Research Article

Sulfur Dioxide Enhances Endogenous Hydrogen Sulfide Accumulation and Alleviates Oxidative Stress Induced by Aluminum Stress in Germinating Wheat Seeds

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Aluminum ions are especially toxic to plants in acidic soils. Here we present evidences that SO₂ protects germinating wheat grains against aluminum stress. SO₂ donor (NaHSO₃/Na₂SO₃) pretreatment at 1.2 mM reduced the accumulation of superoxide anion, hydrogen peroxide, and malondialdehyde, enhanced the activities of guaiacol peroxidase, catalase, and ascorbate peroxidase, and decreased the activity of lipoxygenase in germinating wheat grains exposed to Al stress. We also observed higher accumulation of hydrogen sulfide (H₂S) in SO₂-pretreated grain, suggesting the tight relation between sulfite and sulfide. Wheat grains germinated in water for 36 h were pretreated with or without 1 mM SO₂ donor for 12 h prior to exposure to Al stress for 48 h and the ameliorating effects of SO₂ on wheat radicles were studied. SO₂ donor pretreatment reduced the content of reactive oxygen species, protected membrane integrity, and reduced Al accumulation in wheat radicles. Gene expression analysis showed that SO₂ donor pretreatment decreased the expression of Al-responsive genes TaWali1, TaWali2, TaWali3, TaWali5, TaWali6, and TaALMT1 in radicles exposed to Al stress. These results suggested that SO₂ could increase endogenous H₂S accumulation and the antioxidant capability and decrease endogenous Al content in wheat grains to alleviate Al stress.

1. Introduction

Aluminum ions (Al³⁺) together with silicon and iron are the three most abundant mineral elements in soil. Whereas silicon and iron are required for plant growth, Al is toxic. Many different mechanisms have been advanced to explain Al toxicity in plants [1, 2]. One of the primary causes of Al toxicity is oxidative stress due to accumulation of reactive oxygen species (ROS), such as the superoxide anion (O₂⁻⁻) and hydrogen peroxide (H₂O₂), bringing about lipid peroxidation in plant cells [3–5]. Plants have developed several strategies to counteract oxidative stress caused by Al, such as activation of antioxidants, and exudation of organic acids as a mechanism for Al exclusion [6]. Recently, a range of signaling molecules, such as inositol 1,4,5-triphosphate (IP₃), salicylic acid, hydrogen peroxide (H₂O₂) and nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfide (H₂S), were found to participate in plant’s resistance to Al-induced oxidative stress [4, 7–10].

Sulfur dioxide (SO₂) is a colorless, nonflammable gas with a penetrating odor. Low concentrations of SO₂ have been found to play a physiological role in vivo in animal models, participating in various biological processes [11]. The physiological processes regulated by SO₂ in animals include cardiac function [11], inhibition of L-calcium channels in cardiomyocytes [12], and improvement in pulmonary vascular structural remodeling [13]. In plants, the toxic effects of SO₂ on growth and development have been extensively studied [14, 15]. Exposure to high concentrations of SO₂ can cause visible foliar damage, a decline in photosynthesis, an inhibition of plant growth, and structural disorganization and cell death [16–19]. On the other hand, many reports show
that low levels of atmospheric SO2 might be beneficial to plants [20]. SO2 can be metabolized and used as a sulfur source for plant growth, especially when the sulfur supply in soil is insufficient for normal growth [20]. Recently, low concentrations of SO2 were found to induce transcriptome reprogramming associated with oxidative signaling and biotic defence responses in plants, suggesting a physiological role of SO2 in plant [21].

In plants, sulfate is taken up from soil by high-affinity transporters. Sulfate is largely transported to shoots where it can be activated by ATP via ATP sulfurylase in the leaves. The product is reduced by 5'-adenylylsulfate (APS) reductase to sulfite which can be reduced to H2S by sulfite reductase [22]. SO2 can also be produced endogenously from sulfur-containing amino acids [23]. The endogenous production of SO2 also suggests that it has a physiological role in plants.

In order to establish the role of SO2 in alleviating Al stress, we investigated the effects of SO2 pretreatment on H2S and ROS accumulation and the antioxidant system in whole wheat grains and in wheat radicles. We also analyzed endogenous H2S and Al content as a means of understanding the mechanism of the role of SO2. We speculated that SO2 might act as an antioxidant molecule to alleviate Al toxicity during wheat grain germination.

2. Materials and Methods

2.1. Materials and Treatments. Wheat (Triticum aestivum L.) grains were supplied by the Anhui Aidi Agricultural Technology Co., Ltd., Anhui Province, China. Sodium bisulfite (NaHSO3) and anhydrous sodium sulfite (Na2SO3) were used as sulfur dioxide (SO2) donors according to Laisk et al. [24]. Wheat grains were sterilized by 0.1% HgCl2 for 3 min and washed extensively with H2O and then dried with filter papers. Wheat grains of similar size were selected and allocated randomly in Petri dish (9 cm diameter × 1.2 cm depth, 50 grains per dish). Wheat grains were germinated in H2O or aqueous solutions of AlCl3 at 5, 10, 15, 20, 25, 30, 60, and 90 mM for 48 h at 25°C and the length of coleoptiles and radicles and radicle number were recorded. To test the protective role of SO2 on germination and seedling growth of wheat grains under Al stress, grains were pretreated with H2O or 0.4, 0.8, 1.2, 1.6, or 2.0 mM SO2 donor for 12 h and subsequently subjected to a semi-inhibitory AlCl3 concentration (15 mM). AlCl3 solutions were renewed every 12 h and germinating grains were sampled every 12 h for further analysis.

2.2. Determination of MDA, O2^−, and H2O2. The contents of MDA, O2^−, and H2O2 were determined by the method of Zhang et al. [25].

2.3. Assays of LOX, CAT, APX, and POD Activities. Activity of lipoxynase (LOX, EC 1.13.11.12) was determined following the description by Surrey [26] and those of catalase (CAT, EC1.11.1.6), ascorbate peroxidase (APX, EC 1.11.1.11), and guaiacol peroxidase (POD, EC 1.11.1.7) were assayed according to Hu et al. [27]. Wheat grains were homogenized in ice-cold 50 mM phosphate buffer (pH 7.8) containing 1.0 mM EDTA. The homogenate was centrifuged at 15,000 g at 4°C for 10 min. The supernatant was used for activity determination.

2.4. Assays of Reducing Sugars and Soluble Protein. Wheat grains (0.5 ± 0.05 g) were ground in 5 mL of phosphate buffer (pH 7.0, 200 mM), the homogenate was centrifuged at 10,000 g for 30 min, and the supernatant was used for detection of reducing sugars and soluble protein content. Reducing sugar content was measured according to Miller [28].

For detection of soluble protein, 0.1 mL supernatant was mixed with 0.9 mL H2O and 5 mL Coomassie brilliant blue for 5 min and the absorbance recorded at 595 nm using the method described by Bradford [29].

2.5. Preparation of Wheat Radicles. Wheat grains were germinated in H2O for 36 h in the dark at 25°C and the average of radicle length was approximately 1.0 cm. The germinated wheat grains were pretreated with or without 1 mM SO2 donor for 12 h and then exposed to 0 or 400 μM AlCl3 for 48 h.

2.6. Detection of Plasma Membrane Integrity, Al Accumulation, and ROS Production in Radicles. Plasma membrane integrity of wheat radicles was detected following the method of Yamamoto et al. [30]. Radicles were stained with Evans blue solution (0.025% [w/v] Evans blue in 100 μM CaCl2, pH 5.6) for 10 min, then washed three times with 100 μM CaCl2 solutions, and photographed. Staining intensity of Evans blue is positively correlated with more damaged plasma membrane.

Al content in radicles was visualized by staining tissues with hematoxylin. Hematoxylin stain was prepared as described by Polle et al. [31]. Wheat radicles were washed with H2O for 30 min and then stained with solution of 0.2% hematoxylin and 0.02% NaIO3 for 30 min at room temperature. Radicles were then immersed in H2O for 30 min to remove excess stain and photographed. Staining intensity of hematoxylin is positively correlated with Al uptake.

ROS distribution in radicle tips was detected by 2',7'-dichlorofluorescin diacetate (DCFH-DA) following the method of LeBel et al. [32]. Radicle tips were incubated in a solution containing 100 μM CaCl2 and 10 μM DCFH-DA for 20 min and then washed three times with H2O. The fluorescence was detected with a Nikon 80i microscope (excitation at 488 nm and emission at 525 nm). For each treatment, ten individual roots from ten seedlings were examined and similar results were obtained.

2.7. Real-Time Quantitative RT-PCR Analysis in Wheat Radicles. Radicle tips were prepared for RNA extraction according to Li et al. [33]. Total RNA was isolated by grinding with liquid nitrogen according to the manufacturer’s instructions (CWBio, Beijing, China). cDNA was generated from total RNA with a reverse transcription kit (Prime Script RT Master Mix, Takara, Kyoto, Japan). Quantitative PCR was performed using a StepOnePlus Real-Time PCR
Table 1: Inhibitory effect of Al stress on the germination of wheat grains. Wheat grains were exposed to 0, 5, 10, 15, 20, 25, 30, 60, or 90 mM AlCl₃ for 48 h.

<table>
<thead>
<tr>
<th>Al³⁺ concentration (mM)</th>
<th>Germination percentage (%)</th>
<th>Radicle length (cm)</th>
<th>Coleoptile length (cm)</th>
<th>Radicle number (50 grains)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>64 ± 1.2⁸</td>
<td>3.1 ± 0.8⁸</td>
<td>1.5 ± 0.3⁹</td>
<td>178 ± 7.8⁸</td>
</tr>
<tr>
<td>5</td>
<td>66 ± 1.1⁴</td>
<td>2.7 ± 0.5³⁹</td>
<td>1.6 ± 0.2⁴</td>
<td>168 ± 8.9⁴</td>
</tr>
<tr>
<td>10</td>
<td>51 ± 2.3⁸</td>
<td>1.9 ± 0.3⁹</td>
<td>1.3 ± 0.3⁹</td>
<td>162 ± 7.6⁴</td>
</tr>
<tr>
<td>15</td>
<td>35 ± 3.8⁸</td>
<td>1.1 ± 0.2⁴</td>
<td>1.1 ± 0.2⁹</td>
<td>142 ± 6.3⁹</td>
</tr>
<tr>
<td>20</td>
<td>28 ± 4.2⁵⁸</td>
<td>0.7 ± 0.3⁴</td>
<td>0.8 ± 0.2³⁹</td>
<td>80 ± 5.6⁵</td>
</tr>
<tr>
<td>25</td>
<td>21 ± 5.1⁷⁹</td>
<td>0.4 ± 0.3⁹</td>
<td>0.6 ± 0.2³</td>
<td>68 ± 6.1³</td>
</tr>
<tr>
<td>30</td>
<td>15 ± 4.7⁷⁹</td>
<td>0.2 ± 0.2³</td>
<td>0.3 ± 0.3³</td>
<td>45 ± 5.3²</td>
</tr>
<tr>
<td>60</td>
<td>8 ± 5.2⁸</td>
<td>0.1 ± 0.1³²</td>
<td>0.3 ± 0.2³</td>
<td>22 ± 3.5⁰</td>
</tr>
<tr>
<td>90</td>
<td>7 ± 6.3⁸</td>
<td>0 ± 0⁰</td>
<td>0.2 ± 0.2⁴</td>
<td>0 ± 0⁰</td>
</tr>
</tbody>
</table>

Values are the means ± SD (n = 6). Different letters mean significance of difference between different treatments (P < 0.05).

3. Results

3.1. Inhibitory Effect of Al on Wheat Grain Germination. The effect of Al stress on wheat seedling growth and development was examined following incubation of grain in AlCl₃ with concentrations ranging from 5 mM to 90 mM (Table 1). At concentrations of 5 mM or below, germination percentage, coleoptile length, and radicle number are almost unaffected, but radicle length was reduced by 13%, suggesting that the radicle is the primary target of Al toxicity. At 15 mM Al, germination percentage was almost halved compared with that of control and that concentration was selected for further experiments. At 90 mM Al, radicle growth was completely inhibited, but very stunted coleoptile growth was still observed.

3.2. SO₂ Donor Ameliorates Al Stress in Germinating Wheat Grain. To establish whether the SO₂ donor Na₂SO₃ had a toxic effect on wheat grain germination, grains were germinated in different SO₂ donor concentrations ranging from 0.4 to 2.0 mM for 36 h (see Table S1 in Supplementary Material available online at http://dx.doi.org/10.1155/2015/612363). Table S1 shows there was no significant change in germination percentage, coleoptile length, radicle length, or radicle number between water control and SO₂ donor treatment, establishing that the concentrations of SO₂ donor used in this work exhibited no visible toxic effects. To test the ability of SO₂ donor to alleviate Al stress, wheat grains were pretreated with SO₂ donor concentrations ranging from 0.4 to 2.0 mM for 12 h prior to incubation with 15 mM Al (Table 2 and Figure 1). At all SO₂ donor concentrations used, SO₂ pretreatment was effective in alleviating the toxic effects of Al in a dose dependent manner. The optimal SO₂ donor concentration for alleviating Al stress was 1.2 mM, a concentration where the germination percentage was increased by 51%, radicle and coleoptile length by 28% and 26%, respectively, compared with those exposed to Al. This result clearly shows that SO₂ alleviates Al-induced inhibition of wheat grain germination and seedling growth.

3.3. Effect of SO₂ Donor on the Contents of Reducing Sugars and Soluble Protein in Al-Stressed Wheat Grain. Figure 2(a) shows the changes in reducing sugars in germinating wheat grains preincubated in SO₂ donor or H₂O for 12 h followed by incubation in Al for 48 h. Within 12 h pretreatment in H₂O and 24 h of Al treatment, the content of reducing sugar decreased gradually, whereas reducing sugar in the SO₂ donor pretreatment remained stable and slightly increased at 24 h. Thereafter reducing sugar content increased steadily in both treatments followed by a slight decrease at 48 h. The content of reducing sugars in SO₂ donor pretreated grain was always significantly higher than the counterpart of only Al treatment.

The content of soluble protein increased gradually and peaked on 24 h of Al stress followed by a slight decrease (Figure 2(b)). Though the mean values of soluble protein in...
Table 2: Effects of SO$_2$ donor pretreatment on wheat grain germination under 15 mM Al$^{3+}$ stress. Wheat grains were pretreated with 0, 0.4, 0.8, 1.2, 1.6, and 2.0 mM SO$_2$ for 12 h and subsequently subjected to 15 mM AlCl$_3$ for further 48 h, and then germination was investigated.

<table>
<thead>
<tr>
<th>SO$_2$ donor concentration (mM)</th>
<th>0.0</th>
<th>0.4</th>
<th>0.8</th>
<th>1.2</th>
<th>1.6</th>
<th>2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germination percentage (%)</td>
<td>37 ± 3.3$^a$</td>
<td>42 ± 2.7$^a$</td>
<td>44 ± 3.5$^a$</td>
<td>56 ± 3.8$^a$</td>
<td>48 ± 2.7$^a$</td>
<td>47 ± 3.1$^a$</td>
</tr>
<tr>
<td>Length of radicle (cm)</td>
<td>1.42 ± 0.6$^a$</td>
<td>1.72 ± 0.4$^a$</td>
<td>1.80 ± 0.7$^a$</td>
<td>1.82 ± 0.7$^a$</td>
<td>1.78 ± 0.8$^a$</td>
<td>1.62 ± 0.4$^a$</td>
</tr>
<tr>
<td>Length of coleoptile (cm)</td>
<td>4.64 ± 0.4$^a$</td>
<td>4.70 ± 0.6$^a$</td>
<td>5.20 ± 0.5$^a$</td>
<td>5.83 ± 0.8$^a$</td>
<td>5.40 ± 0.8</td>
<td>5.23 ± 0.6$^a$</td>
</tr>
<tr>
<td>Radicle number (50 grains)</td>
<td>127 ± 7.3$^a$</td>
<td>135 ± 8.1$^a$</td>
<td>139 ± 8.1$^a$</td>
<td>148 ± 7.9$^a$</td>
<td>130 ± 6.7$^a$</td>
<td>119 ± 7.1$^a$</td>
</tr>
</tbody>
</table>

Values are the means ± SD (n = 6). Different letters mean significance of difference between different treatments ($P < 0.05$).

During the 12 h pretreatment time, no significant difference was observed in MDA content in wheat grains whether pretreated with SO$_2$ donor or H$_2$O (Figure 3(d)). After exposure to Al, the content of MDA in water pretreated grains increased rapidly till 48 h of Al stress. An increase of MDA content was also observed in SO$_2$ pretreatment at 12 h of Al stress, but thereafter MDA content remained stable until 36 h. SO$_2$ pretreatment dramatically reduced the amount of MDA from 24 h to 48 h of Al stress in comparison with grains pretreated in water.

3.5. Effects of SO$_2$ Donor Pretreatment on POD, CAT, APX, and LOX Activities. Activities of POD, CAT, APX, and LOX were determined with time in SO$_2$ donor and H$_2$O-pretreated grains exposed to Al (Figure 4). Figure 4(a) shows the time course of POD activity following pretreatment in SO$_2$ donor or H$_2$O for 12 h when POD activity showed almost a twofold increase. During Al stress, POD activity exhibited a gradual increase in both treatments, but SO$_2$ pretreatment maintained significantly higher level of POD activity during Al stress.

The activity of CAT increased almost twofold during 12 h pretreatment with H$_2$O or SO$_2$ donor (Figure 4(b)). After exposure to Al, CAT activity in water pretreatment decreased gradually till 48 h of Al stress, suggesting that CAT activity is very sensitive to Al stress. In contrast, CAT activity in SO$_2$ pretreatment increased steadily and decreased only slightly at 48 h of Al stress.

As shown in Figure 4(c), SO$_2$ pretreatment enhanced APX activity in Al-stressed wheat grain. A rapid increase in APX activity occurred during the pretreatment time in H$_2$O and SO$_2$. Within the first 12 h of Al stress, APX activity in H$_2$O-pretreated grains decreased sharply, whereas SO$_2$ donor pretreatment enhanced APX activity slightly. Thereafter APX activity increased steadily in water pretreated grain, whereas its activity in SO$_2$ donor pretreatment fluctuated slightly. The APX activity in SO$_2$ donor pretreated grains was always significantly higher than the counterpart of water pretreatment.

An increase in LOX activity was observed during the first 24 h of Al stress in SO$_2$ and H$_2$O-pretreated grains (Figure 4(d)). However, the increase of LOX activity in water pretreatment was more rapid than after SO$_2$ pretreatment. Thereafter LOX activity in water pretreatment showed a sharp decrease at 36 h of Al stress, while its activity in SO$_2$ pretreatment decreased at 48 h. At 12 and 24 h of Al stress, SO$_2$ pretreatment maintained significantly lower level of 

SO$_2$ donor pretreatment were higher than those pretreated in H$_2$O, they are not significantly different.

3.4. Effect of SO$_2$ Donor Pretreatment on Contents of Endogenous H$_2$S, O$_2$**, H$_2$O$_2$, and MDA. H$_2$S, which can be produced from sulfite, is involved in plant growth regulation including various abiotic stresses [8, 22]. To investigate whether exogenous SO$_2$ application can induce endogenous H$_2$S production, we measured the concentration of H$_2$S in Al-stressed wheat grain. Generally, H$_2$S accumulated during wheat grain germination following pretreatment with water or SO$_2$, but SO$_2$ donor pretreatment significantly enhanced H$_2$S concentration at 12 h of pretreatment and 12 h, 36 h of Al stress (Figure 3(a)).

To study the protective role of SO$_2$ in the Al-stressed wheat grain, reactive oxygen species O$_2$**, H$_2$O$_2$, and malondialdehyde (MDA) were determined with time. As shown in Figure 3(b), a rapid accumulation of O$_2$** was observed when H$_2$O-pretreated grains were exposed to Al. During the first 12 h of Al exposure, the increase in O$_2$** content was very rapid, but this was followed by a slow decrease. In contrast, the content of O$_2$** in SO$_2$ pretreatment increased slowly till 36 h of Al stress followed by a decrease. SO$_2$ pretreatment maintained significantly lower level of O$_2$** in Al-stressed wheat grains compared with grains incubated in H$_2$O and exposed to Al.

H$_2$O$_2$ in both treatments increased gradually during pretreatment time and 36 h of Al stress and decreased at 48 h (Figure 3(c)). However, H$_2$O$_2$ content in SO$_2$ pretreatment was significantly lower than that in water pretreatment when exposed to Al stress.

Figure 1: Effects of SO$_2$ pretreatment on wheat grain germination under 15 mM Al$^{3+}$ stress. Wheat grains were pretreated with 0, 0.4, 0.8, 1.2, 1.6, and 2.0 mM SO$_2$ for 12 h, subsequently subjected to 15 mM AlCl$_3$ for further 48 h, and then photographed.

Figure 2: Values are the means ± SD (n = 6). Different letters mean significance of difference between different treatments ($P < 0.05$).

Figure 3: (a) APX activity in H$_2$O-pretreated and SO$_2$-pretreated grains during the 12 h pretreatment time. APX activity in SO$_2$ donor pretreatment increased gradually till 36 h of Al stress, whereas its activity in H$_2$O-pretreated grains remained stable throughout the 48 h of Al stress. (b) CAT activity in H$_2$O-pretreated and SO$_2$-pretreated grains during the 12 h pretreatment time. CAT activity in SO$_2$ donor pretreatment increased steadily till 36 h of Al stress, whereas its activity in H$_2$O-pretreated grains decreased gradually till 36 h of Al stress. (c) LOX activity in H$_2$O-pretreated and SO$_2$-pretreated grains during the 12 h pretreatment time. LOX activity in SO$_2$ donor pretreatment increased sharply till 36 h of Al stress, whereas its activity in H$_2$O-pretreated grains decreased gradually till 36 h of Al stress. (d) MDA content in wheat grains under 15 mM Al$^{3+}$ stress. Wheat grains were pretreated in water or SO$_2$ donor for 12 h, exposed to Al for further 48 h, and then MDA content was determined.
**3.6. Effects of SO₂ Donor Pretreatment on Localization of Al, Lipid Peroxidation, and ROS Production.** To detect ROS production in the radicle tips, we used DCFH-DA fluorescence to indicate ROS accumulation. As shown in Figure 5(a), Al treatment induced higher level of ROS in radicle as intense DCFH-DA fluorescence, while SO₂ donor pretreatment for 12 h followed by Al stress significantly reduced fluorescence. Figure 5(b) shows DCFH-DA fluorescence in maturation zone in radicles. Similarly, intense fluorescence in SO₂ donor pretreatment followed by Al stress was much weaker than that in water pretreated plus Al-stressed radicles, suggesting that SO₂ donor was effective in alleviating oxidative stress in radicles. SO₂ donor treatment alone showed comparable fluorescence intensity as observed in water control.

The radicles were stained with Evans blue to show membrane integrity. The radicles treated with Al alone were stained extensively with Evans blue, while Al-stressed radicles pretreated with SO₂ donor for 12 h were less stained (Figure 5(c)), suggesting SO₂ donor serves to protect cell membrane from Al-induced damage. SO₂ donor treatment alone showed similar Evans blue staining to water control, suggesting no visible damaging effect of SO₂ on radicles.

The hematoxylin staining was used to detect Al accumulation in radicles. As shown in Figure 5(d), the radicles of water control and SO₂ treatment incubated with hematoxylin showed no dark staining but wheat radicles treated with Al alone were stained intensively. In contrast, radicles pretreated with SO₂ donor for 12 h and then exposed to Al for 48 h showed much weaker staining compared with Al stress, especially in the elongation zone.

**3.7. Effect of SO₂ Donor Pretreatment on the Relative Expressions of Aluminum Stress Related Genes.** We determined the changes in gene expression of aluminum stress related genes in wheat radicles. Radicles were pretreated with or without 1 mM SO₂ donors for 12 h and then exposed to Al for 48 h. As shown in Figure 6, Al stress induced higher expression of TaWali1, TaWali2, TaWali3, TaWali5, and TaWali6 (wheat aluminum induced) in radicles, while pretreatment with SO₂ donor for 12 h followed by Al stress alleviated such expression increase. Besides, the gene expression of TaALMT1 (Al-activated malate transporter) was also attenuated by SO₂ pretreatment.

**4. Discussion**

In solution, SO₂ is dissociated from its sulfite derivatives (NaHSO₃/Na₂SO₃ 1:3 M/M) [34]. Thus NaHSO₃/Na₂SO₃ (1:3 M/M) was chosen as an SO₂ donor in our study. Similar to the observation that H₂S could promote wheat grain germination and alleviate oxidative damage against Al stress [8], our results show that SO₂ donor pretreatment alleviates Al stress in germinating wheat seedlings. Wheat grains pretreated for 12 h with the SO₂ donor show an increase in germination percentage, coleoptile length, radicle length, and radicle numbers of wheat. The increase in the contents of reducing sugars and soluble protein suggests that nutrients in wheat grains pretreated with SO₂ donor are rapidly mobilized to provide energy to grain germination. SO₂ donor maintained lower level of H₂O₂, O₂⁻, and MDA probably
by activation of the antioxidant system. These results suggest that SO₃ acts as an antioxidant and may function in a way that is similar to what the effects of H₂S, CO, and NO do in plants exposed to heavy metal stress [10, 35].

Sulfite can be reduced by sulfite reductase to H₂S, which is incorporated into O-acetylsereine via O-acetyl(thiol)lyase to form cysteine [22]. In RNA interfered mutant of sulfite reductase (SiR), sulfide synthesis in younger leaves was decreased by the impaired SiR activity [36]. In the present study, exogenous SO₂ application can induce endogenous H₂S production in Al-stressed wheat grains (Figure 3(a)), suggesting the interplay between sulfite and the formation of H₂S.

Consistent with previous observations [7], our results show that Al stress caused overproduction of ROS in wheat. To mitigate and repair oxidative damage, plants have evolved an efficient antioxidant system that includes enzymes such as SOD, CAT, and APX that function to scavenge ROS [37]. SOD catalyzes the dismutation of the superoxide radical O₂⁻⁻ and H⁺ into H₂O₂. CAT, APX, and POD are responsible for the elimination of H₂O₂ generated by SOD. Al stress brings about a dramatic increase in H₂O₂ and O₂⁻⁻. The elevated levels of H₂O₂ and O₂⁻⁻ suggest that antioxidant enzymes in Al-stressed wheat do not efficiently scavenge the overproduction of ROS, and this can result in lipid peroxidation or plasma membrane inhibiting grain germination and seedling growth [8]. Our data show that pretreatment of wheat with SO₂ donor activates antioxidant enzymes including POD, CAT, and APX.

**Figure 3:** Effects of SO₂ pretreatment on the accumulation of endogenous H₂S (a), superoxide anion (O₂⁻⁻) (b), hydrogen peroxide (H₂O₂) (c), and malondialdehyde (MDA) (d) in germinating wheat grains under Al stress. The numbers (0', 12'/0, 12, 24, 36, and 48) or letters (CK or SO₂) presented are the same as mentioned in Figure 2. Al: Al stress without SO₂ pretreatment; S + Al: Al stress with SO₂ pretreatment.
LOX, which catalyzes oxygenation of polyunsaturated fatty acids into lipid hydroperoxides, is considered an indicator of oxidative stress during responses to various environmental stresses [9]. Pretreatment with SO$_2$ donor lowers LOX activity in Al-stressed wheat radicles compared to seedlings pretreated with H$_2$O and exposed to Al. The lowering of LOX by SO$_2$ pretreatment also helps to explain the lower MDA content of Al-stressed grain. Taken together, these data suggest that SO$_2$ donor reduced oxidative stress by modulation of the antioxidant system.

Our data indicate that the radicle is the primary target for Al toxicity. DCFH-DA fluorescence assay shows that Al incubation induces higher accumulation of ROS in radicle tips and maturation zone. SO$_2$ donor pretreatment effectively reduces ROS content in subsequent Al stress, suggesting the role of SO$_2$ in alleviating oxidative stress. Correspondently, Al stress causes membrane injury to radicles, while SO$_2$ donor effectively alleviates such injury. To understand whether SO$_2$ donor helps to reduce Al accumulation in radicles, hematoxylin staining was used to indicate Al and the results show that SO$_2$ donor obviously reduces Al content in radicles, implying a potential role of SO$_2$ donor treatment as a strategy to reduce Al uptake.

In response to Al stress, many gene expressions are activated, for instance, TaWali (wheat aluminum induced), aluminum-activated malate transporter (TaALMT1) [38–41].

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**Figure 4:** Effect of SO$_2$ donor pretreatment on the activities of POD (a), CAT (b), APX (c), and LOX (d) in germinating wheat grains under 15 mM Al stress. Grains were treated and the number or letters presented are the same as mentioned in Figure 2. Al: Al stress without SO$_2$ pretreatment; S + Al: Al stress with SO$_2$ pretreatment.
Figure 5: ROS staining ((a) on radicle tips; (b) on maturation zone; bar: 200 μm), Evans blue staining (c), and hematoxylin staining (d) in wheat radicles. Initially, wheat grains were germinated in water for 36 h. Then four treatment groups were done as follows, control, 60 h in \( \text{H}_2\text{O} \); Pre-S, pretreatment with 1 mM \( \text{SO}_2 \) donor for 12 h, and then exposed to \( \text{H}_2\text{O} \) for 48 h; Al, 12 h in \( \text{H}_2\text{O} \) prior to exposure to 400 μM \( \text{AlCl}_3 \) for 48 h; Pre-S + Al, 12 h in 1 mM \( \text{SO}_2 \) donor pretreatment followed by 400 μM \( \text{AlCl}_3 \) for 48 h.
Relative gene expression analysis shows that Al treatment induces higher expression of TaWali, while these gene expression levels are reduced by SO$_2$ donor pretreatment, suggesting the response to Al stress is attenuated in SO$_2$ donor pretreatment.

5. Conclusion

In the present study, SO$_2$ acts as an antioxidant signal to reduce ROS damage in wheat grains and radicles caused by Al stress. Besides, SO$_2$ also decreases Al uptake. The induced higher level of H$_2$S suggests an intricate interplay of SO$_2$ and H$_2$S in plants. Exogenous application of SO$_2$ may be reduced to H$_2$S by sulfite reductase, thus contributing to H$_2$S production. H$_2$S in itself acts as an antioxidant signaling molecule in plants’ response to abiotic stress. Thus the nature of SO$_2$/sulfite functions in alleviating Al stress still needs further research.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.
**Authors’ Contribution**

Dong-Bo Zhu, Kang-Di Hu, and Xi-Kai Guo contributed equally to this work.

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**References**


