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Sulfur dioxide promotes wheat seed germination under drought stress and regulates antioxidant metabolism in wheat

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Abstract

Sulfur dioxide (SO_2) is a potential signaling molecule, playing a crucial role in regulating multiple physiological processes in organisms. In the present study, we investigate the impact of SO_2 on the germination rate of wheat seed subjected to drought stress. Drought stress was stimulated using polyethylene glycol 6000, and the findings revealed that SO_2 pretreatment significantly enhanced the germination rate of wheat grain. Additionally, SO_2 pretreatment resulted in increased levels of reducing sugars and soluble proteins, as well as elevated amylase activity. Furthermore, SO_2 pretreatment of wheat grain significantly reduced the content of superoxide anion, hydrogen peroxide, and malondialdehyde, while increasing the activities of peroxidase (POD), ascorbate peroxidase and catalase. Additionally, SO_2 pretreatment was associated with a decrease in lipoxygenase activity and an increase in the levels of endogenous hydrogen sulfide. Principal component analysis revealed that POD is the most influential factor in the seed germination process. These findings suggest that SO_2 pretreatment may enhance the germination of wheat grain under drought conditions by facilitating the mobilization of storage materials and improving antioxidant capacity during the germination phase.

Keywords: antioxidants, drought stress, germination, sulfur dioxide, *Triticum aestivum*.

Introduction

With the drastic changes in global climate and the increasing frequency of extreme weather events, abiotic stress poses a significant threat to future food crop production and is a major contributor to crop yield reduction (Ahmad et al., 2010). Wheat, one of the world's primary food crops, is susceptible to both abiotic and biotic stresses at all stages of its growth and development. Numerous studies have indicated that drought stress severely limits the growth and productivity of wheat (Xue et al., 2014; Faran et al., 2019). Therefore, understanding the effects of drought stress on wheat grain germination and developing strategies to mitigate the inhibition of germination caused by drought are crucial for enhancing wheat yield.

Seed germination is the beginning stage of a plant's life cycle, during which seeds exhibit heightened sensitivity to changes in the external environment (Finch-Savage

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Abbreviations: ABA - abscisic acid; AsA-GSH - ascorbate-glutathione; APX - ascorbate peroxidase; CAT - catalase; Cys - cysteine; D-CDes - D-cysteine dehydrogenase; EDTA - ethylene diamine tetraacetic acid; GSH - glutathione; GSH-Px - glutathione peroxidase; L-CDes - L-cysteine dehydrogenase; LOX - lipoxygenase; MDA - malondialdehyde; OASTL - O-acetylserine sulfhydrylase; PAGE - polyacrylamide gel electrophoresis; PCA - principal component analysis; PEG-6000 - polyethylene glycol 6000; POD - peroxidase; ROS - reactive oxygen species; SiR - sulfite reductase; SOD - superoxide dismutase; TCA - trichloroacetic acid.
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and Leubner-Metzger, 2006; Khan et al., 2020). Studies have demonstrated that seed germination is inhibited and seedling emergence is reduced under drought conditions (Sheteiwy et al., 2018). This phenomenon is attributed to the decreased water uptake by the seed during the imbibition stage, which further affects the physiological and metabolic processes essential for germination (Fàbregas and Fernie, 2019; Khan et al., 2019; Patel et al., 2021). During the germination stage, carbohydrates and proteins serve as substrates to supply energy for germination, with amylase being the key enzyme that hydrolyzes amylopectin into soluble sugars. Insufficient external water content negatively impacts the enzyme activity of the plant, subsequently affecting seed germination (Fàbregas and Fernie, 2019; Zhao et al., 2020; Lei et al., 2021). Consequently, seeds adapt to drought stress by accumulating osmoregulatory substances such as sugars, amino acids, and soluble proteins to maintain osmotic balance and enhance water uptake (Sheteiwy et al., 2018; Ozturk et al., 2021; Siddiqui et al., 2021). In addition, under drought conditions, seeds produce excessive reactive oxygen species (ROS) including singlet oxygen species ($^1O_2$), superoxide anion ($O_2^{\bullet-}$), hydroxyl radicals ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2) (Choudhury et al., 2017; Razi and Muneer, 2021). These ROS can cause irreversible damage to plant nucleic acids, proteins, and lipids, leading to mechanical damage, plasma membrane disruption, and cell wall separation, which may ultimately result in cell death. Consequently, various antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) in the seed, play a crucial role in scavenging and defending against ROS (Wang et al., 2022).

Polyethylene glycol (PEG-6000) is a specific polymer characterized by its defined molecular weight. It is recognized for its non-toxic and non-ionic properties, and it is capable of inducing drought stress without inflicting direct cellular or physiological harm. PEG can modulate the osmotic potential of nutrient media in a relatively controllable manner and exhibits significant water absorption capabilities (Murillo-Amador et al., 2002; Almaghrabi, 2012). PEG is extensively utilized to create osmotic stress under controlled experimental conditions (Landjeva et al., 2008; Maulana et al., 2018; Lin et al., 2019; Mahpara et al., 2022). The physiological responses elicited by PEG treatment closely resemble those induced by drought conditions, leading to diminished germination rates, reduced seedling vigor, and overall stunted growth. In the study of wheat drought stress, PEG concentrations ranging from 5 to 15% are commonly employed (Kidokoro et al., 2015; Sallam et al., 2019; Moursi et al., 2020), while concentrations exceeding 25% are utilized to simulate conditions of severe drought stress (Mohamed et al., 2023).

Sulfur dioxide (SO_2) is a common, colorless sulfur oxide that is widely distributed in the environment. SO_2 dissolved in the cytosol generates sulfite (SO_3^{2-}) and bisulfite (HSO_3^-). Additionally, $O_2^{\bullet-}$ produced by chloroplasts oxidizes SO_3^{2-} to sulfate (SO_4^{2-}), simultaneously generating a significant amount of ROS, which can cause oxidative damage to cells (Chung et al., 2011). Once absorbed by the plant, the SO_3^{2-} formed primarily enters the sulfur assimilation pathway, where it is converted to sulfide (S^{2-}) by the enzyme sulfite reductase (SiR). Subsequently, the O-acetylserine sulfhydrylase (OASTL) catalyzes the formation of S^{2-} to the end product, cysteine (Cys) (Yi et al., 2005). Cys plays a crucial role in the conversion of compounds essential for various plant life activities, including biotin, coenzyme A, and glutathione (GSH) (Takahashi et al., 2011). In plant cells, GSH primarily exists in reduced and oxidized forms (Chan et al., 2013). Reduced GSH functions as an antioxidant, directly reacting with ROS *in vivo*, thereby maintaining the redox balance of tissues and cells during stress. Additionally, ROS can be metabolized through the action of enzymes such as glutathione S-transferase (GST) and glutathione peroxidase (GSH-Px) (Chan et al., 2013).

Plants possess the capability to alleviate SO_2 stress through the upregulation of antioxidant enzyme activity and modifying gene expression profiles (Li and Yi, 2012; Zhu, 2016). Empirical studies have demonstrated that prior exposure to SO_2 can induce stomatal closure, enhance antioxidant defense mechanisms, and provide protection against metal ion stress, particularly from Cd^{2+} and Al^{3+}, as well as drought stress (Hu et al., 2015; Zhu et al., 2015; Han et al., 2019). Moreover, SO_2 has been shown to enhance drought resistance in plants by activating the Ca^{2+} signaling pathway (Li et al., 2022a).

Hydrogen sulfide (H_2S) is an endogenous gaseous signal produced by plants, generated from L-cysteine through the catalysis of L-cysteine dehydrogenase (L-CDes) or from D-cysteine *via* D-cysteine dehydrogenase (D-CDes) (Riemenschneider et al., 2005; Hu et al., 2014; Luo et al., 2015). As a signaling molecule, H_2S could inhibit the formation of ROS, reduce cellular senescence, and consequently delay cell death under drought conditions (Jin et al., 2018). Additionally, H_2S may enhance the activity of antioxidant enzymes through the ascorbate-glutathione (AsA-GSH) cycle (Wang et al., 2024). In addition, H_2S mediates plants' responses to various abiotic stresses, including drought, and mitigates drought stress on seed germination by regulating ion homeostasis, enhancing enzyme activities, and inhibiting abscisic acid (ABA) signaling to protect seed germination from damage during drought conditions (Siddiqui et al., 2021).

H_2S functions as a potent reductant that can mitigate oxidative stress, potentially serving as one of the most effective strategies to improve the abiotic stress tolerance in plants (Liu and Xue, 2021; Hao et al., 2025). The study demonstrated that low concentrations of NaHS decrease the content of malondialdehyde (MDA) and hydrogen peroxide in wheat chloroplasts subjected to metal stress. Furthermore, the application of exogenous H_2S facilitates the persulfidation of ascorbate peroxidase (APX) in *Arabidopsis*, thereby enhancing its enzymatic activity (Aroca et al., 2015; Dai et al., 2016).

However, the effect of SO_2 on seed germination under drought conditions is still unclear. SO_2 serves as a precursor to H_2S, and the potential role of SO_2 in mediating drought stress through its influence on H_2S content warrants further examination. The present study indicates that SO_2 pretreatment under drought stress

facilitates the mobilization of storage materials in wheat grain and enhances their antioxidant capacity during germination, thereby contributing to improved seed germination. This work provides a significant theoretical foundation for further research on wheat seed germination under adverse stress conditions.

Materials and methods

The experimental material wheat (*Triticum aestivum* L.) Yannong 19 grains was provided by *Anhui Aidi Agricultural Science and Technology Co*. The combination of $NaHSO_3$ and Na_2SO_3 (1:3 M/M) was used as SO_2 donor (Laisk et al., 1988). The wheat grains were sterilized with 0.1% $HgCl_2$ for 3 min, washed thoroughly with H_2O, and then dried on filter paper. Wheat grains of similar size were selected and randomly assigned to Petri dishes (Zhu et al., 2015). 6 mL of ddH_2O or 0.4, 0.8, 1.2, 1.6, and 2.0 mM of SO_2 donor solution were added to the Petri dishes, and wheat grains were germinated at 25°C for 48 h. The germination percentage, radicle length, coleoptile length, radicle number were recorded. The wheat grains were subjected to a 12-h pretreatment with ddH_2O or 1.6 mM of SO_2 donor, followed by treatment with 25% polyethylene glycol 6000 (PEG-6000) solution. The 25% PEG-6000 solution was replenished every 12 h (Michel and Kaufmann, 1973). Germinated grains were sampled at 12-h intervals for subsequent analysis. The seeds should be placed in a weighing bottle and subsequently stored at 80°C for 12 h in a drying oven to reach a constant dry mass.

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 resulting homogenate was then subjected to centrifugation at 10 000 *g* for 30 min (Zhu et al., 2015). The supernatant was utilized for the determination of reducing sugars and soluble protein content. The reducing sugar content was assessed according to the methodology proposed by Miller (1959). For the soluble protein assay, 0.1 mL of the supernatant was mixed with 0.9 mL of water and 5 mL of Coomassie brilliant blue G250 for 5 min. The absorbance was recorded at 595 nm using the method developed by Bradford (1976).

Amylase and esterase activities: Wheat grains (0.5 ± 0.05 g) were ground in 5 mL of phosphate buffer (pH 7.0, 200 mM) and subjected to centrifugation at 12 000 *g* for 20 min at 4°C. The supernatant was utilized for the detection of amylase and esterase activities by native polyacrylamide gel electrophoresis (PAGE). The activities were determined by the method accordingly Yadav et al. (2024). For the amylase activity assay, the gel plate was submerged in 50 mM pH 7.0 phosphate buffer saline (containing 1.0% soluble starch) and incubated at 25°C for 20 min. The samples were incubated for 20 min and transferred to 0.05 mM pH 7.0 phosphate buffer saline for 10 min. Then they were placed in 0.6% I_2 and 6% KI solutions for gel staining. For the esterase activity assay, 0.05 g of α-naphthyl acetate, 0.05 g of β-naphthyl acetate, and 0.1 g of solid blue B fluoroborate were dissolved in acetone, and then added to 100 mL of 0.05 mM pH 7.0 phosphate buffer saline. The gel was immersed in the solution and incubated at 37°C for 20 min.

Malondialdehyde (MDA), $O_2^{\bullet-}$, and H_2O_2: Wheat grains were ground in a mortar with 5 mL of 4°C acetone, and the homogenate was centrifuged at 3 000 *g* for 10 min to determinate H_2O_2. Wheat grains were ground in 5 mL of 0.05 mM pH 7.8 phosphate buffer saline and quartz sand in a mortar and pestle in an ice bath, and the resulting homogenate was centrifuged at 10 000 *g* for 20 min at 4°C to determinate $O_2^{\bullet-}$. Wheat grains were ground in 5 mL of 10% trichloroacetic acid (TCA) and quartz sand in a mortar and the resulting homogenate was centrifuged at 3 000 *g* for 10 min to determinate MDA content. The contents of MDA, $O_2^{\bullet-}$, and H_2O_2 were determined by the method of Zhang et al. (2011).

Lipoxygenase (LOX), catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD) activities: Activity of LOX was determined in accordance with the methodology described by Surrey (1964) and those of CAT, APX, and POD were assayed according to Hu et al. (2012). The wheat grains were homogenized in ice-cold 50 mM phosphate buffer (pH 7.8) containing 1.0 mM ethylene diamine tetraacetic acid (EDTA). The homogenate was then subjected to centrifugation at 15 000 *g* at 4°C for 10 min, the supernatant was utilized for the determination of the enzyme activity.

Statistical analysis: Statistical significance was assessed using one-way *ANOVA*, and the results are presented as mean values ± SD (standard deviation) from three independent experiments. Significant differences among the parameters ($P < 0.05$) were analyzed by *Student*'s *t*-test or *Duncan*'s multiple tests. The correlation analysis and principal component analysis (PCA) were conducted based on the online tool on *Oebiotech* (https://cloud.oebiotech.com) and *Omicshare* (https://www.omicshare.com).

Results

SO_2 promoted wheat seed germination under drought stress: To determine the optimal concentration of SO_2 for wheat seed germination, a gradient of SO_2 donor concentrations was applied. Compared to the water control, the SO_2-treated grain exhibited a slight increase in all measured indices including germination percentage, radicle length, coleoptile length, and radicle number at each concentration, with the highest germination rate observed at an SO_2 donor concentration of 1.6 mM (Table 1). Additionally, wheat grains were subjected to drought stress at the optimal SO_2 donor concentration to evaluate its impact on germination. As shown in Fig. 1, under the pretreatment conditions, there was no significant difference in grain germination between the two groups. After 12 h of the drought stress simulated by

Table 1. Effects of SO_2 treatment on wheat seed germination under normal condition. Wheat grains were cultured in 0.0, 0.4, 0.8, 1.2, 1.6, and 2.0 mM SO_2 donor for 36 h, and then the parameters of wheat grain germination were investigated. Values are the means ± SD (n = 6). *Different letters* indicate significant differences between the treatments ($P < 0.05$, *Duncan*'s multiple test).

SO_2 donor concentration [mmol/L]	Germination percentage [%]	Radicle length [cm]	Coleoptile length [cm]	Radicle number [50 seeds]
0.0	64.4 ± 2.1^{d}	2.82 ± 0.6^{e}	4.04 ± 0.3^{f}	97 ± 8.7^{e}
0.4	67.7 ± 3.2^{c}	2.92 ± 0.8^{c}	4.20 ± 0.3^{e}	105 ± 8.9^{bc}
0.8	69.2 ± 3.4^{b}	3.04 ± 0.9^{b}	4.60 ± 0.4^{c}	109 ± 7.9^{b}
1.2	71.3 ± 5.3^{a}	2.87 ± 0.7^{d}	4.70 ± 0.4^{ab}	110 ± 8.6^{a}
1.6	73.4 ± 5.6^{a}	3.12 ± 0.9^{a}	5.03 ± 0.7^{a}	115 ± 10.6^{a}
2.0	70.2 ± 4.7^{ab}	2.78 ± 0.7^{ef}	4.43 ± 0.3^{cd}	99 ± 7.5^{e}

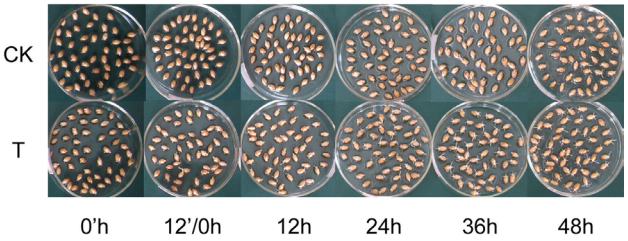

Fig. 1. Effects of SO_2 donor pretreatment on wheat seed germination under drought stress. Wheat grains were pretreated with water (CK) or 1.6 mM SO_2 donor (T) for 12 h (shown from 0' to 12'/0 h of pretreatment time) and then exposed to 25% PEG-6000 for further 48 h (shown as 12'/0, 12, 24, 36, and 48 h), and then photographed.

25% PEG-6000, wheat seeds in the SO_2 donor treatment group exhibited more pronounced germination, with the most notable difference observed at 36 h post-treatment. In contrast, the control group germinated approximately 36 h later than the treatment group (Fig. 1). These results indicate that the SO_2 treatment did not inhibit the germination of wheat grain under controlled conditions, however, SO_2 effectively promoted germination under drought stress.

SO_2 increased reducing sugar and soluble protein content of wheat seeds under drought stress during germination: Reducing sugar and soluble protein are organic nutrients involved in the process of seed germination. Changes in their metabolism could reflect the physiological conditions of plants. As shown in Fig. 2*A*, the reducing sugar content in the control group with water pretreatment increased slightly during drought stress. In contrast, the content of reducing sugar in wheat grain subjected to SO_2 pretreatment exhibited a slight decrease during the initial 24 h of the treatment. Throughout the treatment, the reducing sugar content in the SO_2-treated group remained consistently higher than that of the control group (Fig. 2*A*). Fig. 2*B* shows the alterations in soluble protein content between the two groups, suggesting that SO_2 pretreatment is beneficial for preserving soluble protein content during the germination of wheat seeds. These results imply that SO_2 pretreatment not only aids in maintaining reducing sugar content during the germination of wheat grains but may also facilitate protein synthesis.

SO_2 increased amylase activity in wheat seeds under drought stress: After that, we assessed the activities of amylase and esterase, which are essential for the mobilization of nutrients during the germination of wheat. The activity of amylase in wheat grains was found to increase in parallel with the germination process, with a notable enhancement observed after 24 h compared to earlier time points. Furthermore, the amylase activity in wheat seeds treated with an SO_2 donor was significantly greater than that of the control group (Fig. 2*C*,*D*). In contrast, esterase activity exhibited a pattern characterized by an initial decline followed by an increase, reaching its peak at 48 h post-treatment. The esterase activity in the SO_2 pretreatment group was not significantly elevated compared to the control group (Fig. 2*E*). These findings suggest that SO_2 pretreatment may enhance amylase activity in germinating wheat grains subjected to drought stress, thereby promoting germination process.

SO_2 reduced ROS content in wheat seeds under drought stress: Drought stress could induce the accumulation of ROS in plants (Jia et al., 2023). Therefore, we examined the content of $O_2^{\bullet-}$, H_2O_2, and MDA in wheat grains to study the potential role of SO_2 in regulating ROS metabolism. During the pretreatment phase, the concentration of $O_2^{\bullet-}$ increased in both the control and treated groups, although it remained lower in the treated group compared to the control. Upon the induction of stress using PEG-6000, the $O_2^{\bullet-}$ content continued to rise in the control group, while the SO_2 treated group exhibited a relative stable $O_2^{\bullet-}$ content at a lower level (Fig. 3*A*). The H_2O_2 content in both groups followed a similar pattern, reaching a peak at 36 h post-treatment before subsequently declining. Notably, throughout the duration of the experiment, the H_2O_2 content in the SO_2 pretreated group consistently remained lower than that in the control group (Fig. 3*B*). In contrast, the MDA content in both groups displayed a steady increasing trend throughout the treatment period, with content stabilizing around 36 h of stress (Fig. 3*C*). However, SO_2 pretreatment caused significantly lower content of MDA since 12 h of drought stress. These findings indicate that SO_2 pretreatment significantly reduces the content of $O_2^{\bullet-}$, H_2O_2, and MDA, thereby alleviating oxidative damage induced by drought stress in wheat grains.

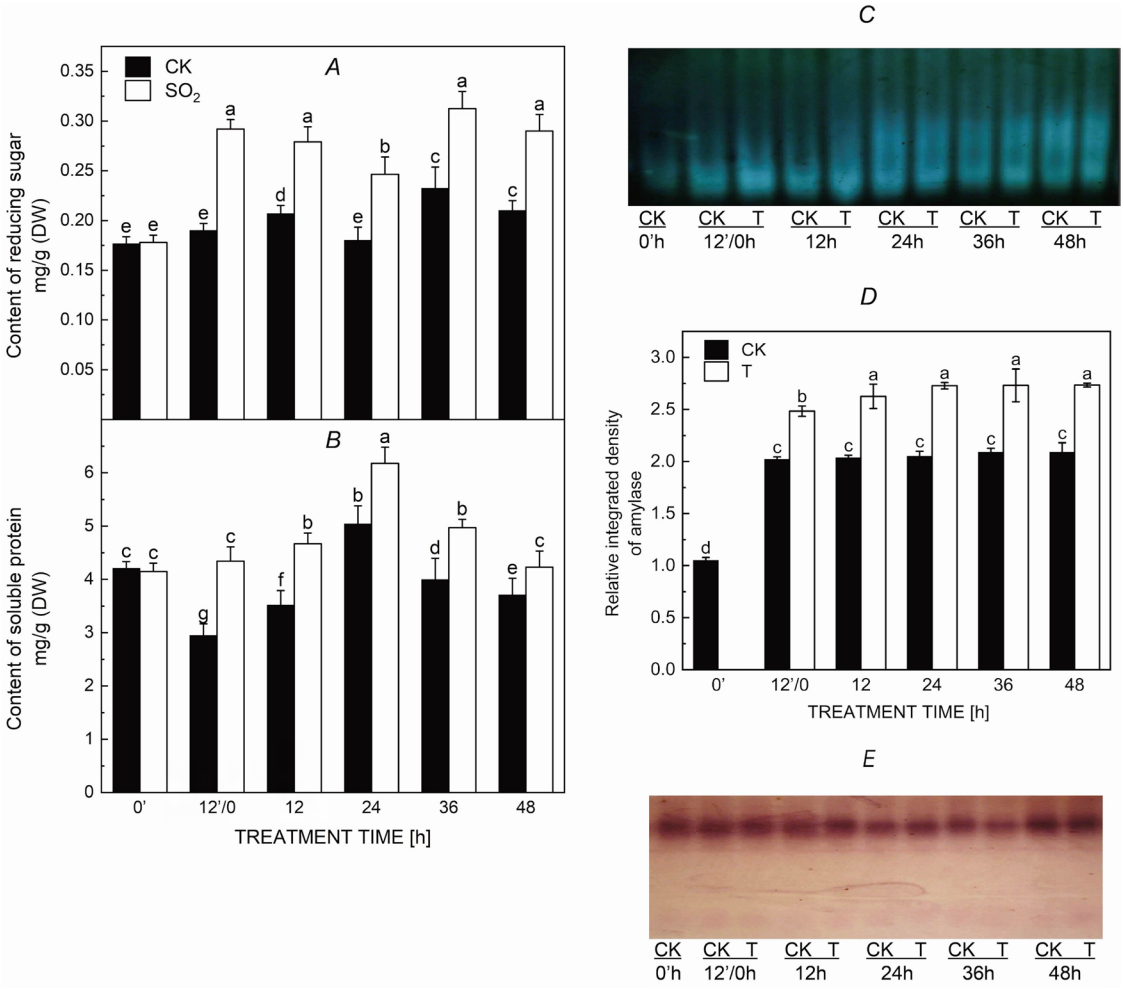

Fig. 2. Effects of SO_2 pretreatment on the accumulation of reducing sugar (*A*) and soluble protein (*B*) in germinating wheat seed under drought stress. (*D*) Relative integrated density of amylase activity. The data was processed using *ImageJ* software. Wheat grains were pretreated with water (CK) or 1.6 mM SO_2 donor (SO_2) for 12 h (shown from 0' to 12'/0 h of pretreatment time) and then exposed to 25% PEG-6000 for further 48 h (shown as 12'/0, 12, 24, 36, and 48 h). DW means dry weight. Data represent average ± SD (n = 3). *Different letters* indicate significant differences between the treatments ($P < 0.05$, *Duncan*'s multiple test). Effects of exogenous SO_2 treatments on the amylase activity (*C*) and esterase activity (*E*) by native PAGE of wheat grain subjected to 12 h of water (CK) or SO_2 (T) treatment followed by 25% PEG-6000 for further 48 h.

Effect of SO_2 on the activities of antioxidative enzymes: Antioxidant enzymes are important components of the plant's antioxidant system and play a significant role in maintaining redox balance in plant by metabolizing excessive ROS. To investigate how SO_2 treatment reduced ROS accumulation, we determined the activities of antioxidant enzymes APX, POD, and CAT. As shown in Fig. 4*A*, during the initial 12-h pretreatment, a significant increase in APX activity was observed compared with the control group. Thereafter, the activity of APX in the SO_2-pretreated group remained elevated and was significantly higher than that of water pretreatment control at 12, 36, and 48 h (Fig. 4*A*). Besides, SO_2 pretreatment could maintain stable level of POD activity during pretreatment time, while its activity reduced in water control compared with the initial time point (Fig. 4*B*). During duration of drought stress, the activity of POD initially showed an upward trend, followed by a slight decline, while its activity was significantly higher in SO_2 pretreated group compared with water control. For both control and SO_2 pretreated group, the activity of CAT gradually increased during drought stress, whereas its activity was always higher in SO_2 pretreated group in comparison to control (Fig. 4*C*). The activity of LOX in the control group peaked after 12 h of drought stress before decreasing and stabilizing. In contrast, the activity of LOX in the SO_2 pretreatment group exhibited inhibition at both the 12 h and 48 h, with a more significant reduction observed after 48 h. Furthermore, the activity of LOX in seeds pretreated with SO_2 exhibited no significant variation when compared to the control group (Fig. 4*D*). These findings suggest that SO_2 pretreatment can enhance the activities of POD, CAT, and APX, while also inhibiting the activity of LOX in wheat grain during germination under drought stress.

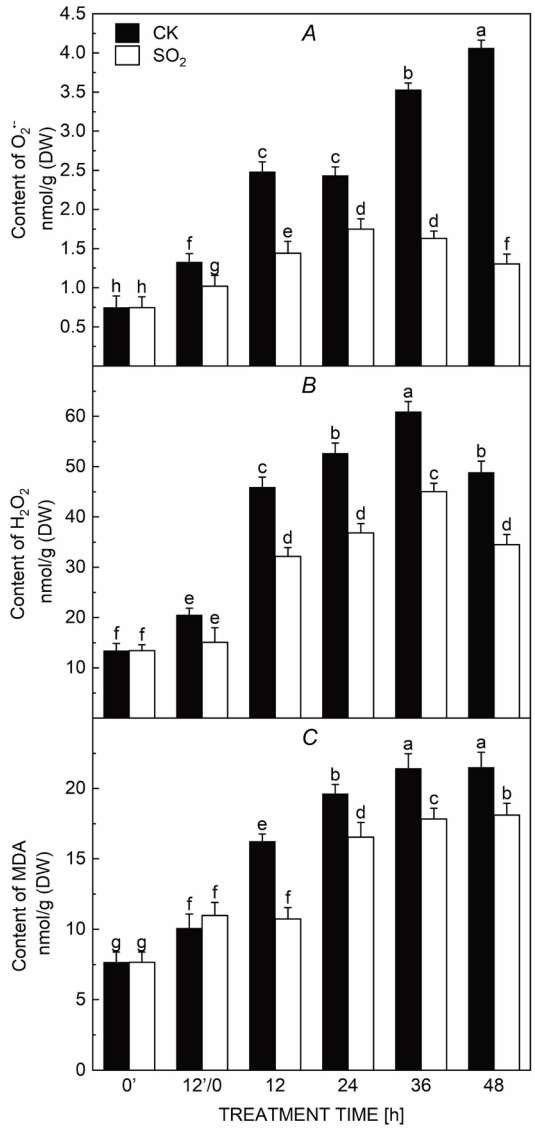

Fig. 3. Effects of SO_2 pretreatment on the accumulation of $O_2^{\bullet-}$ (*A*), H_2O_2 (*B*), and MDA (*C*) in germinating wheat grains under drought stress. Wheat grains were pretreated with water (CK) or 1.6 mM SO_2 donor (SO_2) for 12 h (shown from 0' to 12'/0 h of pretreatment time) and then exposed to 25% PEG-6000 for further 48 h (shown as 12'/0, 12, 24, 36, and 48 h). DW means dry weight. Data represent average ± SD ($n = 3$). *Different letters* indicate significant differences between the treatments ($P < 0.05$, *Duncan*'s multiple test).

SO_2 increased endogenous hydrogen sulfide content in wheat seeds under drought stress: SO_2 and H_2S exhibit a metabolic relationship, wherein sulfur dioxide can be converted into H_2S *via* enzymatic processes within the transsulfuration pathway (Liu et al., 2021). H_2S also signals the drought stress response, and so we observed alterations in H_2S content during the process of seed germination in drought stress. Following the imposition of drought stress on wheat seeds, the H_2S content in the control group remained largely unchanged from 12 to 36 h. However, a significant increase was observed at 48 h. In contrast, the SO_2 pretreatment group exhibited a notable increase of H_2S at 36 h, followed by a significant decline at 48 h. The content of endogenous H_2S in the SO_2 pretreatment group was significantly higher than that in the control group (Fig. 4*E*). These results suggest that SO_2 pretreatment may enhance the content of endogenous H_2S.

Correlation analysis of parameters and PCA: In order to elucidate the relationships among the various physiological indices, the index data were subjected to correlation analysis and principal component analysis. As illustrated in Fig. 5*A*, H_2S exhibited a positive correlation with the antioxidant enzymes POD, CAT, and APX, with correlation coefficients of 0.87, 0.69, and 0.83, respectively. Furthermore, the antioxidant enzymes POD, CAT, and APX demonstrated positive correlations with $O_2^{\bullet-}$, H_2O_2, and MDA. And the variations in $O_2^{\bullet-}$, APX, H_2S, reducing sugar, POD, and CAT were key factors influencing seed germination, with POD identified as the most critical factor in the seed germination process (Fig. 5*B*).

Discussion

During the seed germination, the germination rate of seeds and the subsequent growth of seedlings play a critical role in determining the future growth and yield of plants. Among the various environmental factors that influence plant development, water is of paramount importance (Liu et al., 2016). Research has demonstrated that the leaf water potential in maize can decline to -0.9 and -0.7 MPa, resulting in the cessation of leaf expansion (Boyer, 1970). A deficit in water availability can lead to alterations in the properties of cell wall extension. In staple crops such as rice and wheat, the ability of cell walls to extend is significantly diminished, which subsequently hampers overall plant growth (Nonami and Boyer, 1990; Covarrubias et al., 1995). Consequently, investigating the impact of abiotic stress on seed germination is of considerable significance.

There is an increasing body of evidence indicating that exogenous treatment with SO_2 enhances the expression of numerous genes associated with various physiological functions, including antioxidant activity, osmoregulation, and the synthesis of metabolites that influence seed germination and plant responses to environmental stressors (Li et al., 2022b). This study compared the germination indices of wheat seeds that were pretreated with SO_2 to those of water control, under conditions of drought stress. The findings revealed that treatment with different concentrations of SO_2 donors did not inhibit the germination of wheat seeds; instead, it appeared to significantly promote germination. This phenomenon can be explained by the observation that the increased levels of SO_2 resulted in a decrease in the concentration of ROS in wheat seeds, leading to a lower level of oxidative stress in the plants. Interestingly, this oxidative stress did not exert a toxic effect on the germination of wheat seeds; rather, it was associated with an enhancement in the germination rate (Zhu et al., 2015).

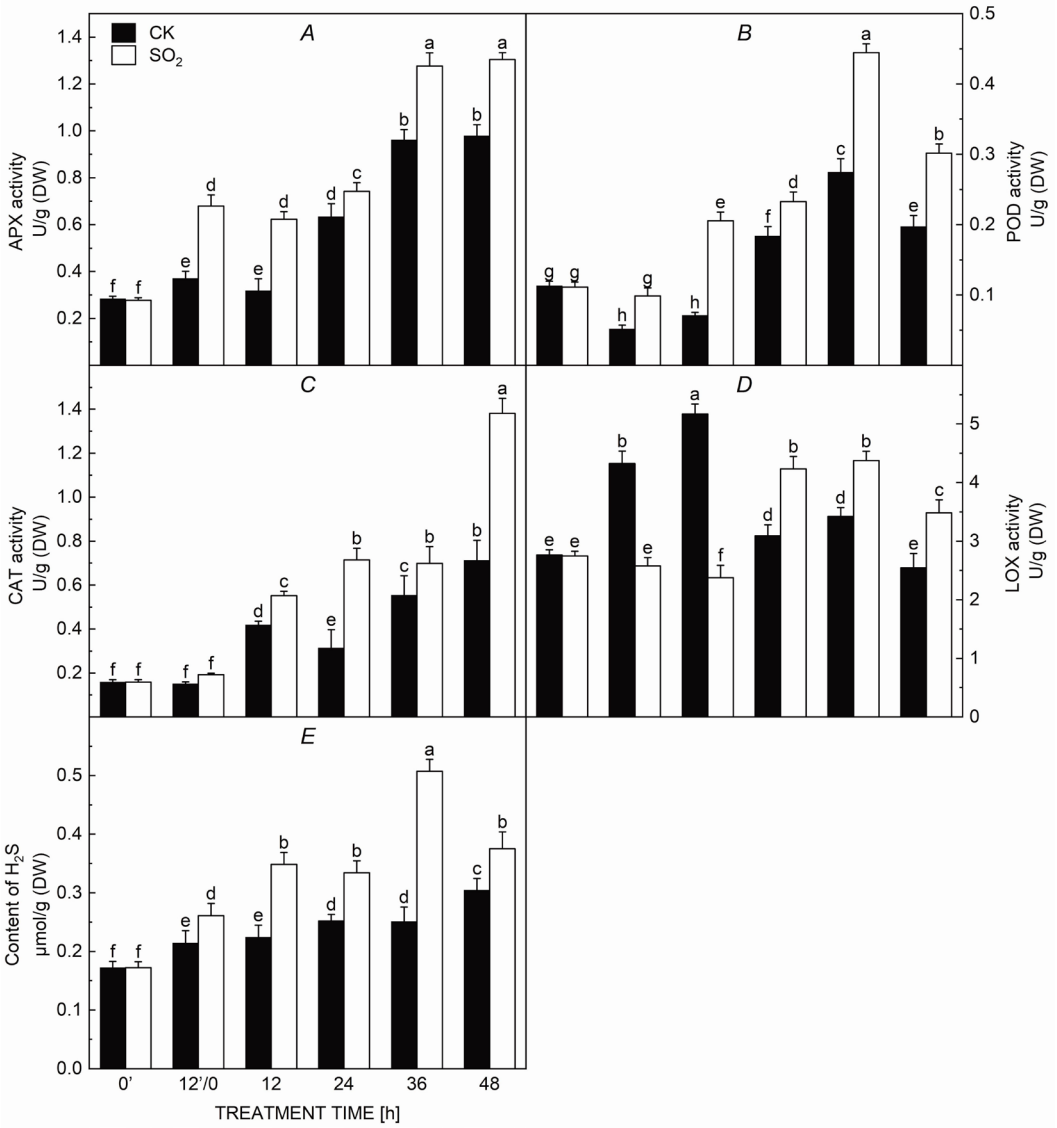

Fig. 4. Effect of SO_2 donor pretreatment on the activity of APX (*A*), POD (*B*), CAT (*C*), LOX (*D*), and endogenous H_2S (*E*) in germinating wheat grains under drought stress. Wheat grains were pretreated with water (CK) or 1.6 mM SO_2 donor (SO_2) for 12 h (shown from 0' to 12'/0 h of pretreatment time) and then exposed to 25% PEG-6000 for further 48 h (shown as 12'/0, 12, 24, 36, and 48 h). DW means dry weight. Data represent average ± SD (n = 3). *Different letters* indicate significant differences between the treatments ($P < 0.05$, *Duncan*'s multiple test).

In response to environmental stressors, plants modulate the synthesis of reducing sugars and soluble proteins, thereby improving their adaptability to such conditions. Consequently, this regulation serves as a significant indicator of the plant's physiological state (Janeček and Ševčík, 1999). The results showed that SO_2 pretreatment significantly increased the content of reducing sugars and soluble proteins in wheat seeds, which increased the plant's resistance to stress to a certain extent. Amylase is an important enzyme that is indispensable in the seed germination process. The results of the study showed that SO_2 pretreatment enhanced the activity of amylase. $O_2^{\bullet-}$, H_2O_2, and MDA content can be used as a measure of oxidative stress suffered in plants (Yu et al., 2024). The results showed that SO_2 pretreatment was able to reduce the accumulation of ROS. POD, CAT, APX, and LOX are antioxidant enzymes that resist adversity stress in plants. SO_2 can alleviate abiotic stresses by stimulating the antioxidant defense and enhancing the enzymatic activity of antioxidant enzymes. The results of the study showed that SO_2 pretreatment of wheat seeds could increase the activities of POD, CAT, and APX and effectively reduce the activity of LOX, thus alleviating lipid peroxidation. By PCA analysis, POD was determined to be the most critical factor for wheat seed germination under SO_2 pretreatment and drought stress conditions.

Correlation analysis indicated that H_2S exhibited a positive correlation with the antioxidant enzymes POD, CAT, and APX. The antioxidant enzymes POD, CAT, and APX demonstrated to be positively correlated with

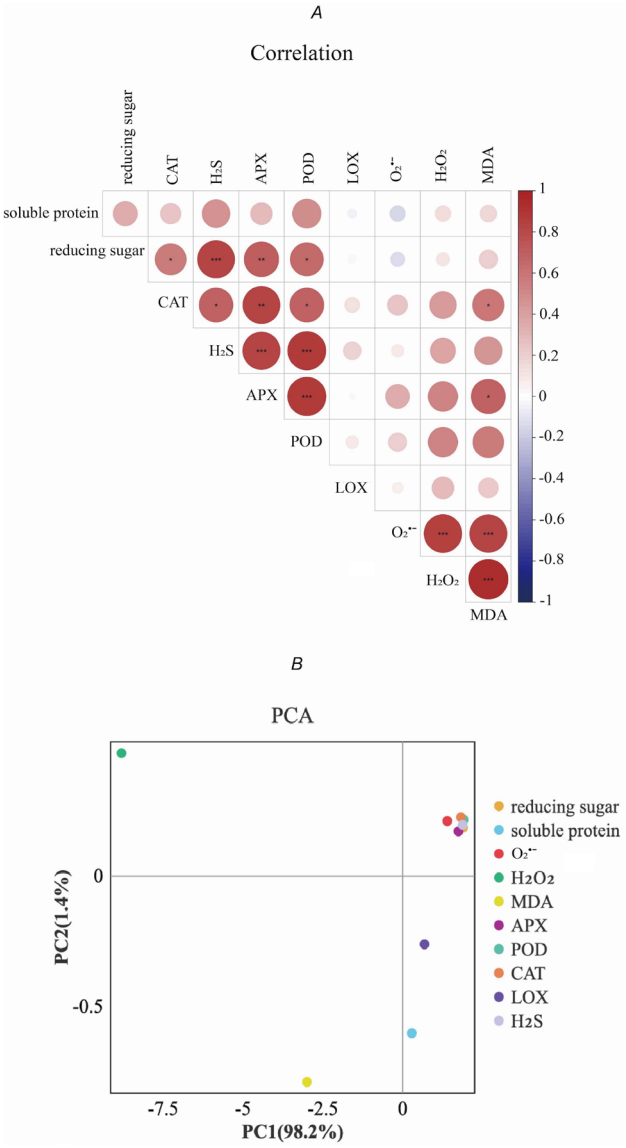

Fig. 5. Correlation analysis (*A*) and principal component analysis (PCA) (*B*). The indicators include reducing sugar, soluble protein, $O_2^{\bullet-}$, H_2O_2, MDA, APX, POD, CAT, LOX, and endogenous H_2S. Correlation analysis was produced by *Oebiotech*, while PCA was conducted by *Omicshare*.

$O_2^{\bullet-}$, H_2O_2, and MDA. The results suggested that during the process of seed development, wheat seeds generate a significant quantity of ROS as a response to drought stress. This production of ROS subsequently activates antioxidant enzymes within the wheat seeds, thereby increasing their activity. This enhanced enzymatic activity serves to mitigate the detrimental effects of drought stress on seed germination. H_2S is the third endogenous gas signaling molecule after NO and CO, and H_2S regulates multiple physiological functions and coordinates physiological processes and defense mechanisms. H_2S can alleviate the oxidative damage suffered by plants under stress (Siddiqui et al., 2021; Choudhary et al., 2022). The study measured the content of endogenous H_2S during wheat grain germination in drought stress, and the results showed that SO_2 pretreatment could increase the content of endogenous H_2S. H_2S plays a significant role in increasing plant antioxidant enzymes, among other functions, thereby promoting plant resistance to drought induced oxidative stress.

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