

The Enhancement of Drought Tolerance in *Arabidopsis* Plants Induced by Pretreatment with Sulfur Dioxide

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Research Article

Keywords: Sulfur dioxide, Drought tolerance, Photosynthesis, Sulfur assimilation, Osmotic adjustment

Posted Date: September 21st, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-627672/v1>

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Abstract

Sulfur dioxide (SO₂) is a common air pollutant that has multiple effects on plants. Here, the effect of prior exposure to SO₂ on the improvements of drought tolerance and possible regulation mechanisms were investigated in *Arabidopsis* plants. The experimental results showed that pre-exposure to 30 mg/m³ SO₂ for 72 h could reduce leaf water loss, and enhance the drought tolerance of *Arabidopsis* plants. SO₂ pre-exposure decreased leaf stomatal conductance (Gs) and transpiration rate (Tr) but increased net photosynthetic rate (Pn), water use efficiency (iWUE) and photosynthetic pigment contents under drought conditions. Importantly, the activities of superoxide dismutase (SOD) and peroxidase (POD) were significantly increased, while the contents of hydrogen peroxide (H₂O₂) and malondialdehyde (MDA) were decreased in SO₂-pretreated *Arabidopsis* plants under drought stress. Additionally, the activity of o-acetylserine(thio)lyase (OASTL) and the content of cysteine (Cys), the rate-limiting enzyme and the first organic product of sulfur assimilation, were increased significantly in drought-stressed plants after SO₂ pretreatment, along with the increases of other thiol-containing compounds glutathione (GSH) and non-protein thiol (NPT). Meanwhile, SO₂ pre-exposure induced a higher level of proline accumulation, accompanied by the increased activity of proline synthase P5CS, the decreased activity of proline dehydrogenase ProDH and the corresponding alteration of gene transcription. Collectively, the enhanced drought tolerance afforded by SO₂ might be related to the improvement of plant photosynthesis, antioxidant defense, sulfur assimilation and osmotic adjustment. These findings provide new insights in understanding the role of SO₂ in plant adaptation to environmental stress.

Introduction

Sulfur dioxide (SO₂) is one of the important gaseous pollutants that have an irreparable impact on plants, animals and ecosystem. Airborne SO₂ mainly enter plants through stomata and rapidly hydrolyzed into sulfite and bisulfite (Hänsch and Mendel 2005). Sulfite can be oxidized to sulfate accompanied by reactive oxygen species (ROS) production, or be fed into sulfur assimilation pathway to form cysteine (Cys) (Zhao and Yi 2014). The phytotoxicity of SO₂ is strongly dependent on its concentration and duration. High concentrations of SO₂ could cause leaf necrosis, growth inhibition and even plant death (Li et al. 2008; Muneer et al. 2014; Lee et al. 2017).

As sessile organisms, plants have evolved an array of defense mechanisms to cope with the environmental stress (Zandalinas et al. 2018; Peck and Mittler 2020; Zhao et al. 2021). In our previous studies, exposure to 30 mg/m³ SO₂ for 72 h triggered a series of defense responses, including stomatal closure, antioxidant response, and defense-related gene expression in *Arabidopsis* plants, associated with the increase of intracellular ROS levels (Li et al. 2008; Li et al. 2010; Li and Yi 2012a; Li and Yi 2012b). The ROS, which have emerged as signal molecules to activate the stress/defense responses (Baxter et al. 2014; Mittler 2017; Choudhury et al. 2017), might play some important roles in modulating plant physiology and biochemistry process under SO₂ stress.

Plants might be challenged by various environmental stimuli. The responses of plants to one environmental stress are capable of alleviating environmental stress and eliciting significant tolerance to other stresses (Achuo et al. 2006; Alcázar and Parker 2011; Hatmi et al. 2014; Xue and Yi 2018; Liu et al. 2021). Water deficit is the major abiotic stress threatening plant growth and productivity in arid and semiarid regions of the world (Wang et al. 2018; Marín-de la Rosa et al. 2019). Drought stress could cause stomatal closure, antioxidant defense, and osmolytes accumulation (Cruz de Carvalho 2008; Blum 2017; Choudhury et al. 2017; Saradadevi et al. 2017). The drought-induced physiological changes are similar with the responses of plants to SO₂ stress, implying that SO₂-induced defense responses might provide a specific plant physiological status contributing to drought tolerance. There were several studies reporting the enhanced drought tolerance in SO₂-exposed *Arabidopsis* and foxtail millet plants (Wang and Yi 2017; Han et al. 2019). However, the exact molecular mechanisms of SO₂-induced drought tolerance still remain largely unknown. Therefore, the objective of this study was to explore the effects of SO₂ pretreatment on the physiology and biochemistry of drought resistance in *Arabidopsis* plants to reveal the possible regulatory mechanisms.

Materials And Methods

Plant cultivation and treatments

The seeds of *Arabidopsis thaliana* wild type Columbia-0 (Col-0) were vernalized at 4°C for 24 h, and then sown on a culture medium of high-nutrient soil (KlasmannDeilmann, Germany) and vermiculite at a ratio of 3:1 in square pots. The cultures were maintained in a controlled growth room under a 60–70% relative humidity, temperature 23 ± 2°C, light/dark regime of 16/8 h and light intensity of 140 μmol m⁻² s⁻¹. These plants were watered regularly and watered with a nutrient solution after three weeks.

Four-week-old *Arabidopsis* plants were randomly divided into four groups: control, SO₂, drought and SO₂ + drought. (1) control: the plants with neither SO₂ nor drought treatment; (2) SO₂: the plants exposed with SO₂ only; (3) drought: the plants were exposed with filtered pollutant-free air and subsequently treated by drought stress. (4) SO₂ + drought: the plants were fumigated with SO₂ and subjected to drought stress later. For the control and SO₂ treatment, the plants were exposed with pollutant-free air or 30 mg/m³ SO₂ for 72 h in a chamber and subsequently watered regularly. Drought stress were performed by withholding irrigation.

Measurement of relative water content (RWC)

The detection of leaf RWC was performed according to the method of Nawaz et al. (2015). The RWC was calculated as follows: RWC (%) = (FW-DW) / (TW-DW) × 100%. FW, DW and TW represent fresh weight, dry weight and turgid weight, respectively.

Determination of leaf gas exchange and photosynthetic pigment contents

The photosynthetic gas exchange parameters were measured with a portable photosynthetic apparatus (SY-1020, Shiyakeji, Shijiazhuang), as described previously (Li and Yi 2020). The contents of leaf chlorophylls and carotenoids were determined by measuring the absorbances at 440 nm, 663 nm, 645 nm and 652 nm in 80% anhydrous acetone extracts according to Lichtenthaler (1987).

Measurement of relative electrical conductivity, malondialdehyde (MDA) and H₂O₂ contents

The relative electrical conductivity of *Arabidopsis* leaves was measured using DDS-307 Conductivity Meter (LEICI Company, China). Simply, twenty round leaf sections were cut from different individual plants and immediately immersed in 5 mL deionized water, followed by vacuum infiltration. The initial electrical conductivity (A_1) of the samples was detected. Then the samples were boiled for 30 min to release all electrolytes. The ultimate electrical conductivity (A_2) was measured again when the samples had cooled to 25°C. The relative electrical conductivity = $A_1/A_2 \times 100\%$.

MDA content was assayed by the thiobarbituric acid (TBA) reaction according to the method of Draper and Hadley (1990). H₂O₂ contents were measured using the procedure according to the H₂O₂ assay kit (Nanjing Jiancheng Bioengineering Institute, China).

Assay of antioxidant enzyme activities

The activities of antioxidant enzymes including superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) were measured using the method reported by Li and Yi (2012b). SOD activity was assayed by recording the decrease in optical density of nitroblue tetrazolium (NBT) at 560 nm. POD activity was measured by using guaiacol as a hydrogen donor and monitoring the increase in absorbance at 470 nm. CAT activity was determined by the rate of H₂O₂ decomposition at 240 nm.

The glutathione peroxidase (GPX) activity was determined by the rate of oxidation of reduced glutathione (GSH) by H₂O₂ oxidation following previously described method of Wendel (1981). The glutathione reductase (GR) activity was determined by the rate of reduction of oxidized glutathione (GSSG) by NADPH oxidation according to the method of Foyer and Halliwell (1976).

Determination of o-acetylserine(thio)lyase (OASTL) activity and the contents of cysteine (Cys), glutathione (GSH) and non-protein thiol (NPT)

The OASTL activity was assayed by quantification of the reaction product cysteine according to the method of Riemenschneider et al. (2005) with minor modification. Briefly, the plant shoots were homogenized with 20 mM Tris-HCl (pH 8.0) and centrifugated for the assay of enzyme activity. The reaction mixture, containing 20 mM Tris-HCl (pH 8.0), 50 mM sodium sulfide (Na₂S), 50 mM dithiothreitol

(DTT), 50 mM o-acetylserine (OAS) and the supernatant. One unit (U) of OASTL activity was defined as the amount of enzyme required to catalyze the formation of 1 μ mol of Cys per min at 25°C.

The contents of Cys, GSH and NPT were determined according to previously described methods (Li and Yi 2020). The assay of Cys level was performed by acid ninhydrin method and the absorbance of reaction mixture was measured at 560 nm. The contents of GSH and NPT were detected by the reaction of the supernatant and 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB), and the absorbance of reaction mixture was measured at 412 nm. The supernatants of GSH and NPT were extracted by 3% (w/v) trichloroacetic acid and 5% sulfosalicylic acid followed by centrifugation, respectively.

Measurement of the contents of proline and soluble sugar, and the activities of P5CS and ProDH

The determination of proline content was performed by using the ninhydrin method described by Bates et al. (1973). The *Arabidopsis* shoots were ground with 3% (w/v) sulfosalicylic acid and then the supernatant collected by centrifugation was mixed with acid-ninhydrin reagent for the analysis of proline content. The soluble sugar was extracted with distilled water and then incubated at 50°C for 15 min, followed by centrifugation. The supernatant was reacted with anthrone reagent and the absorbance of reaction mixture at 630 nm was measured for the assay of soluble sucrose content.

The P5CS activity was measured following the previously described method (Lei et al. 2007). The crude enzyme was extracted with 50 mM phosphate buffer (pH 7.0) containing 1% (w/v) PVP, 1 mM EDTA, 0.6 M KCl and 5 mM MgCl₂, followed by centrifugation. The reaction mixture contained 50 mM Tris-HCl (pH 7.5), 50 mM glutamic acid, 10 mM ATP, 2 mM MgCl₂, 1 mM NADH and the supernatant. One unit (U) of P5CS activity was defined as a change in absorbance of 0.01 at 440 nm per min.

The ProDH activity was determined from the change of NAD reduction at 340 nm according to previously described methods (Ren et al. 2018). The samples were extracted with 50 mM Tris-HCl buffer (pH 7.5) containing 5% (w/v) PVP, 7 mM MgCl₂, 3 mM EDTA, 1 mM DTT and 0.6 M KCl. The supernatant collected by centrifugation was used to measure the ProDH activity.

RNA isolation and quantitative RT-PCR analysis

RNAs were isolated from *Arabidopsis* shoots using Trizol reagent (TransGen Biotech, China) and were used as templates to synthesize cDNAs with a PrimeScript RT reagent kit (TaKaRa, Japan) according to the manufacturer's protocol. The transcription levels of genes were detected by quantitative RT-PCR with specific primers (Table 1). The changes of gene expression under different treatments were analyzed by using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001). The *Arabidopsis Actin2* was used as a standard control.

Statistical analysis

Each experiment was repeated with at least three times and the values have been subjected to statistical analysis. One-way analysis of variance (ANOVA) and Duncan's multiple range test were performed to compare the means of different data sets. For all the analyses, the significant difference was considered at $P < 0.05$.

Results

SO₂ pre-exposure enhanced the tolerance of *Arabidopsis* plants to drought stress

The morphology of *Arabidopsis* plants did not show obvious differences between SO₂ and control groups after 72 h of SO₂ exposure except a few transparent small spots appeared on the leaves of SO₂-fumigated plants. Under drought condition, leaf curling and wilting were occurred and gradually aggravated with prolonged drought stress. However, after SO₂ pre-exposure, the progress of leaf wilting and curling was slowed down under drought condition (Fig. 1A), and obvious differences were occurred at 8th days of drought treatment. The leaf RWCs constantly decreased with prolonged drought treatment, while the leaf RWCs in SO₂ + drought group were significantly higher than in drought group. The results of leaf RWCs were consistent with the change of plant morphology under drought stress, indicating that SO₂ pre-exposure improved the leaf RWCs and enhanced the adaptability of *Arabidopsis* plants to drought stress. Therefore, the *Arabidopsis* plants at 8th day of drought stress was selected as the materials to further explore the mechanisms underlying improvements in drought adaptability afforded by SO₂.

SO₂ pre-exposure enhanced photosynthetic rate and photosynthetic pigment contents in *Arabidopsis* plants under drought stress

Photosynthesis, the basic process of green plants, is very sensitive to environmental stresses. As shown in Fig. 2, drought stress decreased leaf gas exchange in *Arabidopsis* plants. Compared with the drought group, Gs and Tr decreased markedly by 28.6% and 20.0%, respectively (Fig. 2B and C), meanwhile Pn and iWUE increased significantly in SO₂ + drought group (Fig. 2A and E). No significant difference was found in Ci between drought and SO₂ + drought groups (Fig. 2D). These results indicated that pre-exposure to 30 mg/m³ SO₂ affected photosynthetic performance of *Arabidopsis* plants under drought stress.

Photosynthetic pigments are the important molecules responsible for photosynthesis. As shown in Fig. 2, the contents of photosynthetic pigments, including chlorophyll a, chlorophyll b and carotenoid, were significantly decreased under drought stress. Compared with drought treatment alone, the contents of chlorophyll a, chlorophyll b and carotenoid were increased by 14.03%, 8.15% and 12.40%, respectively, in

SO₂ + drought group (Fig. 2). These results indicated that SO₂ pre-exposure could efficiently inhibit the decline of photosynthetic pigments in *Arabidopsis* plants to support effective photosynthetic performance under drought stress.

SO₂ pre-exposure induced antioxidant defense responses to alleviate drought stress

Drought stress could induce ROS accumulation and disrupt the redox balance in plants. As shown in Fig. 3, the activities of SOD and POD were increased with the increases of H₂O₂ and MDA levels in drought-stressed *Arabidopsis* plants. Compared to drought group, the activities of SOD and POD in SO₂ + drought group were significantly increased, meanwhile the contents of H₂O₂ and MDA were markedly decreased, although no significant effect on CAT activity was found. Similarly, drought treatment caused the increase of the relative electrical conductivity, while SO₂ pre-exposure decreased the increment of relative electrical conductivity in drought-treated *Arabidopsis* plants. These results indicated that SO₂ pretreatment could induce antioxidant defense responses and effectively alleviate oxidative stress caused by drought stress.

SO₂ pre-exposure promoted sulfur assimilation and the biosynthesis of thiol-containing compounds in *Arabidopsis* plants under drought stress

Drought tolerance is associated with enhanced sulfur metabolism. As shown in Fig. 4, the activity of OASTL, the rate-limiting enzyme in sulfur assimilation pathway, was increased significantly in *Arabidopsis* plants under drought stress. Compared to drought stress alone, SO₂ pretreatment further enhanced the activity of OASTL in drought-treated *Arabidopsis* plants. Cys, the first organic product of sulfur assimilation, increased significantly under drought conditions, along with the increases of GSH and NPT contents. The contents of Cys, GSH and NPT were increased markedly in SO₂ + drought group as compared with drought stress alone (Fig. 4). The activities of GPX and GR (Fig. 4E and F) increased significantly under drought stress and showed a noticeably higher increment in SO₂ + drought group, which could accelerate turnover of the GSH redox cycle to reduce ROS accumulation under drought stress. Collectively, these results indicated that SO₂ pretreatment promoted sulfur assimilation and the biosynthesis of thiol-containing compounds, which could enhance the ROS-scavenging capacity to help maintain the cellular redox status under drought stress.

SO₂ pre-exposure enhanced osmotic adjustment of *Arabidopsis* plants under drought stress

Osmotic adjustment is a prime adaptive response to drought stress (Blum 2017). As shown in Fig. 5, the contents of proline and soluble sugar, the important osmoregulatory substances, were significantly increased under drought stress. And more, the increment was higher in SO₂ + drought group than in drought stress alone (Fig. 5A and B). These results demonstrated that SO₂ pretreatment could enhance the osmotic adjustment capacity to cope with drought stress by accumulation of osmoregulatory substances.

To further explore the responses of free proline accumulation, the activities of P5CS and ProDH, which are key enzymes functioning at proline synthesis and decomposition pathways, respectively, were measured. As shown in Fig. 5, the activities of P5CS and ProDH were increased markedly in drought-stressed *Arabidopsis* plants, while P5CS activity was significantly increased by 43.0% and ProDH activity was decreased by 27.2% in SO₂ + drought group as compared with drought treatment alone. Our findings indicated that SO₂ pretreatment promoted the proline accumulation for osmotic adjustment by increasing proline biosynthesis and decreasing proline decomposition in *Arabidopsis* plants.

SO₂ pre-exposure influenced the transcription of drought-responsive genes in *Arabidopsis* plants under drought stress

Transcriptional regulation is a key step in plant responses to environmental stress. The results showed that the transcription levels of genes encoding antioxidant enzymes (SOD, POD and CAT) were significantly up-regulated in *Arabidopsis* under drought condition. Moreover, the transcription levels of these genes were higher in SO₂ + drought group than in drought group. Similarly, the transcriptional levels of *P5CS1*, *P5CS2* (coding for Δ^1 -pyrroline-5-carboxylate synthetase) and *P5CR* (coding for pyrroline-5-carboxylate reductase) were up-regulated significantly in drought group and with much high increase in SO₂ + drought group, accompanied with the down-regulation of *ProDH* (coding for proline decomposing enzyme). The changes in gene expression were consistent with the alterations of their corresponding enzymes activities. Therefore, SO₂ pretreatment could influence gene expression regulating the physiological and cellular processes to cope with drought-induced oxidative stress, water deficit and other related changes in plant cells.

Discussion

Plants are often challenged with various environmental stresses simultaneously or consecutively. Cross-adaptation is the phenomenon that the adaptive changes caused by one stressor may make the organism more fit to resist the adverse effects of another type of stressor, which plays important roles in plant adapting to complex and changeable environments (Alcázar and Parker 2011; Hatmi et al. 2014; Xue and Yi 2018; Liu et al. 2021). In the present study, we report that SO₂ pre-exposure resulted in an enhanced tolerance of *Arabidopsis* plants to drought stress. The enhanced drought tolerance is mainly

attributed to the improving photosynthetic performance, the increasing biosynthesis of osmotic adjustment substances, and the enhancing antioxidant capability.

Photosynthesis of green plants is easily affected by drought stress (Chaves et al. 2009). Under water deficit conditions, the reduction of leaf RWC, the alteration of leaf gas exchange and the decrease of photosynthetic pigment contents could cause inhibition of photosynthesis (Degl'Innocenti et al. 2009; Misson et al. 2010; Hejnák et al. 2015). Here, SO₂ pretreatment effectively decreased the Gs in drought-stressed plant leaves, which may be beneficial for the reduction of Tr and the improvement of RWC under drought stress (Fig. 2B and C). However, the decreases of Gs and Tr in SO₂ + drought group did not directly lead to a lower Pn, which may be due to the enhanced iWUE in SO₂ pre-exposed *Arabidopsis* plants (Fig. 2E). The higher leaf RWCs and the improvement of iWUE induced by SO₂ pretreatment were of great relevance for drought tolerance of *Arabidopsis* plants under drought stress. Additionally, the increases of chlorophylls and carotenoid contents in SO₂ pretreatment group (Fig. 2), could promote the light harvesting and photosynthetic efficiency of drought-stressed *Arabidopsis* plants, consistent with the higher Pn under drought stress. Taken together, the high photosynthetic performance was contributed to the enhanced drought tolerance of *Arabidopsis* plants.

Drought stress generally causes high levels of ROS in plant cells, which is considered to be the main cause for the harmful effects of drought stress on plants (Osakabe et al. 2014; Nakabayashi et al. 2014; Wei et al. 2019). Excessive accumulation of ROS would cause oxidative stress to the organisms and seriously affect plant growth and development (Tambussi et al. 2000; Cruz de Carvalho 2008; Noctor et al. 2014; Choudhury et al. 2017). In this study, SO₂ pretreatment significantly decreased the contents of H₂O₂ and MDA in drought-stressed *Arabidopsis* plants, indicating that SO₂ could alleviate oxidative stress and oxidative damage caused by drought stress. These positive effects were dependent on the SO₂ pretreatment-triggered increases of antioxidant capability in *Arabidopsis* plants. The increased activities of SOD and POD, associated with high levels of antioxidant substances, could provide an enhanced ROS scavenging capacity to eliminate drought-induced ROS and alleviate oxidative damage.

It has been reported that ROS could act as signal molecules mediating the responses of plants to biotic and abiotic stresses, such as drought stress and high concentrations of SO₂ (Cruz de Carvalho 2008; Yi et al. 2017; Mahmood et al. 2020). The increased ROS evoked by SO₂ might trigger a series of responses including antioxidant defense, sulfur assimilation activation and proline synthesis contributing to drought tolerance of *Arabidopsis* plants, although the exact mechanisms need further study.

The sulfur assimilation and downstream metabolic pathways play important roles in plant response to drought stress (Chan et al. 2013; Stanislav et al. 2019). In the present study, SO₂ pre-exposure promoted the sulfur assimilation in the leaves of drought-stressed *Arabidopsis* plants, as evidenced by the enhanced OASTL activity and the increased Cys content. The increased Cys content is beneficial for the biosynthesis of other thiol-containing compounds, including GSH and NPT (Fig. 4). High GSH level together with increased GPX and GR activities could enhance the ROS scavenging capacity of GSH-GSSG

cycle in drought-treated *Arabidopsis* plants, and reduce drought-caused damage. Taken together, SO₂ pretreatment enhanced the sulfur assimilation and further promoted the synthesis of GSH and NPT to complete the detoxification under drought stress.

The accumulation of osmoprotectants (such as soluble sugar, proline, and betaine) is a significant strategy for plants to maintain water potential and membrane stability to avoid drought-induced damage (Fang and Xiong 2015). In this study, drought stress resulted in a significant increase of the contents of proline and soluble sugar in *Arabidopsis* plants. Moreover, SO₂ pretreatment induced a higher level of proline accumulation in drought-stressed *Arabidopsis* plants, consistent with the increased P5CS activity and decreased ProDH activity. In accordance with this result, the alteration of the transcription level of key genes involved in proline biosynthesis pathway (*P5CS1*, *P5CS2* and *P5CR*) and degradation pathway (*ProDH*) showed the similar tendencies, indicating the involvement of gene expression and cellular metabolism in SO₂ pretreatment-induced drought tolerance. In addition to its accepted role as an osmolyte, proline can act as a potent nonenzymatic antioxidant induced by ROS, contributing to overall antioxidant activity in plant response to limited water supply (Ben Rejeb et al. 2014). Therefore, proline accumulation induced by SO₂ pretreatment might protect plant cells by osmotic adjustment, and also promote redox balance by scavenging ROS under drought stress, which contribute to the improvement of drought tolerance in *Arabidopsis* plants.

In conclusion, this study provides a systematic description of key events leading to enhanced drought tolerance in SO₂-pretreated *Arabidopsis* plants (Fig. 7). SO₂ pretreatment significantly reduced leaf water loss and maintained higher RWC, photosynthetic activity and proline levels, as well as higher P5CS activity under water deficit conditions. Importantly, SO₂-induced antioxidant enzymes, together with high levels of nonenzymatic antioxidants increased the ROS scavenging capacity to recover drought-induced cellular redox imbalance. Taken together, SO₂ exposure could enhance drought tolerance in *Arabidopsis* grown in water shortage conditions by increasing the photosynthesis performance, antioxidant defense, sulfur assimilation, and osmoprotectant accumulation. The present study reveals the molecular mechanism of cross-adaptation of plants under SO₂ and drought stress, and provides a new perspective for future researches on stress-resistance mechanisms. However, more detailed analyses might be still needed to better understand the role of SO₂ in plant adaptation to drought stress.

Declarations

Author Contribution

Lijuan Li measured photosynthetic parameters, analysed antioxidant indexes and gene expression, and wrote the manuscript. Huilan Yi conceived and guided the project, and reviewed and edited manuscript. All authors read and approved the final manuscript.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 30870454; No. 31371868; No. 31972132).

Compliance with Ethical Standards

This article does not contain any studies with human participants or vertebrate animals performed by any of the authors.

Conflict of interest

The authors declare that they have no conflict of interest.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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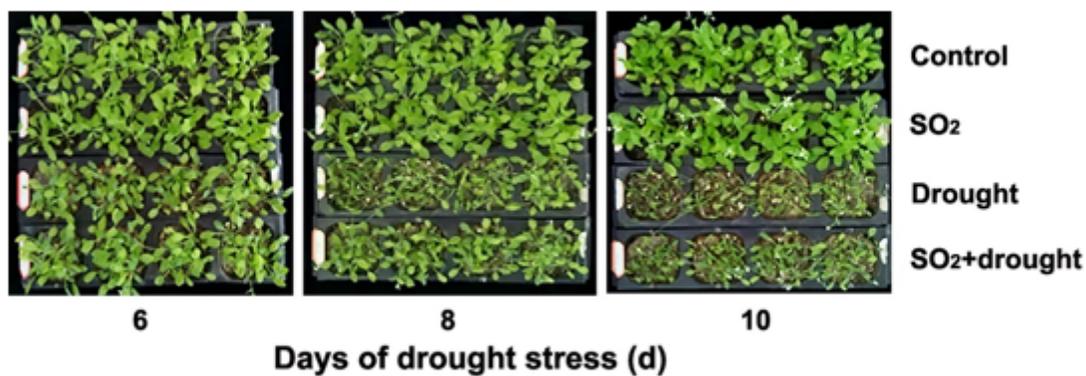
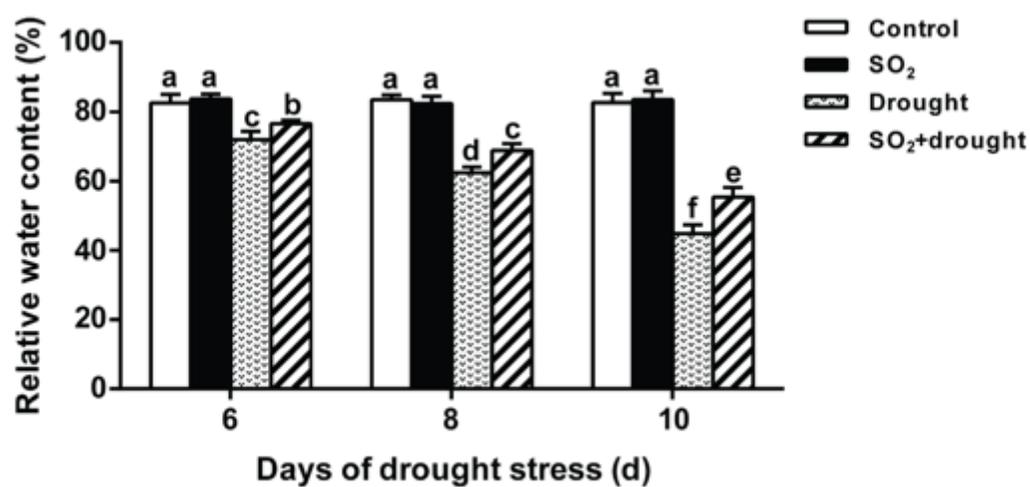
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Tables

Table 1 Primer sequences used in real-time PCR

Gene	Primer sequence
<i>CSD1</i>	5'-AGACGAAGCAAAAACATTTCAGAGA-3' 5'-GGCCAGAAACTGTTCCACTC-3'
<i>CSD2</i>	5'-ATGGCTGCCACCAACACAATCC-3' 5'-TTAGAGCGGCGTCAAGCCAATC-3'
<i>CAT</i>	5'-TCACAGCCACGCCACTAA-3' 5'-AGAACCAAGCGACCAACC-3'
<i>POD</i>	5'-TCCGGGAGCCACACCATTGG-3' 5'-TGGTCGGAATTCAACAG-3'
<i>P5CS1</i>	5'-AACGCCAGCACAAGATTC-3' 5'-CCTCTCATTATCCATCTCGTTGT-3'
<i>P5CS2</i>	5'-ATGATCTTATTTATGTTCTGC-3' 5'-CACTATCTTCCGTCACTAT-3'
<i>P5CR</i>	5'-CACAGACCGTTCTTGGAGCTG-3' 5'-GTGTTGCCCGGAAAGAGCCTTT-3'
<i>ProDH</i>	5'-CGCCAGTCCACGACACAATTCA-3' 5'-CGAATCAGCGTTATGTGTTGCG-3'
<i>Actin2</i>	5'-TGCGATAATGGAAGTGGTATGG-3' 5'-AAGACAGCCCTGGGCGCATCA-3'

Figures

A**B****Figure 1**

Effects of SO₂ pretreatment on morphology (A) and leaf relative water content (B) of four-week-old *Arabidopsis* plants under drought stress conditions. The plants were exposed to 30 mg/m³ SO₂ for 72 h and then subjected to drought stress by withholding watering. Columns labelled with different letters (a, b, c, d, e) indicate significant differences at P<0.05.

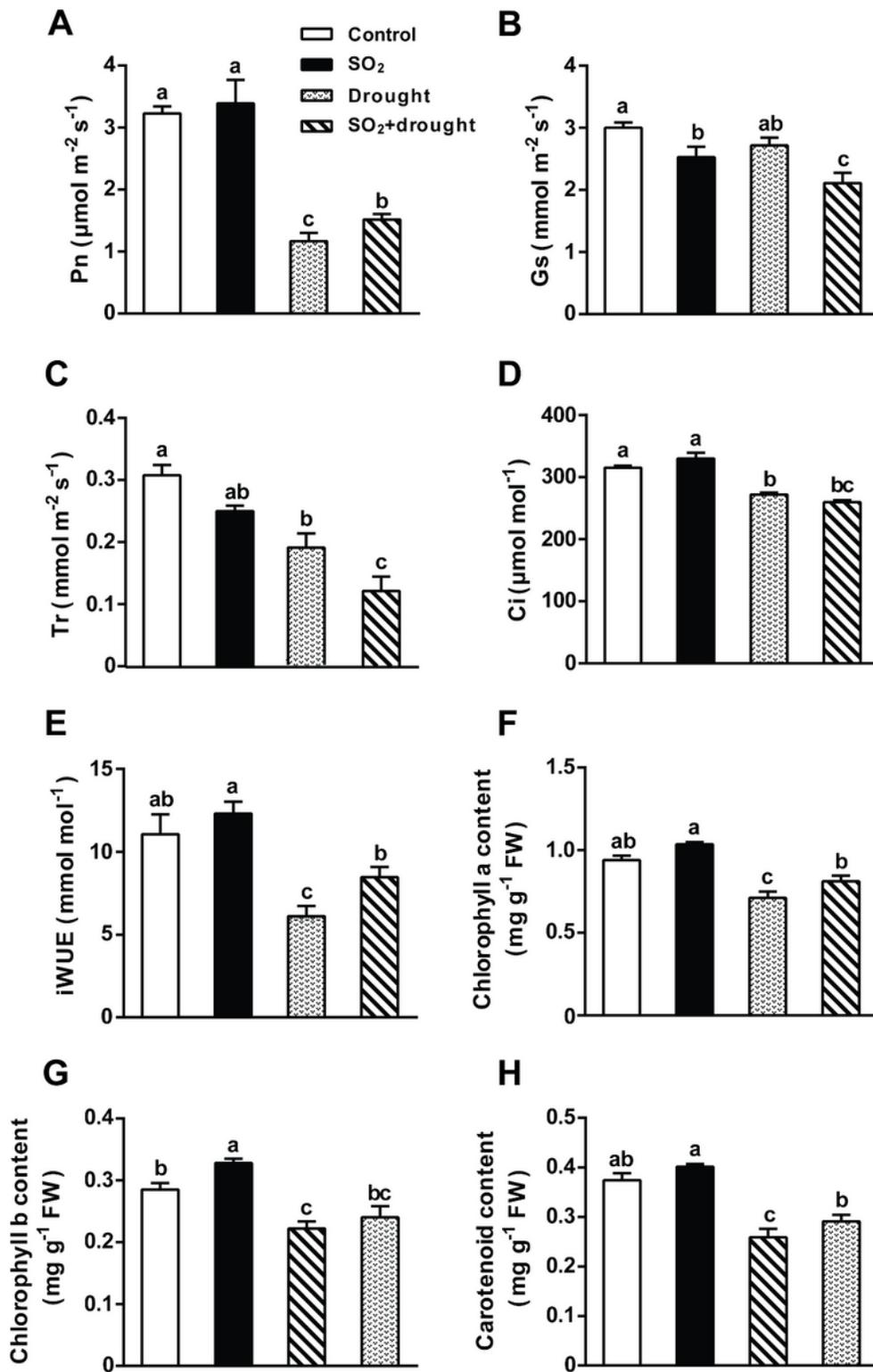


Figure 2

Effects of SO₂ pretreatment on leaf gas exchange and photosynthetic pigments of four-week-old Arabidopsis plants under drought stress. The plants were exposed to 30 mg/m³ SO₂ for 72 h and then subjected to drought stress for 8 days. A, Net photosynthetic rate (Pn); B, Stomatal conductance (Gs); C, Transpiration rate (Tr); D, Intercellular CO₂ concentration (Ci); E, Intrinsic water use efficiency (iWUE); F,

Chlorophyll a content; G, Chlorophyll b content; H, Carotenoids content. Columns labelled with different letters (a, b, c) indicate significant differences at $P < 0.05$.

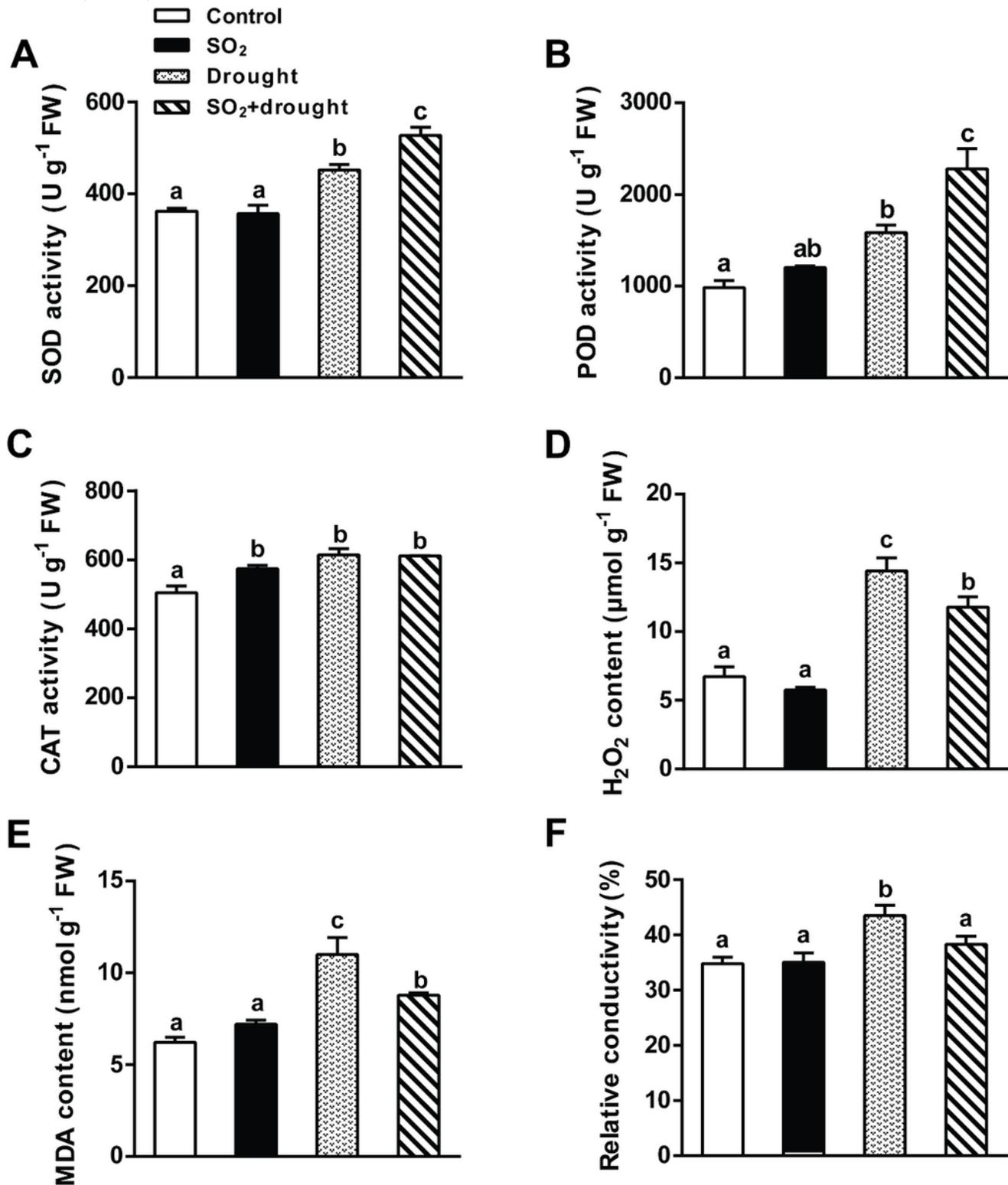


Figure 3

Effects of SO₂ pretreatment on the antioxidant responses of four-week-old *Arabidopsis* plants to drought stress. The plants were selected after 0 or 8 days of drought stress with or without SO₂ pretreatment. Bars with different letters (a, b, c) indicate significant differences at $P < 0.05$.

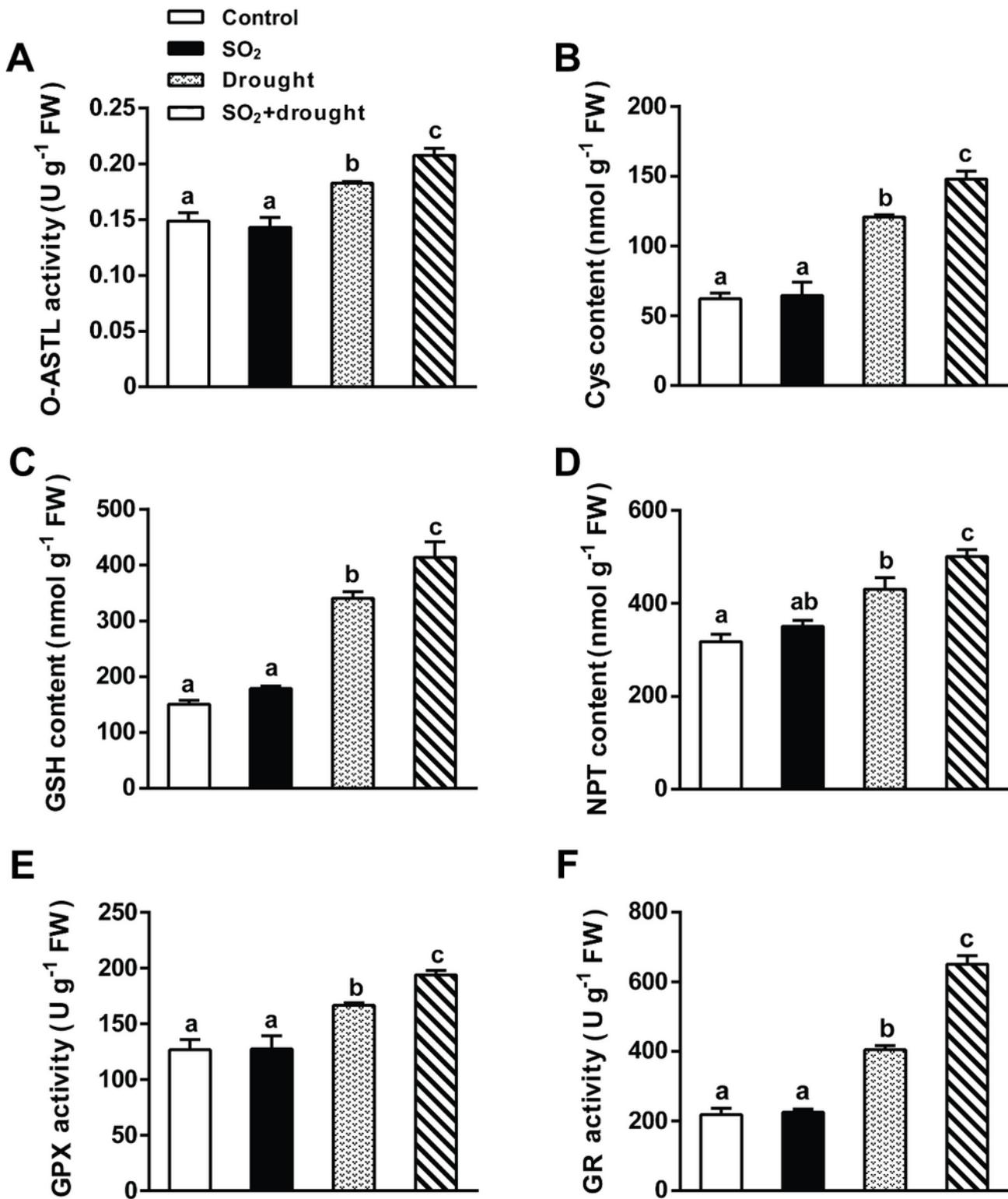


Figure 4

Effects of SO₂ pretreatment on the activity of OASTL and the contents of Cys, GSH and NPT in four-week-old *Arabidopsis* plants under drought stress. The plants were selected after 0 or 8 days of drought stress with or without SO₂ pretreatment. Columns labelled with different letters (a, b, c) indicate significant differences ($P < 0.05$).

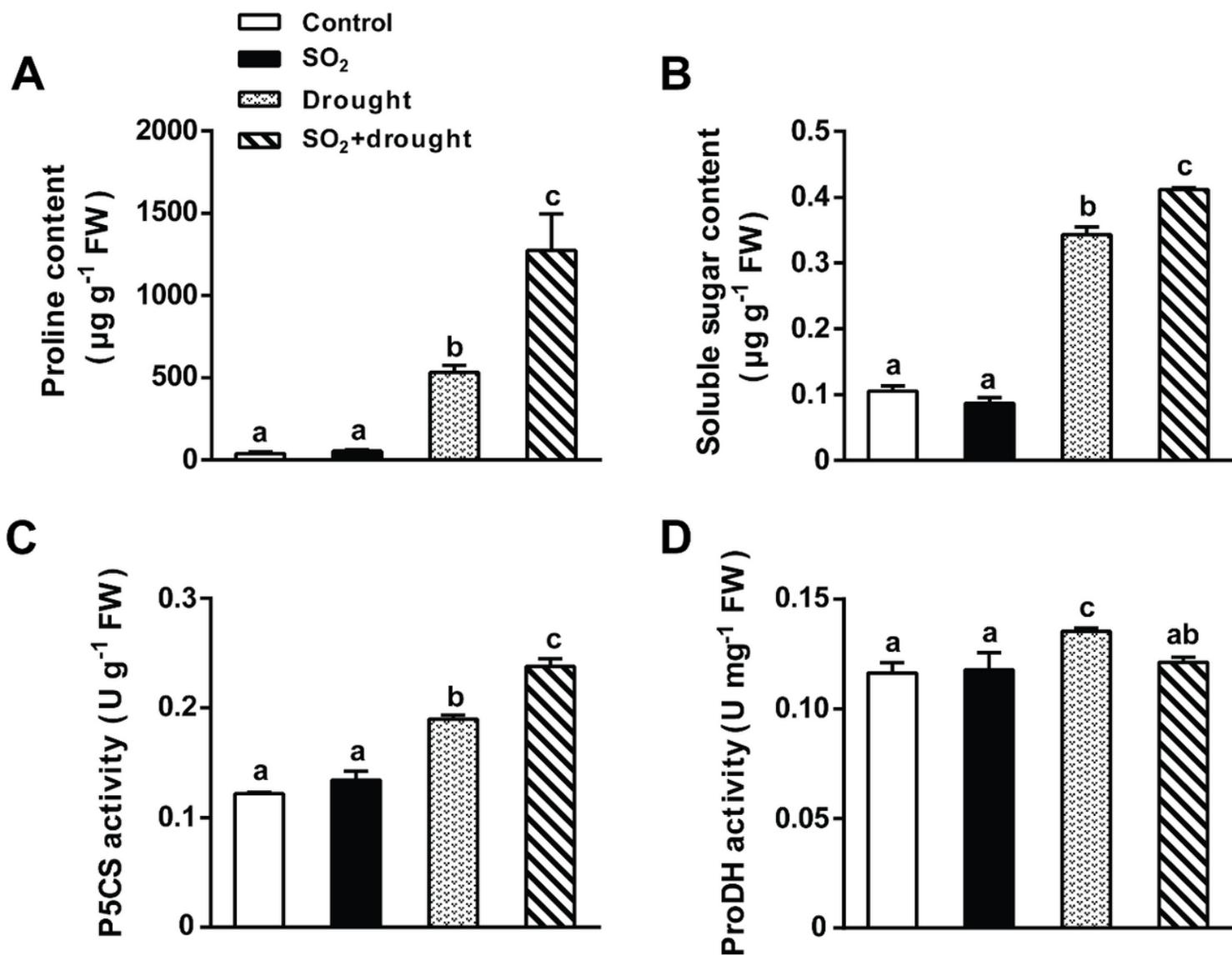


Figure 5

Effects of SO₂ pretreatment on the contents of proline and soluble sugar, the activities of P5CS and ProDH in four-week-old *Arabidopsis* plants under drought stress. The plants well-watered and drought-stressed for 8 days were used for the measurements after SO₂ pretreatment. Bars with different letters indicate significant differences ($P < 0.05$).

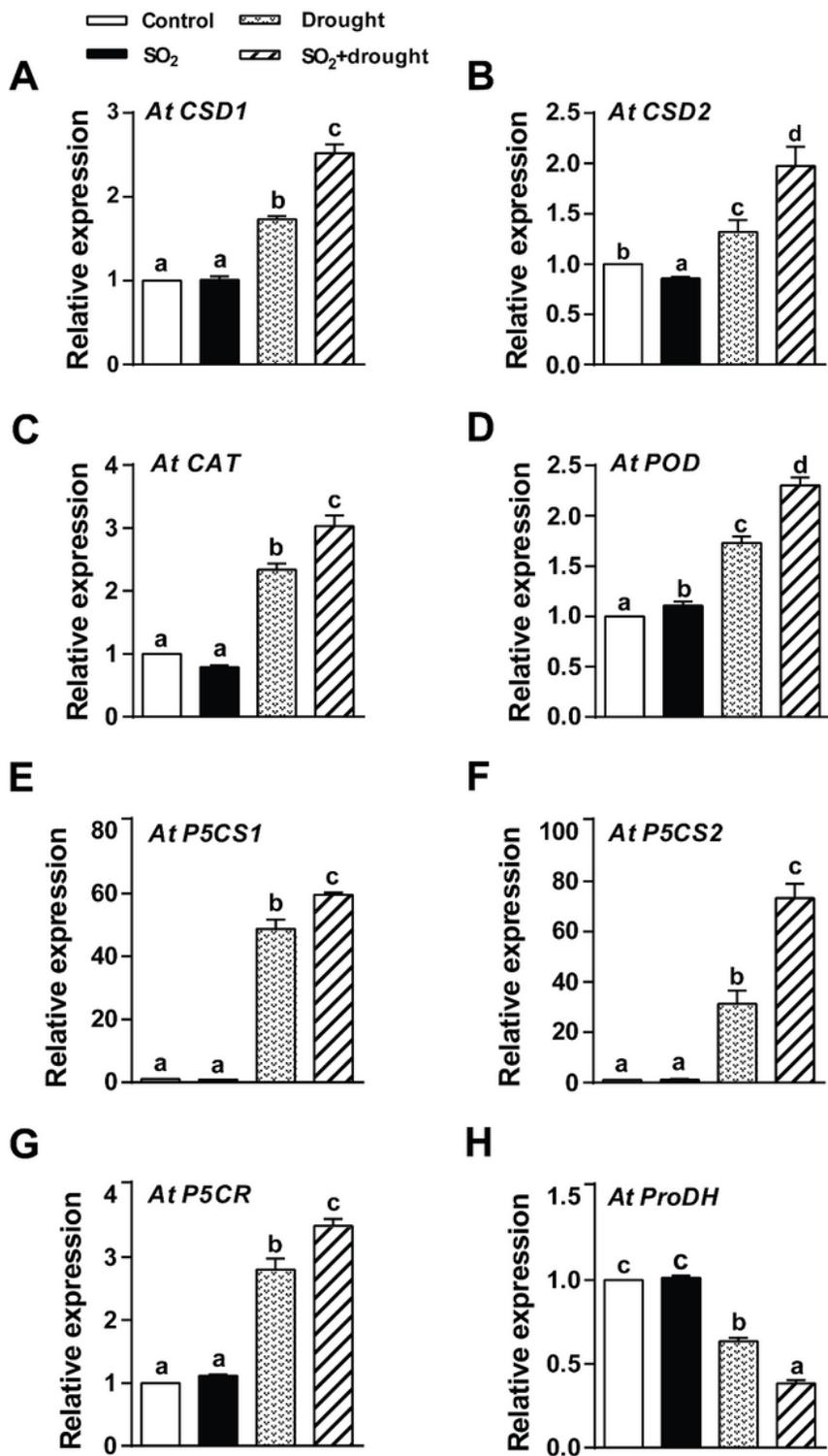


Figure 6

Effects of SO₂ pretreatment on the expression of genes in four-week-old Arabidopsis plants under drought stress. The plants were pre-exposed to 30 mg/m³ SO₂ for 72 h and then were subjected to drought treatment for 8 days. Different letters (a, b, c, d) indicate significant differences at P < 0.05.

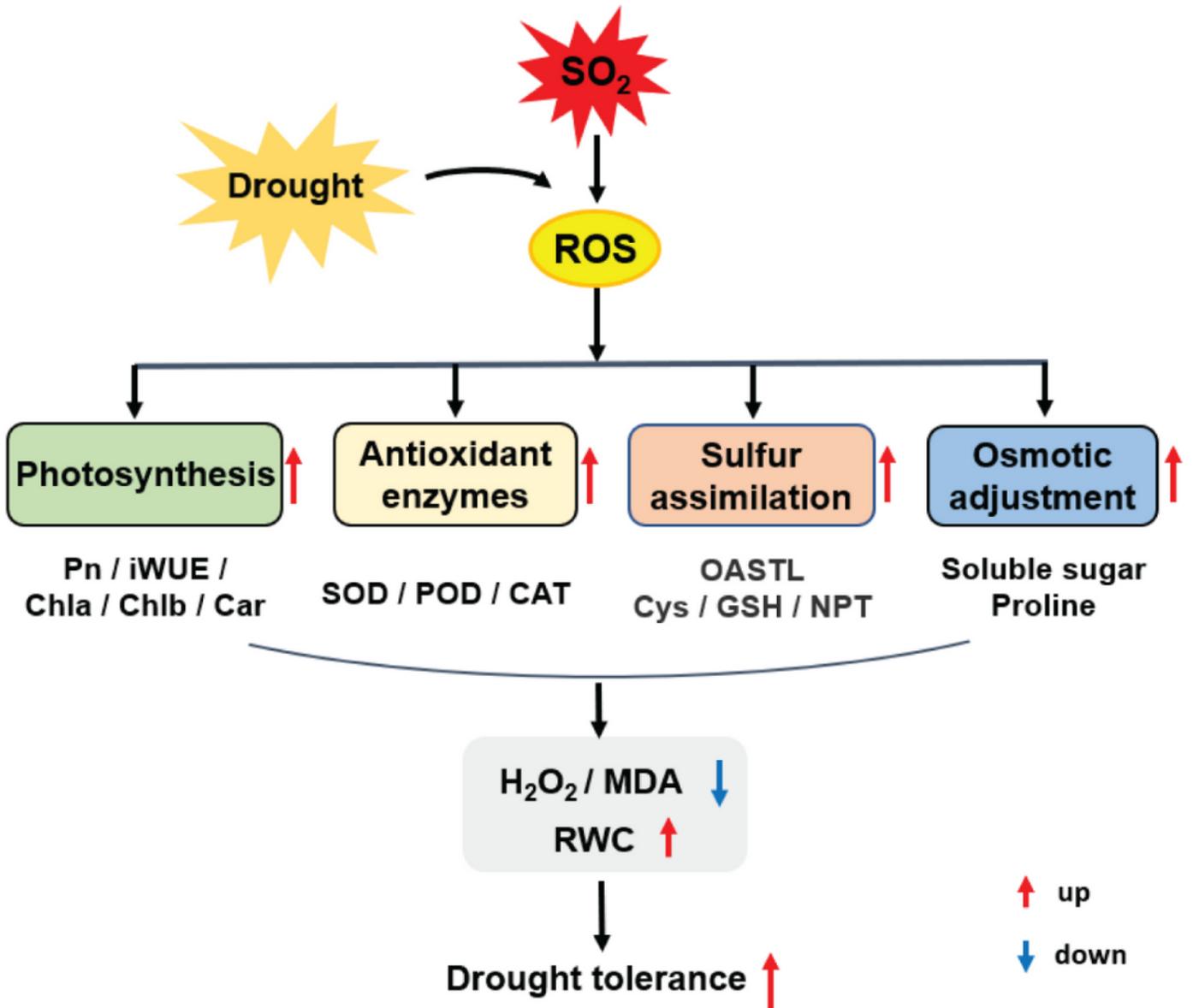


Figure 7

A schematic model for a systematic description of key events leading to enhanced drought tolerance in SO₂-pretreated Arabidopsis plants.