA and 0.2 M ascorbic acid. Homogenate was mixed in a test tube containing 0.5 mL of 1 M HCl to emit H_2S at room temperature, and the released H_2S was absorbed in 1% (w/v) zinc acetate (0.5 mL) trap at the bottom of the test tube. After 30 min of reaction, 0.3 mL of 5 mM dimethyl-*p*-phenylenediamine dissolved in 3.5 mM H_2SO_4 was added to the trap, immediately followed by injecting 0.3 ml of 50 mM ferric ammonium sulphate in 100 mM H_2SO_4 into the trap. After incubating the mixture for 15 min at room temperature, the mass of H_2S was measured spectrophotometrically at 667 nm (*LV-1800, MAPADA Co., Ltd*., Shanghai, China). Blanks were prepared by same procedures with unused zinc acetate solution.

Statistical analysis: The experiments were designed in a completely randomized design. Three replicates were performed for each experiment. All values were expressed as means ± SD. Statistical analyses were performed by *SPSS 22* software (*IBM Corp.*, Chicago, IL, USA). Data were evaluated by *Shapiro-Wilk*'s test to verify normality and *Levene*'s test to verify homoscedasticity. Then, they were analyzed by one-way analysis of variance (*ANOVA*) followed by *Duncan*'s multiple range tests at $p<0.05$. All figures were drawn using the *SigmaPlot* software (*version 12.5*, *SYSTAT Software Inc*., Richmond, CA, USA).

Results

Optimal concentration of NaHS: Since the effects of H_2S on plants are concentration dependent, seedlings were supplied with different concentrations of NaHS (100, 300, 500; 1,000; and 1,500 μM) for 7 d to determine the optimal concentration. Results showed that 500 μM of NaHS significantly increased turf quality, dry mass, Chl content, and alleviated MDA content. Hereafter, 500 μM of NaHS was used in the following experiments as H_2S donor (Table 1).

Growth and morphology: Significant differences were observed in the growth and morphology parameters of tall fescue among all treatments. The growth after 7 d under LL stress was found to be suppressed, showing a reduction in plant height, leaf width, tiller number, dry mass, and turf quality of tall fescue by 23.2, 18.5, 30.4, 57.6, and 42.1%, respectively, compared with the control plants. In addition, exogenous application of H_2S partially reversed the impacts of LL stress. Application of exogenous H_2S significantly increased plant height, leaf width, tiller number, dry mass, and turf quality of tall fescue by 14.4, 11.4, 31.3, 48.0, and 22.7%, respectively. Further, under LL stress, treating plants with HT (H_2S scavenger) decreased the plant height, leaf width, tiller number, dry mass, and turf quality of tall fescue compared to LL stress alone. Similarly, treatment with AOA (H_2S-biosynthesis inhibitor) also significantly reduced above-mentioned parameters when compared to LL stress seedlings (Table 2).

Photosynthetic pigment contents: After 7 d of growth under LL stress, concentrations of Chl *a*, Chl *b*, Chl (*a*+*b*), and Car decreased by 35.0, 29.1, 33.7, and 33.9%, respectively, when compared with the control seedlings (Table 3). Moreover, diminution of contents of Chl *a*, Chl *b*, Chl (*a*+*b*), and Car were significantly eliminated when leaves of tall fescue were treated with exogenous H_2S. However, the application of HT or AOA evidently reduced the production of Chl *a*, Chl *b*, Chl (*a*+*b*), and Car under LL stress (Table 3).

Gas-exchange parameters: When leaves of tall fescue

Table 1. Effects of different NaHS concentrations (0, 100, 300, 500; 1,000; and 1,500 μM) on low light (LL) stress-induced changes in turf quality, dry mass, Chl content, and malondialdehyde (MDA) content in leaves of tall fescue seedlings at 7 d after treatments. CK – treatment with distilled water; LL – treatment with distilled water under LL; LL + 100 – treatment with 100 μM NaHS under LL; LL + 300 – treatment with 300 μM NaHS under LL; LL + 500 – treatment with 500 μM NaHS under LL; LL + 1,000 – treatment with 1,000 μM NaHS under LL; LL + 1,500 – treatment with 1,500 μM NaHS under LL. Each value is the mean ± SD of three replicates (n = 3). Means marked with *different letters* indicate statistically significant differences according to *Duncan*'s multiple range tests (p<0.05).

Treatment	Turf quality	Dry mass [g per plant]	Chl (a+b) [mg g^{-1}(FM)]	MDA [nmol g^{-1}(FM)]
CK	8.07 ± 0.13^{a}	0.59 ± 0.08^{a}	3.36 ± 0.06^{a}	2.29 ± 0.39^{c}
LL	4.67 ± 0.13^{cd}	0.25 ± 0.06^{c}	2.23 ± 0.01^{d}	3.40 ± 0.13^{a}
LL + 100	4.93 ± 0.07^{c}	0.27 ± 0.02^{c}	2.32 ± 0.16^{d}	3.31 ± 0.17^{a}
LL + 300	5.40 ± 0.12^{b}	0.33 ± 0.02^{bc}	2.65 ± 0.18^{bc}	3.15 ± 0.09^{a}
LL + 500	5.73 ± 0.24^{b}	0.37 ± 0.02^{b}	2.80 ± 0.11^{b}	2.91 ± 0.14^{b}
LL + 1,000	4.27 ± 0.13^{d}	0.30 ± 0.01^{bc}	2.53 ± 0.06^{c}	2.99 ± 0.06^{b}
LL + 1,500	3.80 ± 0.12^{e}	0.28 ± 0.03^{bc}	2.27 ± 0.03^{d}	3.16 ± 0.13^{a}

Table 2. Effects of exogenous H_2S on low light (LL) stress-induced changes in plant height, leaf width, tiller number, dry mass, and turf quality in leaves of tall fescue seedlings at 7 d after treatments. CK – treatment with distilled water; LL – treatment with distilled water under LL; LL + NaHS – treatment with 500 μM NaHS under LL; LL + HT – treatment with 300 μM hypotaurine under LL; LL + AOA – treatment with 300 μM aminooxy acetic acid under LL. Each value is the mean ± SD of three replicates (n = 3). Means marked with *different letters* indicate statistically significant differences according to *Duncan*'s multiple range tests (p<0.05).

Treatment	Plant height [cm]	Leaf width [mm]	Tiller number	Dry mass [g per plant]	Turf quality
CK	22.08 ± 1.04^{a}	5.88 ± 0.24^{a}	4.60 ± 0.40^{a}	0.59 ± 0.08^{a}	8.07 ± 0.13^{a}
LL	16.94 ± 0.71^{c}	4.79 ± 0.14^{c}	3.20 ± 0.20^{b}	0.25 ± 0.06^{bc}	4.67 ± 0.13^{c}
LL + NaHS	19.38 ± 0.28^{b}	5.34 ± 0.10^{b}	4.20 ± 0.37^{a}	0.37± 0.02^{b}	5.73 ± 0.24^{b}
LL + HT	14.50 ± 0.73^{d}	3.87 ± 0.22^{d}	2.40 ± 0.24^{c}	0.14 ± 0.02^{d}	2.60 ± 0.12^{d}
LL + AOA	13.30 ± 0.93^{d}	4.22 ± 0.08^{d}	2.60 ± 0.24^{c}	0.20 ± 0.01^{c}	3.13 ± 0.35^{d}

Table 3. Effects of exogenous H_2S on low light (LL) stress-induced changes in chlorophyll (Chl) and carotenoids (Car) contents in leaves of tall fescue leaves seedlings at 7 d after treatments. CK – treatment with distilled water; LL – treatment with distilled water under LL; LL + NaHS – treatment with 500 μM NaHS under LL; LL + HT – treatment with 300 μM hypotaurine under LL; LL + AOA – treatment with 300 μM aminooxy acetic acid under LL. Each value is the mean ± SD of three replicates (n = 3). Means marked with *different letters* indicate statistically significant differences according to *Duncan*'s multiple range tests (p<0.05).

Treatment	Chl a [mg g^{-1}(FM)]	Chl b [mg g^{-1}(FM)]	Chl (a+b) [mg g^{-1}(FM)]	Chl a/b	Car [mg g^{-1}(FM)]
CK	2.57 ± 0.10^{a}	0.79 ± 0.04^{a}	3.36 ± 0.06^{a}	3.25 ± 0.05^{a}	1.27 ± 0.07^{a}
LL	1.67 ± 0.03^{c}	0.56 ± 0.02^{c}	2.23 ± 0.01^{c}	3.00 ± 0.04^{ab}	0.84 ± 0.02^{c}
LL + NaHS	2.13 ± 0.12^{b}	0.67 ± 0.01^{b}	2.80 ± 0.11^{b}	3.17 ± 0.04^{a}	1.02 ± 0.01^{b}
LL + HT	1.30 ± 0.08^{d}	0.46 ± 0.01^{d}	1.76 ± 0.08^{d}	2.82 ± 0.15^{b}	0.71 ± 0.01^{d}
LL + AOA	1.34 ± 0.10^{d}	0.48 ± 0.03^{d}	1.81 ± 0.07^{d}	2.80 ± 0.15^{b}	0.72 ± 0.02^{d}

were exposed to LL regime for 7 d, gas-exchange parameters including P_N, g_s, and E were reduced by 50.8, 63.0, and 80.7%, respectively, when compared with control seedlings. Moreover, treatment of plants with exogenous H_2S significantly increased P_N, g_s, and E in leaves of tall fescue when compared to LL treatment alone. However, the application of exogenous H_2S significantly reduced C_i by 9.2% compared to LL treatment alone. Further, when endogenous H_2S was scavenged (HT addition), C_i value increased significantly (Fig. 1).

Chl fluorescence parameters: Compared to control plants, seedlings grown in LL showed lower F_v/F_m, Φ_{PSII}, q_P, and ETR values with significant differences throughout the experimental period. In addition, when compared to LL treatment only, significant increments of 3.8, 12.5, 16.1, and 24.5% were recorded in the values of F_v/F_m, Φ_{PSII}, q_P, and ETR, respectively, under exogenous H_2S treatment. However, plants supplied with HT showed reduced fluorescence parameters and the variation trend was similar to that of AOA-treated plants (Fig. 2).

Total soluble sugar content: After 7 d of LL treatment, severe inhibition was observed in the total soluble sugar content of leaves of tall fescue seedlings, in contrast with the control plants. In addition, exogenous H_2S treatment

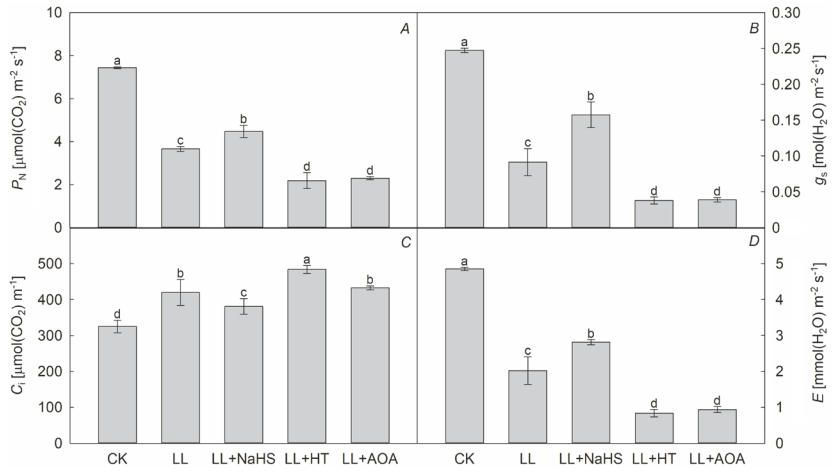

Fig. 1. Effects of exogenous H_2S on low light stress-induced changes in the net photosynthetic rate (P_N) (*A*), stomatal conductance (g_s) (*B*), intercellular CO_2 concentration (C_i) (*C*), and transpiration rate (*E*) (*D*) in leaves of tall fescue seedlings at 7 d after treatments. CK – treatment with distilled water; LL – treatment with distilled water under LL; LL + NaHS – treatment with 500 μM NaHS under LL; LL + HT – treatment with 300 μM hypotaurine under LL; LL + AOA – treatment with 300 μM aminooxy acetic acid under LL. Each histogram denotes a mean value of three replicates and vertical bars represent SD (*n* = 3). Means marked with *different letters* above the bars indicate statistically significant differences according to *Duncan*'s multiple range tests.

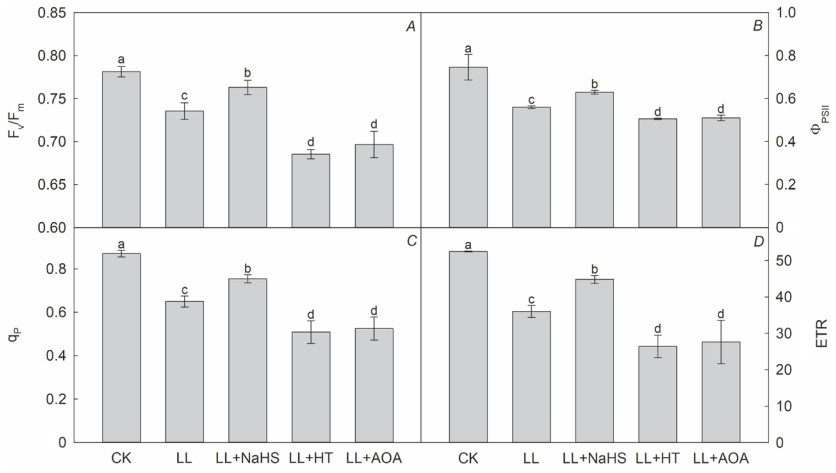

Fig. 2. Effects of exogenous H_2S on low light stress-induced changes in maximal quantum yield of PSII photochemistry (F_v/F_m) (*A*), effective quantum yield of PSII photochemistry (Φ_{PSII}) (*B*), photochemical quenching coefficient (q_P) (*C*), and electron transport rate (ETR) (*D*) in leaves of tall fescue seedlings at 7 d after treatments. CK – treatment with distilled water; LL – treatment with distilled water under LL; LL + NaHS – treatment with 500 μM NaHS under LL; LL + HT – treatment with 300 μM hypotaurine under LL; LL + AOA – treatment with 300 μM aminooxy acetic acid under LL. Each histogram denotes a mean value of three replicates and vertical bars represent SD (*n* = 3). Means marked with *different letters* above the bars indicate statistically significant differences according to *Duncan*'s multiple range tests.

significantly improved the accumulation of soluble sugar content in leaves when compared with LL stress only. Further, the application of HT or AOA did not significantly affect total soluble sugar content in tall fescue leaves, compared to the LL-stressed seedlings only (Fig. 3*A*).

Soluble protein content was significantly reduced in leaves of tall fescue subjected to LL stress, compared with the control plants. Moreover, an increase of soluble protein concentration was observed in LL-treated leaves with exogenous H_2S application. However, HT or AOA addition was found to decrease soluble protein concentration in plants when compared with LL treatment alone (Fig. 3*B*).

Activities of Rubisco enzyme: Value of Rubisco activity of tall fescue in LL was lower than those in the control plants. However, photosynthetic enzyme activity inhibition was considerably ceased by the application of exogenous H_2S under LL conditions. Further, compared to LL treatment only, when endogenous H_2S was scavenged or endogenous H_2S biosynthesis was inhibited, Rubisco activity of tall fescue leaves was significantly reduced (Fig. 3*C*).

Membrane lipid peroxidation: When compared with control seedlings, MDA accumulation significantly increased by 48.6% in tall fescue after 7-d LL treatment. In addition, the decreasing MDA content was found when tall fescue leaves were supplied with exogenous H_2S, compared to the LL stress alone. However, MDA was remarkably enhanced in tall fescue leaves treated with HT or AOA, respectively (Fig. 4*A*).

Activities of antioxidant enzymes: LL stress showed significant increment in the activity of POD in tall fescue leaves when compared with control plants. The treatment of tall fescue seedlings with exogenous H_2S remarkably increased POD activity with significant difference when compared to LL treatment alone. The response was reversed in POD activity of tall fescue leaves treated with HT or AOA (Fig. 4*B*).

Compared to control seedlings, a significant increase of SOD activity was found in LL-stressed tall fescue leaves. In addition, application of exogenous H_2S evidently increased the activity of SOD. Further, treating plants with HT or AOA greatly reduced SOD activity of tall fescue seedlings, compared with LL-stressed plants (Fig. 4*C*).

After 7 d of growth under LL stress, the activity of APX was remarkably enhanced in leaves of tall fescue

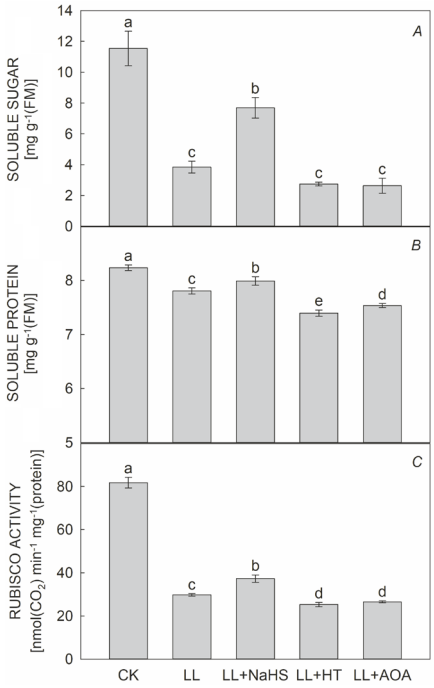

Fig. 3. Effects of exogenous H_2S on low light stress-induced changes in soluble sugar (*A*), soluble protein (*B*), and Rubisco activity (*C*) in leaves of tall fescue seedlings at 7 d after treatments. CK – treatment with distilled water; LL – treatment with distilled water under LL; LL + NaHS – treatment with 500 μM NaHS under LL; LL + HT – treatment with 300 μM hypotaurine under LL; LL + AOA – treatment with 300 μM aminooxy acetic acid under LL. Each histogram denotes a mean value of three replicates and vertical bars represent SD (n = 3). Means marked with *different letters* above the bars indicate statistically significant differences according to *Duncan*'s multiple range tests.

plants. Moreover, when compared to LL treatment only, exogenous H_2S treatment significantly improved the activity of APX. However, plants applied with HT or AOA showed decreased APX activity, compared with the LL-stressed seedlings alone (Fig. 4*D*).

Endogenous H_2S concentration: To explore the potential role of endogenous H_2S in response to LL stress of tall fescue, H_2S contents of leaves were measured. Tall fescue seedlings under LL stress resulted in increased H_2S production when compared to control plants. In addition, treatment with exogenous H_2S led to a significant increase in endogenous H_2S content when compared to LL treatment alone. However, HT or AOA treatment severely reduced the production of endogenous H_2S in tall fescue leaves (Fig. 5).

Discussion

The intensity and quantity of light have a strong influence on growth and development of tall fescue seedlings. Severe LL conditions can suppress the turfgrass capability to perform efficiently photosynthesis, triggering a harmful effect on various physiological and biochemical processes of plants that can exhibit diminishing survival rates (Zhang *et al*. 2018). H_2S, as the third gasotransmitter, may play an important role in physiological and metabolic processes in plants (Guo *et al*. 2016). In the present study, H_2S concentration significantly increased under LL treatment compared to the control, which indicated that H_2S is possibly involved in LL responses in tall fescue. Previous studies have demonstrated that exogenous H_2S showed considerable impacts on plant leaf photosynthesis to multiple abiotic stresses (Duan *et al*. 2015). In our study, higher concentrations of soluble sugar and soluble protein in tall fescue after the application of NaHS might indicate that photosynthesis was elevated. We proposed that H_2S can alleviate the damage caused by LL stress through improving P_N exposure in tall fescue.

Photosynthetic efficiency could be influenced by many factors, such as stomatal or nonstomatal limitations (Hu *et al*. 2010). However, there are contrasting conclusions regarding stomatal movements affected by H_2S. One study demonstrated that low concentration of H_2S enhances stomatal aperture, by decreasing the expression level of ABA receptor candidates (Jin *et al.* 2013). In contrast, another study showed that low content of H_2S represses stomatal opening through ABA-dependent signaling network (García-Mata and Lamattina 2010). In agreement with the findings of Sui *et al*. (2012) on responses to low light stress in *Capsicum annuum*, the results of the present

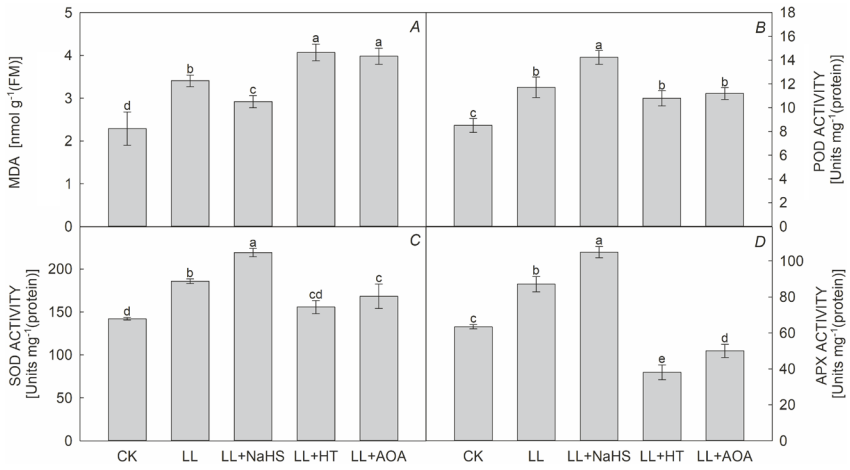

Fig. 4. Effects of exogenous H_2S on low light stress-induced changes in malondialdehyde (MDA) (*A*), peroxidase (POD) (*B*), superoxide dismutase (SOD) (*C*), and ascorbate peroxidase (APX) (*D*) in leaves of tall fescue seedlings over 7 d after treatments. CK – treatment with distilled water; LL – treatment with distilled water under LL; LL + NaHS – treatment with 500 μM NaHS under LL; LL + HT – treatment with 300 μM hypotaurine under LL; LL+AOA – treatment with 300 μM aminooxy acetic acid under LL. Each histogram denotes a mean value of three replicates and vertical bars represent SD (n = 3). Means marked with *different letters* above the bars indicate statistically significant differences according to *Duncan*'s multiple range tests.

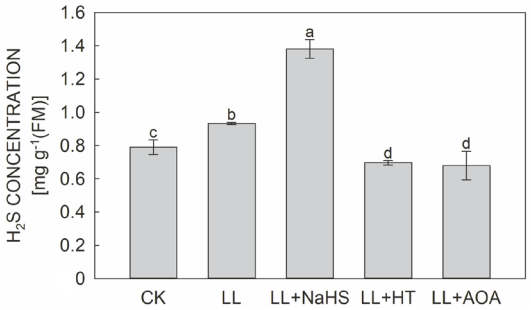

Fig. 5. Effects of exogenous H_2S on low light stress-induced changes in endogenous H_2S content in leaves of tall fescue seedlings at 7 d after treatments. CK – treatment with distilled water; LL – treatment with distilled water under LL; LL + NaHS – treatment with 500 μM NaHS under LL; LL + HT – treatment with 300 μM hypotaurine under LL; LL + AOA – treatment with 300 μM aminooxy acetic acid under LL. Each histogram denotes a mean value of three replicates and vertical bars represent SD (n = 3). Means marked with *different letters* above the bars indicate statistically significant differences according to *Duncan*'s multiple range tests.

study demonstrated that g_s was reduced and C_i increased in tall fescue exposed to LL conditions. On the contrary, LL-treated tall fescue seedlings subjected to H_2S donor treatment effectively increased g_s and reduced C_i. These results offered supporting evidence that H_2S was capable to improve the photosynthetic ability under LL stress through sustaining stomatal conductance at a high level. It has been reported that increments in CO_2 assimilation and stomatal conductance were the consequence of increased Rubisco activity (Liang *et al.* 2005), whose level is closely linked to carboxylation efficiency and rate of carbon assimilation (Xu *et al*. 2013). Here, prominent increase of Rubisco activity was monitored at exogenous H_2S addition upon LL stress. We found the similar observation reported by Chen *et al*. (2011) in *Spinacia oleracea*. These results indicated H2S might modulate photosynthesis through increasing stomatal conductance and improving Rubisco activity.

Besides carboxylation, Chl fluorescence is also widely recognized as a determination of PSII function and light-harvesting efficiency. Chl fluorescence parameters, such as F_v/F_m, Φ_{PSII}, q_P, and ETR, were differentially attenuated in tall fescue seedlings when subjected to LL stress. In addition, HT or AOA application increased this tendency, which is similar to the findings described by Cheng *et al.* (2018). The decrements of F_v/F_m and Φ_{PSII} suggested that LL stress generated photoinhibition by preventing the PSII electron transfer from Q_A to Q_B (Kumar and Prasad 2015). The apparent increments of F_v/F_m, Φ_{PSII}, q_P, and ETR in leaves under the combined application of NaHS and LL regime indicated that exogenous H_2S promoted photochemical reaction in PSII and photochemical conversion efficiency (Dai *et al*. 2016), which was consistent with enhanced Chl production. Chl was responsible for PSII function because intrinsic Chl-binding proteins could capture light energy to catalyze the water oxidation and reduction of plastoquinone (Eshaghi *et al*. 1999). H_2S showed effective recovery potential in enhancing electron transport efficiency and photochemical activity of PSII. In addition, changes in synthesis of photosynthetic pigment demonstrated improvement in photosynthetic efficiency (Yang *et al*. 2018). In this study, increased Chl content [Chl *a*, Chl *b*, and Chl (*a*+*b*)] in response to LL suggested the important role of exogenous H_2S in enhancing photosynthesis (Kaya *et al*. 2018). By contrast, the diminution of the Chl content was observed with application of HT or AOA, which was consistent with the decrease of H_2S concentration in tall fescue seedlings. These results further implied that H_2S served as a signaling molecule to regulate photosynthesis.

Environmental stresses, including LL stress, stimulated chloroplasts to generate ROS by direct transfer of excitation energy from Chl (Gill and Tuteja 2010). It can be assumed that the accumulation of cytotoxic ROS subsequently caused lipid peroxidation, overproduction of MDA, and enzyme inactivation in chloroplasts, affecting carbon assimilation of photosynthesis (Li *et al*. 2017). Moreover, excessive MDA might reflect that the apparatus of photosynthesis was damaged and the content of Chl was remarkably reduced (Hou *et al*. 2015). In the current study, LL stress increased the MDA content, implying that the functionality and integrity of membranes were severely repressed, which was similar to the finding reported in cucumber (Zhang *et al*. 2011). Moreover, application of HT or AOA further elevated the content of MDA in plants exposed to LL, coinciding with previous research description in wheat (Shan *et al.* 2017). Reinforcement of antioxidant enzyme system is regarded as an adaptive way to reduce the susceptibility to LL stress (Yang *et al*. 2014). It was observed that tall fescue plants supplied with NaHS under LL stress conditions demonstrated further increments in the activities of SOD, POD, and APX, which is in accordance with diminution in the content of MDA under identical treatment. The higher activities of above antioxidant enzymes might increase the resistance to oxidative damage and protected the function of photosynthetic machinery from LL-triggered damage (Dai *et al*. 2016). These results suggested that H_2S might act as a beneficial conductor in protecting chloroplasts and Chl pigment from cellular oxidative damage through increased activities of antioxidant enzyme in tall fescue.

In conclusion, LL was responsible for various morphological and physiological disruptions in tall fescue and could result in shade injury of turf aesthetic value. However, application of exogenous H_2S demonstrated improved LL tolerance of tall fescue. Our results showed that H_2S, as an important signaling molecule, effectively maintained a high net photosynthetic rate through increased stomatal conductance, carboxylation efficiency, Chl content, higher activity of PSII, and alleviated ROS damage under LL stress in tall fescue seedlings. However, further studies are required to understand the underlying molecular mechanism of H_2S in regulating leaf photosynthesis.

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