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BRIEF COMMUNICATION

Hydrogen sulfide alleviated chromium toxicity in wheat

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Abstract

Effects of H_2S on seed germination under chromium (Cr) stress were investigated in wheat (*Triticum aestivum* L.). Under Cr stress, the percentage of germination of wheat seeds decreased, but this decrease could be alleviated by pretreatment with NaHS, an H_2S donor, in a dose-dependent manner. Furthermore, NaHS significantly enhanced the activities of amylase, esterase, superoxide dismutase, catalase, ascorbate peroxidase, and guaiacol peroxidase in Cr-stressed germinating seeds, whereas reduced the Cr-induced increase in lipoxygenase activity and over-production of malondialdehyde (MDA) and H_2O_2, and sustained slightly higher content of endogenous H_2S.

Additional key words: amylase, antioxidative enzymes, esterase, lipid peroxidation, seed germination, *Triticum aestivum.*

Chromium (Cr), which can be a toxic carcinogen, released in soil due to leather tanning, textile, carpet and steel plating industries (Vajpayee *et al*. 2002). It is not essential for higher plants and can disturb plant metabolism, leading to chlorosis, depression of ATP formation, reduced growth, and even plant death (Bishnoi *et al*. 1993a,b, Sharma *et al*. 1995, Gupta *et al*. 2009). Cr induced the excessive accumulation of reactive oxygen species (ROS) in many plants (Dixit *et al*. 2002, Panda and Khan 2003) resulting in the damages of DNA, RNA, proteins and lipids (Vajpayee *et al*. 2002). Plant growing under Cr stress utilizes its antioxidant system to defend against the Cr-induced oxidative stress. For example, studies in green gram roots exposed to Cr^{6+} stress showed that superoxide dismutase (SOD) and ascorbate peroxidase (APX) activities increased to ameliorate Cr-induced oxidative damages (Shanker *et al*. 2004).

It has been shown that hydrogen sulfide (H_2S) can act as the third gaseous signaling molecule after nitric oxide (NO) and carbon monoxide (CO) in animals (Hosoki *et al*. 1997). In plant kingdom NO and CO have already been identified as signaling molecules involving anti-oxidative defense (Delledonne 2005, Huang *et al*. 2006, Han *et al*. 2007, Sa *et al*. 2007). However, whether H_2S serves as a signal molecule in plants remains unclear, though H_2S emission has already been observed in many species (Wilson *et al*. 1978, Winner *et al*. 1981, Sekiya *et al*. 1982a,b, Rennenberg and Filner 1982, 1983, Rennenberg 1983). In plants, H_2S can be released from cysteine *via* a reversible O-acetylserine (thiol) lyase (OAS-TL) reaction or by the action of desulfhydrases localized in various cellular compartments (Hällgren and Fredriksson 1982, Leon *et al*. 2002, Riemenschneider *et al*. 2005, Rausch and Wachter 2005). More recently, we demonstrated that H_2S is involved in the plants' antioxidant response against copper and osmotic stresses (Zhang *et al*. 2008, 2009). The aim of this research was to examine the possible roles of H_2S in promoting wheat seed germination and alleviating oxidative damage under Cr stress.

Wheat (*Triticum aestivum* L., Yangmai 158) seeds, which were supplied by Jiangsu Academy of Agricultural Sciences, were sterilized with 0.1 % $HgCl_2$ for 3 min and washed extensively with distilled water. To investigate the toxic effect of chromium on seed germination, seeds were allocated randomly in Petri dishes (9 cm diameter × 1.2 cm depth, 50 seeds per dish) at 25 °C with 0, 0.5, 1.0,

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Abbreviations: APX - ascorbate peroxidase; CAT - catalase; LOX - lipoxygenase; MDA - malondialdehyde; POD - peroxidase; ROS - reactive oxygen species; SOD - superoxide dismutase.
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2.0, 4.0, 6.0 mM Na_2CrO_4 for 48 h. A seed was regarded as germinated when the radicle protruded through the seed coat and the length of radicle reached over 50 % of seed length. The semi-inhibitory Cr stress (4.0 mM), under which the germination percentage dropped to about 50 % as compared with the control, was found and selected for following experiments. To examine the possible promotive roles of hydrogen sulfide (H_2S) on seed germination under Cr stress, seeds were pretreated with 0, 0.4, 0.8, 1.2, 1.6, 2.0 mM sodium hydrosulfide (NaHS, H_2S donor, *Sigma,* St. Louis, USA) for 12 h, and subsequently subjected to 4.0 mM Na_2CrO_4 for further 48 h. The germination percentage was calculated and the length of coleoptiles and radicles and number of radicles were recorded to find the optimum NaHS concentration. The seeds pretreated in water (CK) served as control. Germinating seeds were sampled for further analyses.

Activities of amylase (EC 3.2.1.1/2) and esterase (EC 3.1.1.3) were measured by starch-iodine method and by hydrolysis of *p*-nitrophenylbutyrate, respectively, as was described previously (Zhang *et al*. 2008). Activity of lipoxygenase (LOX, EC 1.13.11.12) was determined according to Surrey (1964) and activities of superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), ascorbate peroxidase (APX, EC 1.11.1.11) and guaiacol peroxidase (POD, EC 1.11.1.7) were assayed according to García-Limones *et al*. (2002). Contents of MDA and H_2O_2 were determined according to Heath and Packer (1968) and Patterson *et al*. (1984), respectively. Endogenous H_2S content in seeds was determined by formation of methylene blue from dimethyl-*p*-phenylenediamine in H_2SO_4 according to the method described by Sekiya *et al*. (1982a).

All values were expressed as the mean values ± SD of at least three independent experiments, the significance of differences was analysed using Student's *t*-test.

With the enhancement of Cr stress, the germination percentage dropped gradually and length of coleoptiles and radicles and number of radicles decreased sharply (data not shown). However, when NaHS was applied to seeds prior to 4.0 mM Cr, the protective role of NaHS on wheat seed germination under Cr stress was observed. The most suitable NaHS concentration was 1.2 mM (Table 1).

To get insight of the promoting effect of NaHS on wheat seed germination under Cr-stress, the activities of amylase and esterase were investigated. During germination, amylase activities in both water and NaHS pretreated wheat seeds increased gradually up to 24 h and than decreased. During the first 12 h of Cr treatment, the amylase activities showed no significant difference between the NaHS pretreatment and the water control. Thereafter, the amylase activity in NaHS pretreated seeds was higher than that of water control (Fig. 1*A*). Similarly, there was no significant difference in esterase activities between the NaHS pretreatment and the control at the early stage of germination, but from 24 to 48 h NaHS pretreatment significantly enhanced the esterase activity (Fig. 1*B*).

As concerns antioxidant enzymes, NaHS maintained a higher SOD activity as compared with that of control during the whole germination time. At 12 h of pretreatment before Cr exposure, a burst of SOD activity occurred in both (water and NaHS) pretreatments and SOD activity in NaHS pretreated seeds was 30 % higher than that in control seeds. After exposure to Cr, SOD activity in NaHS pretreated seeds remained high, whereas that of control dropped sharply at 12 h after Cr stress, thereafter restored after 24 h of Cr exposure but was still lower than that in NaHS pretreated seeds (Fig. 1*C*). CAT activity increased during 12 h of the both pretreatments. CAT activity in NaHS pretreated seeds increased steadily with time, while that of control seeds decreased slightly during 24 h after Cr stress and then increased at 36 h of Cr exposure, but it was still significantly lower than that of NaHS pretreated seeds (Fig. 1*D*). The activities of APX and POD in NaSH pretreated seeds were not significantly different as compared with those in water pretreated seeds up to 24 and 12 h, respectively, of Cr stress. However, NaSH pretreatment could effectively up-regulate APX and POD activities in the later stages of germination (Fig. 1*E,F*).

A rapid increase in LOX activity, which is an indicator of lipid peroxidation, occurred in seeds after NaHS pretreatment for 12 h and exposure to Cr for further 12 h, whereas that in the control was lower. After prolonged exposure to Cr stress, LOX activity in the water pretreated seeds rose gradually. However, the activity of LOX in seeds pretreated with NaHS remained on the initial level till the end of experiment, which was significantly lower as compared with that in control (Fig. 1*G*). During the pretreatments, MDA contents were low in seeds treated with NaHS or water, indicating that NaHS did not negatively affect the early stage of germination. After exposure to Cr stress, MDA content in control seeds increased dramatically, while it increased gradually in NaHS pretreated seeds (Fig. 1*H*).

The time course of H_2O_2 accumulation showed similar pattern as that of MDA. A burst of H_2O_2 in seeds occurred during the both pretreatments. The H_2O_2 content increased during the Cr stress but less in NaHS pretreated seeds (Fig. 1*I*). Interestingly, at the first 12 h of germination a rapid accumulation of endogenous H_2S was observed in seeds whether pretreated with NaHS or not. After exposure to Cr, the contents of endogenous H_2S decreased gradually, but the endogenous H_2S content in NaHS-pretreated seeds was always slightly higher than that in the controls (Fig. 1*J*).

Cr stress inhibited wheat seed germination and stunted the bud growth and radicle emergence. It was possible that the observed increases in MDA or H_2O_2 in germinating seeds (Fig. 1*H*, *I*) and in radicle tips (data not shown) under Cr stress attributed to the lipid peroxidation or plasma membrane damage, which in turn resulted in the inhibition of seed germination and seedling growth. Wheat seeds pretreated with NaHS under Cr stress exhibited higher germination percentage, prolongation of buds and radicles (Table 1) and lower contents of MDA and H_2O_2 (Fig. 1*H*, *I*). To mitigate and repair the oxidative damage,

plants have developed a complex antioxidant system. Different plant species respond to Cr stress by modulating the antioxidant enzymes differently (Dixit *et al*. 2002, Yu *et al*. 2007). The synchronous modulation of various antioxidant enzymes, such as SOD, CAT and APX, is one of the most efficient mechanisms to scavenge ROS (Clijsters *et al*. 1999, Matés 2000). A higher SOD activity in NaHS-pretreated seeds than in controls was observed after exposure to Cr for 48 h (Fig. 1*C*). Besides, CAT, APX and POD activities in NaHS-pretreated seeds were higher than those of water pretreated seeds (Fig. 1*D*,*E*,*F*). Thus the modulation of antioxidant enzymes enabled a more

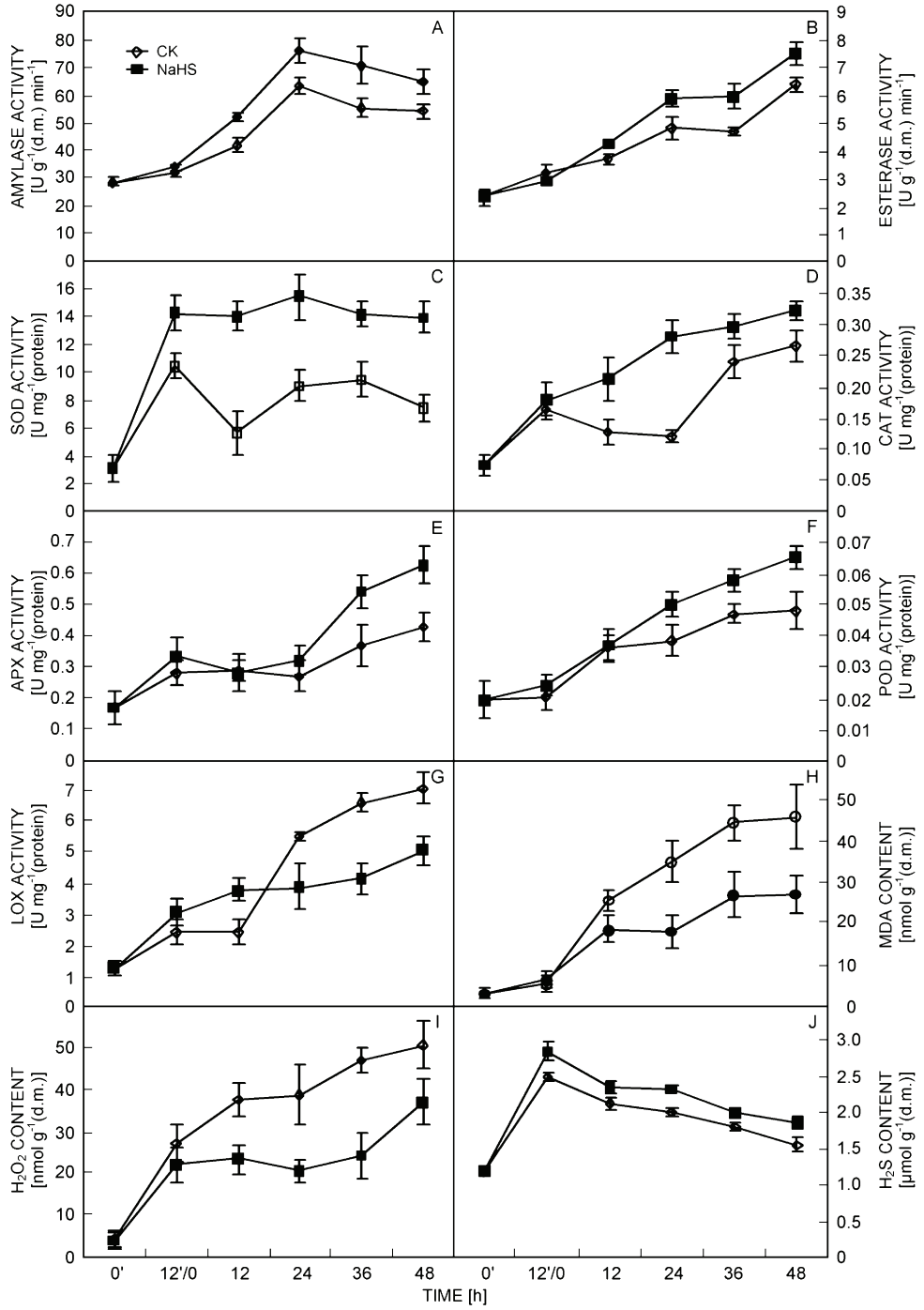

Fig. 1. Effect of H_2S donor NaHS on amylase activity (*A*), esterase activity (*B*), SOD activity (*C*), CAT activity (*D*), APX activity (*E*), POD activity (*F*), LOX activity (*G*), MDA content (*H*), H_2O_2 content (*I*), and endogenous H_2S content (*J*) in wheat seeds under Cr stress. Wheat seeds were pretreated with water (CK) and 1.2 mM NaHS (NaHS) for 12 h (shown as from 0' to 12'/0 h) prior to exposure to 4.0 mM Cr stress for further 48 h (shown as 12, 24, 36, 48 h, respectively). *Vertical bars* represent the SD of the mean (n = 6).

Table 1. Effects of NaHS pretreatment on wheat seed germination under Cr stress. Wheat seeds were pretreated with 0, 0.4, 0.8, 1.2, 1.6 or 2.0 mM NaHS for 12 h, and subsequently subjected to 4.0 mM Cr for further 48 h, and then the germination percentage was investigated. Values are the means ± SD (n = 6). Different letters mean significance of difference among the treatments ($P < 0.05$).

Concentration of NaHS [mM]	0	0.4	0.8	1.2	1.6	2.0
Germination [%]	51.7 ± 2.9a	71.7 ± 6.3b	80.5 ± 2.5b	85.5 ± 3.6c	80.7 ± 6.3c	49.2 ± 2.9d
Length of coleoptile [mm]	7.1 ± 0.5a	9.4 ± 0.7b	10.6 ± 0.6b	13.1 ± 0.8c	10.6 ± 0.6bc	8.2 ± 0.6ab
Length of radicle [mm]	4.3 ± 0.2a	5.3 ± 0.1b	5.4 ± 0.1b	6.7 ± 0.3c	5.7 ± 0.1bc	5.5 ± 0.2b
Radicle number [seed^{-1}]	0.9 ± 0.1a	1.1 ± 0.1b	1.2 ± 0.2b	1.7 ± 0.1c	1.3 ± 0.0b	1.0 ± 0.1ab

balanced redox state in Cr-stressed seeds. Alternatively, LOX was considered as an indicator of oxidative stress, which catalyzed oxygenation of unsaturated fatty acids into lipid hydroperoxides. Lower LOX activity (Fig. 1*G*) may enable a more balanced redox state in NaHS pretreated seeds, and further explain why MDA was kept at a lower level (Fig. 1*H*).

NaHS dissociates to Na^+ and HS^- in solution, then HS^- associates with H^+ and produces H_2S (Hosoki *et al*. 1997). It has been widely used for exogenous H_2S applied in solutions. Na^+ and other sulfur-containing components, such as S^{2-}, SO_4^{2-}, SO_3^{2-}, HSO_4^-, and HSO_3^- were not able to improve the seeds germination under Cr stress as NaHS did (data not shown). It indicates that H_2S or HS^-, rather than other compounds derived from NaHS, plays a potential role in promoting seed germination under Cr stress. Furthermore, a rapid accumulation of endogenous H_2S in seeds was observed at the early stage of germination, and the H_2S content in NaHS-pretreated seeds was slightly higher than that in controls (Fig. 1*J*). It may be interpreted as H_2S involvement in the mechanism of seed germination. Alternatively, the mobilization of reserve material in endosperm by hydrolytic enzymes is essential for wheat seed germination. As expected, the activation of amylase and esterase by NaHS (Fig. 1 *A*,*B*) can contribute to promotion of seed germination under Cr stress. In our previous paper, we showed that H_2S stimulates wheat seed germination and antioxidant metabolism under copper stress (Zhang *et al*. 2008). More recently we have proved, that H_2S counteracted chlorophyll loss in sweet potato seedling leaves and alleviates oxidative damage under osmotic stress (Zhang *et al*. 2009). The present research further supports the hypothesis that H_2S can be a signal molecule involved in plant response to abiotic stresses.

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