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ORIGINAL PAPER

Hydrogen sulfide improves plant drought tolerance by regulating the homeostasis of reactive oxygen species

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Abstract

Drought stress severely impedes plant growth and development, and hydrogen sulfide (H_2S) actively participates in plant drought responses. To comprehensively understand the regulatory mechanism of H_2S in drought tolerance, an RNA-Seq analysis was conducted to investigate the drought-responsive genes modulated by H_2S. The study identified 3,043 differentially expressed genes (DEGs) that displayed dual responses to 'drought stress-H_2S signaling'. Subsequent enrichment analyses using Gene Ontology and the Kyoto Encyclopedia of Genes and Genomes pathways identified the enrichment of several DEGs associated with 'ribosome biogenesis', 'hormone response', 'drought transcription factors', and 'antioxidant properties'. DEGs closely linked to ribosome biogenesis yield products, such as ribonucleases, nucleolar proteins, and others, which play crucial roles in ribosome biogenesis. Among the eight hormone classes, abscisic acid acts as a positive regulator in response to H_2S signaling. H_2S mainly enhanced plant drought resistance by regulating three key factors in the double-negative regulatory signal pathway of abscisic acid. Under drought stress, five types of transcription factor families, basic helix-loop-helix, NAM-ATAF$_{1/2}$-CUC$_{1/2}$, myeloblastosis, MYB_related, and ethylene responsive factor, were actively regulated by H_2S signals. In addition, H_2S enhanced the cell's ability to remove reactive oxygen species by increasing superoxide dismutase and peroxidase activities, as well as glutathione peroxidase expression. In conclusion, the H_2S signals improved plant drought resistance through the regulation of ribosome biogenesis, plant hormone signal transduction, transcription factors, and antioxidant capacity. This research provides an important reference that aids in our comprehensive understanding of plant H_2S-related drought resistance mechanisms.

Keywords Hydrogen sulfide (H_2S) · Drought stress tolerance · Reactive oxygen species (ROS) · Homeostasis

Introduction

Plants inevitably face numerous harsh environmental challenges as a result of their sessile growth. Among these challenges, drought is one of the most adverse abiotic stresses. It significantly impedes plant growth and development, posing a threat to sustainable crop production (Zhang et al. 2022a). Furthermore, the detrimental effects of global warming and climate change on agricultural production are progressively becoming more severe. Ultimately, the occurrence of drought stress will significantly impact the food security of the expanding global population (Vicente-Serrano et al. 2022). To withstand the effects of drought stress, plants initiate a series of cellular responses associated with morphological, physiological, and biochemical processes. Plants demonstrate diverse cellular responses, including adjusting root plasticity (Karlova et al. 2021), altering leaf morphology (Chen et al. 2020a), closing stomata (García-Mata and Lamattina 2010), modifying membrane lipids (Kränzlein et al. 2023), synthesizing osmoprotectants (Ozturk et al. 2021), accumulating antioxidants, scavenging reactive oxygen species (ROS) (Jiang and Zhang 2002), activating transcription (Takahashi et al. 2018), and engaging in hormonal crosstalk (Waadt et al. 2022), that allow them to adapt to varying environmental conditions.

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In recent years, hydrogen sulfide (H_2S) has garnered considerable attention as an emerging third gasotransmitter. The investigation of endogenous H_2S in higher plants can be traced back to 1978 (Wilson et al. 1978). However, in the past decade, as the signaling-molecule roles of nitric oxide (NO) and carbon monoxide (CO) became more fully understood, the physiological significance of endogenous H_2S in plant growth, development, and abiotic stress responses was gradually revealed (Pei 2016). The physiological concentration of H_2S has positive effects on both seed germination and seedling growth (Zhou et al. 2018). Moreover, H_2S plays a role in root development (Zhang et al. 2009), photosynthesis (Chen et al. 2011), photomorphogenesis (Liu et al. 2019), and nicotine biosynthesis (Chen et al. 2016). Additionally, H_2S regulates the flowering period (Ma et al. 2021), delays senescence (Jin et al. 2018), retards maturity (Geng et al. 2022), and enhances post-harvest preservation (Liu et al. 2017). Furthermore, H_2S has been implicated in plant responses to various abiotic stresses, including heavy metals (Fang et al. 2017), osmosis (Hao et al. 2020), salt (Ma et al. 2023), temperature (Zhang et al. 2021), and drought (Jin et al. 2013).

H_2S signaling appears to play a crucial role in the development of drought tolerance in plants. The acquisition of plant drought tolerance involves a signal network centered on H_2S, which interacts with signals such as NO, hydrogen peroxide (H_2O_2), abscisic acid (ABA), calcium ions (Ca^{2+}), and others. The network has primarily three pathways: (1) H_2S triggers stomatal closure via the ABA-dependent pathway, thereby reducing water loss; (2) H_2S stimulates the accumulation of osmotic regulating substances, leading to a decrease in cellular water potential; and (3) H_2S activates the antioxidant system to maintain ROS homeostasis in cells. Within the system, H_2S has the ability to directly activate functional proteins or regulate protein activity through a process referred to as 'persulfidation' (Chen et al. 2020b; Shen et al. 2020; Zhou et al. 2021; Zhang et al. 2022b; Jurado-Flores et al. 2023). Additionally, it can indirectly stimulate the transcriptional expression of stress-responsive genes through a mechanism known as 'signal crosstalk' (Wang et al. 2021). As a result, the coordinated regulation at both the transcriptional and post-translational modification levels effectively establishes a regulatory network for plant drought tolerance, which collectively sustains the survival and growth of plants under drought conditions.

RNA-Seq technology has been widely used to study the differential gene expression in plants in response to various abiotic stresses (Bashir et al. 2019; Hasan et al. 2019; Rakkammal et al. 2022). By analyzing differentially expressed genes (DEGs) obtained from paired comparisons of varying levels of H_2S treatments under drought stress, a thorough comprehension of the physiological functions of H_2S in plant drought resistance-related regulation can be attained. Additionally, this analysis enables the construction of a network depicting the involvement of H_2S in plant drought regulation. Consequently, new strategies for enhancing plant drought resistance through the utilization of H_2S can be explored. Furthermore, it is possible to identify the entry point for the regulation of plant drought stress caused by H_2S. In addition, through this research, new evidence regarding the direct role of H_2S in the process of plant drought resistance can be rapidly obtained. These findings will significantly contribute to the early elucidation of the physiological functions of H_2S as a signaling molecule.

Materials and methods

Plant materials and growth conditions

In this study, *Arabidopsis thaliana* plants were cultured at 23 ℃ with 60% relative humidity and 160 μEmm^{-2} s^{-1} light intensity in an automated 16/8 h (light/dark) photoperiod environment for up to 20 d. Both the wild-type (WT) and homozygous double-mutant *lcd/des1* line were used. All the mutants were in the Col-0 background and developed using hybridization of T-DNA insertion mutant *lcd* (SALK-082099) with *des1* (SALK-205358C). *Arabidopsis* seeds were sterilized with 75% ethanol for 30 s followed by 6% sodium hypochlorite for 10 min. Later, they were rinsed three times with sterile water and then transferred onto 1/2 MS (1.0% agar and 1.0% sucrose, pH 6.0) medium.

Treatment of experimental materials

PEG treatment: An equal volume of 1/2 MS liquid medium supplemented with 40% polyethylene glycol 8000 (PEG/P) was poured onto freshly prepared 1/2 MS plates. After equilibration for 2 d, the supernatant was discarded, rendering the plates suitable for subsequent utilization. Detailed procedural instructions can be found in Verslues et al. (2006).

H_2S (NaHS as donor) treatment: The 20-d-old seedlings were transferred into the PEG medium, along with 1.5 mL centrifuge tube lids containing sodium hydrosulfide (NaHS) solution. Then, the petri dish was sealed (H_2S final concentration of 20 μM). PEG (P) stress and H_2S (S) fumigation were performed for 9 h, starting at the same time. Afterward, samples were collected for subsequent experiments.

A total of seven treatment groups were established as follows: WT (T1); *lcd/des1* (T2); WT with 20 μM H_2S (T3, WT + S); WT with 40% PEG (T4, WT + P); *lcd/des1* with 40% PEG (T5, *lcd/des1* + P); WT with 40% PEG and 20 μM H_2S (T6, WT + P + S); *lcd/des1* with 40% PEG and 20 μM H_2S (T7, *lcd/des1* + P + S). The specific details of each treatment, and the key combinations are illustrated in Fig. 1.

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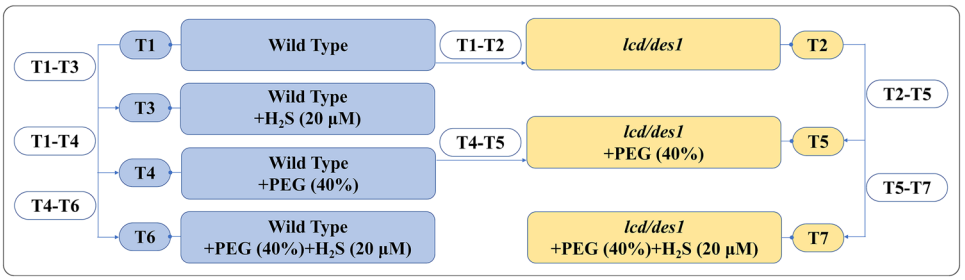


Fig. 1 Experimental treatment conditions and pairwise comparison group settings

Measurement of physiological and biochemical parameters

Twenty-day-old *Arabidopsis* seedlings were subjected to seven distinct treatments (T1–7), and phenotypic, physiological, and biochemical parameters were comprehensively analyzed. These parameters included endogenous H_2S content (Fang et al. 2017), dehydration rate (Hao et al. 2020), chlorophyll content (Hong et al. 2023), oxidative stress indicators, such as malondialdehyde (MDA) and H_2O_2 concentrations (Fang et al. 2014), as well as the activities of various antioxidant enzymes, namely superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX). All the measurements were taken in accordance with the protocols outlined by Li et al. (2002).

RNA extraction, library construction, and RNA-Seq

The total RNAs from the different *A. thaliana* treatment groups were extracted using TRIzol® Reagent (Life Technologies®, USA) following the manufacturer's instructions. The RNA samples were then assessed for integrity and concentration using an Agilent® 2100 Bioanalyzer (Agilent Technologies, Inc.,USA). To obtain mRNA, the qualified RNAs were enriched using Oligo (dT) magnetic beads and subsequently randomly fragmented. The NEBNext® Poly (A) mRNA Magnetic Isolation Module (NEB, E7490, USA) was used to isolate the mRNA. The construction of the cDNA library was carried out using the NEBNext Ultra RNA Library Prep Kit for Illumina (NEB, E7530, USA) and NEBNext Multiplex Oligos for Illumina (NEB, E7500, USA) following the instructions provided in the user's manual. The enriched mRNAs mentioned above were fragmented into approximately 200-nt RNA inserts, which were then used for the synthesis of the first- and second-strand cDNA. The double-stranded cDNA underwent end-repair by adding A-tails and adapter ligation. Subsequently, the segments were size-selected using AMPure XP beads. The appropriate fragments were isolated using Agencourt AMPure XP beads (Beckman Coulter, Inc., USA) and further enriched through PCR amplification. Each constructed cDNA library was validated by Qubit 2.0 and Agilent 2100 to determine the library concentration and insert fragment size. Finally, the cDNA libraries from the *A. thaliana* treatment groups were sequenced on an Illumina HiSeq™ X-ten sequencing platform with a paired-end read length of 150 bp.

Quality control and comparative analysis

The raw image data files obtained from the Illumina HiSeq X-ten were converted into sequencing reads using Illumina's base-calling software, resulting in raw sequences or reads in FASTQ format. To eliminate low-quality reads, which included those with only adaptors, unknown nucleotides exceeding 5%, or a Q20 value below 20%, indicating high sequencing error rates, a Perl script was employed for filtering. The clean reads, which derived from the raw reads, were subsequently aligned to the *A. thaliana* genome (TAIR 9) using Tophat2 (Kim et al. 2013). The resulting alignments were assessed in BAM/SAM formats to remove any potential duplicate molecules. Finally, gene expression levels were estimated from fragments per kilobase of exon per million fragments mapped (FPKM) values using Cufflinks software (Trapnell et al. 2012).

Identification of DEGs

The analysis of DEGs was conducted using EBSeq (Leng et al. 2013). Furthermore, the Benjamini–Hochberg method was applied to correct the false discovery rate based on fold change. To ensure the identification of biologically relevant DEGs, a false discovery rate correction method was utilized to eliminate false positives and false negatives, ensuring the exclusive reporting of biologically significant DEGs. Genes with a log2 fold-change ratio ≥ 1 and a p-adjusted < 0.001 were assigned as the most significant DEGs.

GO and KEGG pathway enrichment analysis

A Gene Ontology (GO) enrichment analysis of the DEGs was conducted with TopGo (R package), which utilizes the Wallenius non-central hyper-genometric distribution (Young et al. 2010) to adjust for gene length bias in DEGs. To perform the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis, KOBAS software (Mao et al. 2005) was utilized. This analysis

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was based on the annotation of *A. thaliana* in the KEGG database (http://www.genome.jp/kegg/). Significantly enriched GO terms and KEGG pathways were identified using a corrected *p*-value threshold of less than 0.05 for the DEGs.

Validation of qRT-PCR analysis

The candidate genes were validated using quantitative reverse transcriptase PCR (qRT-PCR). After RNA extraction, cDNA was synthesized using the PrimeScript™ RT Kit (Takara, JPN). Each qRT-PCR reaction was conducted using the SYBR® Premix Ex Taq™ (Takara, JPN) following the provided instructions. All actions were performed in a CFX96 Real-Time PCR System (Bio-Rad, USA). The primers required for qRT-PCR detection are listed in Table S6. Each qRT-PCR analysis was repeated three times. The relative expression level was calculated using the $2^{-\Delta\Delta CT}$ method, with *Actin* (*AT3G46520*) as the internal control.

Data statistical analysis

Data processing and one-way analysis of variance were performed using Excel and SPSS Statistics 17.0 software. The data are presented as the means ± standard errors, and Duncan's test was used to assess the differences between treatments. SigmaPlot 10.0 and Photoshop 20.0.6 software were used to create graphs.

Results

H_2S enhanced the drought tolerance of Arabidopsis seedlings

Under normal growth conditions (Fig. 2A: T1-T3), both WT and *lcd/des1*, a double mutant that lacks the gene responsible for the major H_2S-producing enzyme, exhibited favorable growth states characterized by erect leaves and similar leaf color (Fig. 2A: T1 vs. T2). Moreover, the application of exogenous H_2S had no significant impact on the WT phenotype (Fig. 2A: T1 vs. T3). However, when subjected to drought stress (Fig. 2A: T4-T7), both materials displayed evident wilting symptoms, with *lcd/des1* being more susceptible to drought stress compared with WT (Fig. 2A: T4 vs. T5). Nevertheless, the exogenous application of H_2S mitigated the damage caused by drought stress to varying degrees in both materials (Fig. 2A: T6 vs. T7). Considering the aforementioned differences in phenotypes, the endogenous H_2S contents, water loss rates, and chlorophyll contents of seedlings were examined further. Drought stress induced varying levels of endogenous H_2S production (Fig. 2B: T1 vs. T4, T2 vs. T5). Additionally, the application of exogenous H_2S under drought stress effectively supplemented the

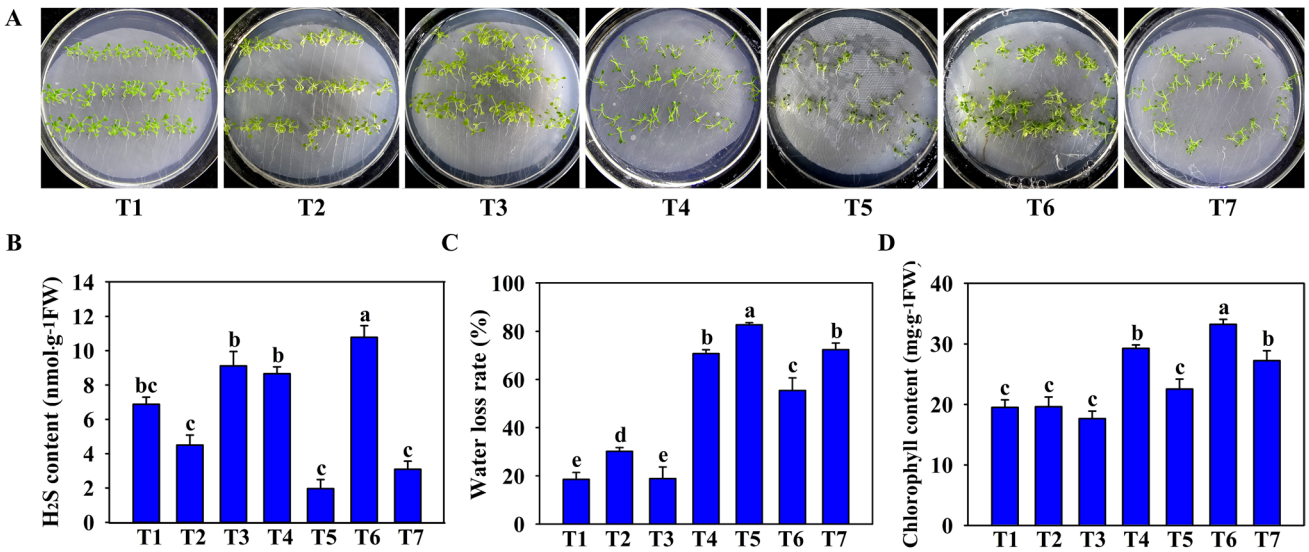


Fig. 2 Effects of H_2S signaling on phenotypic plasticity of *Arabidopsis* seedlings. The seedlings of WT and *lcd/des1* were cultivated on 1/2 MS medium for 20 d and then transferred to the treatment medium (T1–7) using nylon membranes. T1–7: T1, WT; T2, *lcd/des1*; T3, WT + S; T4, WT + P; T5, *lcd/des1* + P; T6, WT + P + S; T7, *lcd/des1* + P + S; P, PEG; S, H_2S. They were further cultured for 9 h before subsequent analyses, which included the assessment of seedling phenotypic traits **A** measurement of seedling endogenous H_2S contents **B** determination of water-loss rates **C** and evaluation of chlorophyll contents **D** Bars with distinct letters indicate significantly different ($p < 0.05$). This notation applies to all subsequent figures

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endogenous H_2S content (Fig. 2B: T4 vs. T6, T5 vs. T7). Furthermore, under drought stress, exogenous H_2S significantly reduced water loss (Fig. 2C: T4 vs. T6, T5 vs. T7) and exerted a comparable regulatory effect on chlorophyll content, effectively mitigating the detrimental effects of drought stress on chlorophyll (Fig. 2D: T4 vs. T6, T5 vs. T7).

Transcriptional sequencing data quality and alignment analysis

To investigate differential gene expression in response to H_2S signaling during drought stress, transcriptome sequencing was conducted across seven experimental treatments (T1–7). Two *A. thaliana* varieties, namely the WT and the double-mutant *lcd/des1*, were utilized in this study. The transcriptome sequencing analyses results are summarized in Table S1. A total of 367.6 million high-quality clean reads were obtained from the seven sequenced libraries. Each sample exhibited a minimum of 97.68% perfect alignment to the *Arabidopsis* genome, with over 95% of the reads uniquely mapped to specific locations. Furthermore, all samples demonstrated a Q30 base percentage of at least 94%, indicating high-quality sequencing data.

Overview of DEGs in response to H_2S regulation

This study focused on two key factors: 'drought stress' and 'H_2S signaling'. In total, seven pairs of combinations and selected DEGs among the groups were analyzed. As shown in Fig. 3A, under normal growth conditions, 883 DEGs (319 up-regulated and 564 down-regulated) were detected when comparing the WT to *lcd/des1* (WT vs. *lcd/des1*). However, the exogenous application of H_2S induced the expression of 8,217 DEGs (2,221 up-regulated, 5,996 down-regulated). Under drought-stress conditions, both the WT and *lcd/des1* showed strong characteristics of drought response, with an increase in the number of DEGs by 8,858 (2,123 up-regulated and 6,735 down-regulated) and 8,478 (2,118 up-regulated and 6,360 down-regulated), respectively, compared with before the drought-stress treatment. If exogenous H_2S was applied concurrently with the drought stress treatment, then the number of DEGs significantly decreased. Both the WT and *lcd/des1* exhibited similar response trends. Thus, the exogenous H_2S application resulted in a reduction of 5,427 DEGs in the WT (669 up-regulated and 4,758 down-regulated genes decreased), and 5,730 DEGs (506 up-regulated genes and 5,224 down-regulated genes decreased) in *lcd/des1*. Under drought stress, the number of DEGs between the WT and *lcd/des1* was very small, consisting of 122 DEGs (50 up-regulated and 72 down-regulated).

Under normal conditions (Fig. 3B), 596 genes exhibited differential expression due to regulation by both endogenous and exogenous H_2S. However, when exposed to drought-stress conditions, the numbers of genes regulated by the exogenous H_2S response in the WT and *lcd/des1* were 1,949 and 1,094, respectively. Importantly, there was an overlap of 675 co-expressed genes, as shown in Fig. 3C.

Functional annotation of DEGs regulated by H_2S under drought stress.

The study aimed to identify the potential biological functions of DEGs through a GO enrichment analysis. Statistical analyses were carried out on all enriched GO categories (Table S2), as well as on the top 20 enriched GO categories (Figures S1, S2), in the four comparative groups: WT vs. WT + PEG, *lcd/des1* vs. *lcd/des1* + PEG, WT + PEG vs. WT + PEG + H_2S, and *lcd/des1* + PEG vs. *lcd/des1* + PEG + H_2S. These comparisons were based on the keywords 'drought stress' and 'H_2S'. The results indicated that H_2S significantly affects 'ribosomal large

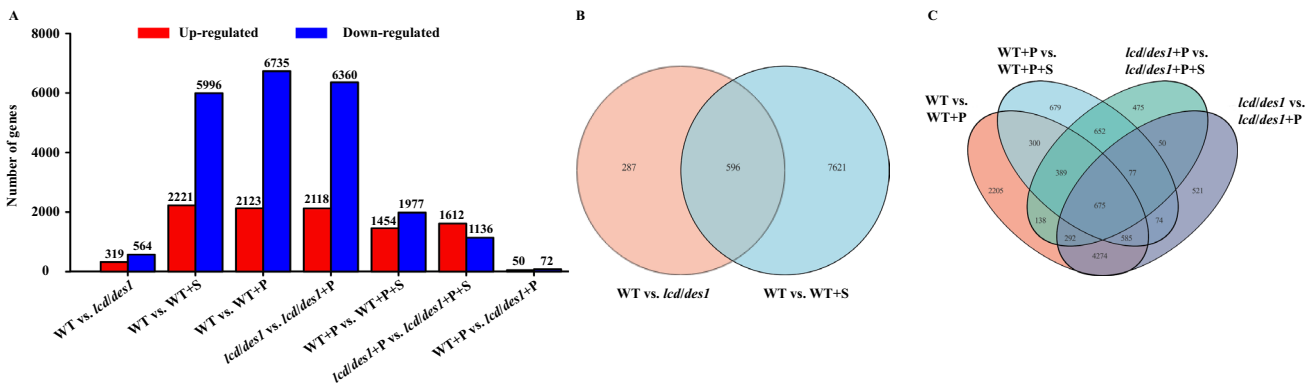


Fig. 3 Distribution of DEGs responding to H2S signaling under drought- or non-drought-stress conditions. Distributions of up- and down-regulated DEGs in pairwise comparison groups **A** Venn diagram analyses of the DEGs regulated by H2S signaling under normal conditions **B** and drought-stress conditions **C**

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subunit biogenesis', 'ribosome biogenesis', 'response to abscisic acid', 'response to oxygen-containing compound', 'glutathione binding', and 'oxidoreductase activity' under drought-stress conditions. Consequently, the subsequent study focused on obtaining relevant information about 'ribosome biogenesis', 'hormone response', 'antioxidant performance', and other pertinent factors within the four comparative groups (Table 1).

Furthermore, KEGG enrichment analyses were conducted on the DEGs from these four comparison groups. As shown in Fig. 4, the comparison groups WT vs. WT + PEG and *lcd/des1* vs. *lcd/des1* + PEG demonstrated substantial enrichments of pathways, such as 'photosynthesis-antenna proteins', 'plant hormone signal transduction', 'photosynthesis', 'porphyrin and chlorophyll metabolism', and 'carbon fixation in photosynthetic organisms'. In contrast, the comparison groups WT + PEG vs. WT + PEG + H_2S and/or *lcd/des1* + PEG vs. *lcd/des1* + PEG + H_2S exhibited notable enrichments of pathways including 'ribosome biogenesis in eukaryotes', 'photosynthesis-antenna proteins', 'indole alkaloid biosynthesis', 'vitamin B6 metabolism', and 'glutathione metabolism'.

DEGs associated with ribosome biogenesis and regulated by H_2S under drought stress

This study used a Venn analysis (Fig. 5A) and type classification (Figure S3, Table S3) to investigate the impact of H_2S on ribosome biogenesis under drought-stress conditions. This enabled the evaluation of DEGs associated with ribosome biogenesis enriched in the three comparative groups: WT vs. WT + H_2S (43 DEGs), WT + PEG vs. WT + PEG + H_2S (50 DEGs), and *lcd/des1* + PEG vs. *lcd/des1* + PEG + H_2S (50 DEGs).

The previously mentioned DEGs were categorized into three groups: First, there were 32 genes that belonged to the 'H_2S-dependent regulation' category. These genes consistently responded to H_2S regardless of the presence of drought stress. Second, under drought-stress conditions, there were 14 DEGs that fell into the 'H_2S-inducible regulation' category. These genes were regulated in response to H_2S

Table 1 Screening of GO enrichment categories significantly affected by drought stress and H_2S

Category	GO accession	Description	Numbers of Genes			
			T1 vs. T4	T2 vs. T5	T4 vs. T6	T5 vs. T7
Biological process	GO:0009768	photosynthesis, light harvesting in photosystem I	20	20	8	6
	GO:0009725	response to hormone	669	530	245	195
	GO:0009737	response to abscisic acid	253	210	116	100
	GO:0009753	response to jasmonic acid	112	90	44	34
	GO:0009751	response to salicylic acid	112	88	43	32
	GO:0000302	response to reactive oxygen species	94	83	42	29
	GO:0006979	response to oxidative stress	239	179	104	81
	GO:0006970	response to osmotic stress	355	276	165	117
	GO:0042542	response to hydrogen peroxide	52	41	26	16
	GO:0042273	ribosomal large subunit biogenesis	14	5	15	13
	GO:0042254	ribosome biogenesis	38	12	40	37
	GO:0022613	ribonucleoprotein complex biogenesis	53	17	50	44
	GO:1,901,700	response to oxygen-containing compound	745	593	308	251
	GO:0033993	response to lipid	307	251	133	113
	GO:0044550	secondary metabolite biosynthetic process	84	70	45	29
Cell component	GO:0030684	preribosome	38	21	58	58
	GO:1,990,904	ribonucleoprotein complex	0	0	187	184
	GO:0005732	small nucleolar ribonucleoprotein complex	15	6	16	14
Molecular function	GO:0020037	heme binding	189	146	74	57
	GO:0048037	cofactor binding	555	409	189	152
	GO:0004601	peroxidase activity	76	57	29	23
	GO:0016491	oxidoreductase activity	753	564	261	217
	GO:0003824	catalytic activity	3777	2766	1401	1145
	GO:0016209	antioxidant activity	84	63	33	21

p-value < 0.05; T1 vs. T4 (WT vs. WT + PEG); T2 vs. T5 (*lcd/des1* vs. *lcd/des1* + PEG); T4 vs. T6 (WT + PEG vs. WT + PEG + H2S); T5 vs. T7 (*lcd/des1* + PEG vs. *lcd/des1* + PEG + H2S)

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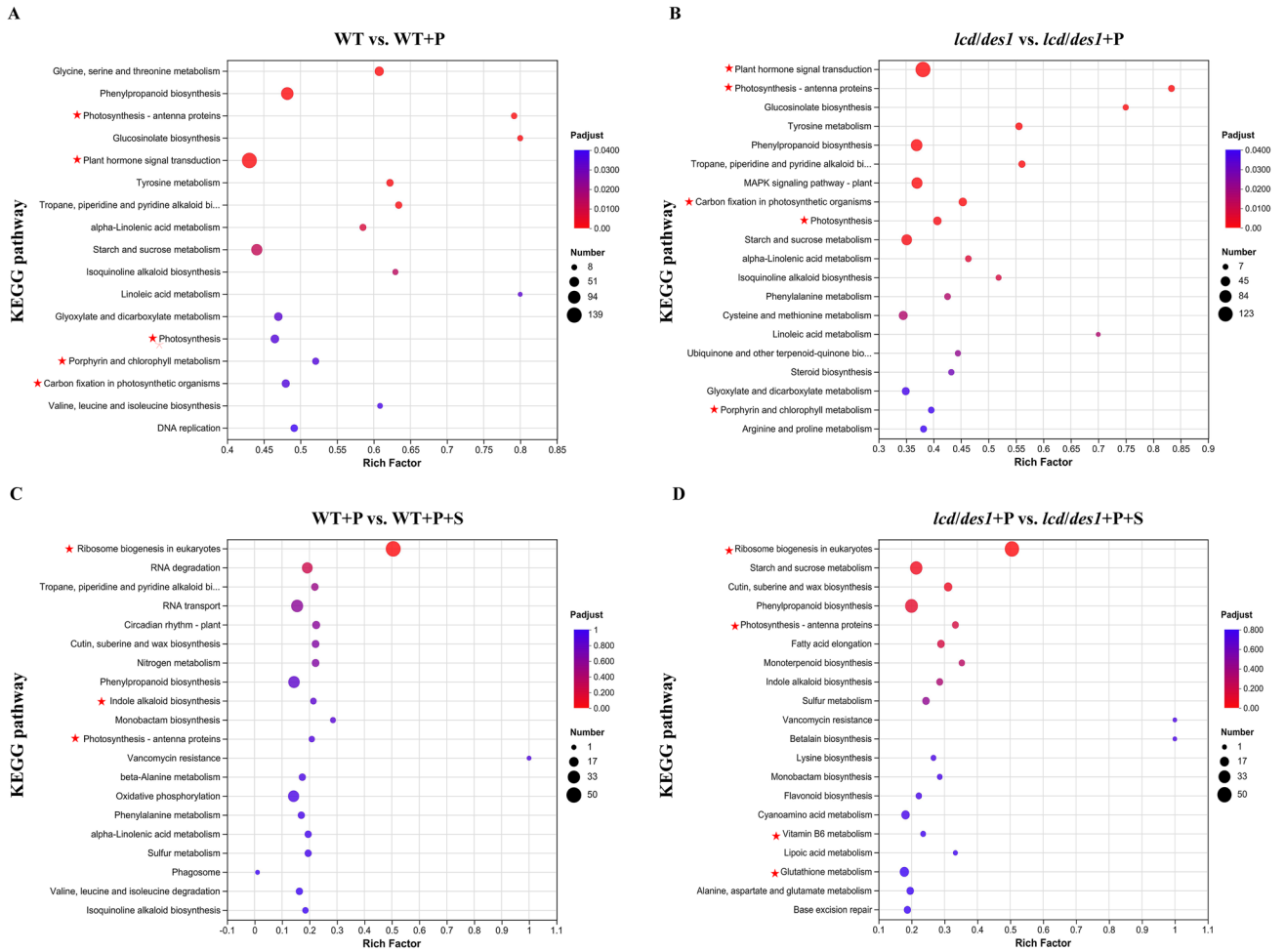


Fig. 4 KEGG pathway enrichment analyses of DEGs from four comparison groups. The *X*-axis indicates the enrichment factor, and the *Y*-axis indicates the KEGG pathways. The asterisks (*) emphasize the signaling pathways associated with 'ribosome biogenesis', 'hormone response', and 'antioxidant capacity' in the pairwise comparison groups. P, PEG; S, H_2S

specifically under drought stress. Third, there were additional DEGs that exhibited diverse H_2S-responsive abilities due to variations in material background.

A hierarchical clustering analysis was conducted on the 14 DEGs belonging to the second category (Fig. 5B). It revealed that only *AT1G27461*, a drought-responsive gene (DRG), was down-regulated, whereas the remaining genes were up-regulated.

H_2S-assisted hormone signaling co-regulates drought-stress responses

A KEGG enrichment analysis was performed to evaluate the distribution of 135 DEGs across various phytohormone signaling transduction pathways in three comparative groups: WT vs. WT + H_2S, WT + PEG vs. WT + PEG + H_2S, and *lcd/des1* + PEG vs. *lcd/des1* + PEG + H_2S (Figure S4A–C). The annotated genes were categorized according to their respective KEGG pathways, and their summaries are presented in Table S4. Furthermore, a hierarchical clustering analysis was employed to classify the genes based on their hormone categories (Fig. 6). As illustrated in Fig. 6, the distribution of DEGs in the signaling pathway was as follows: Auxin ranked first (approximately 48.1%), followed by ABA (approximately 11.9%), whereas the numbers for the other six hormones (brassinosteroid, BR; cytokinin, CTK; ethylene, ETH; gibberellic acid, GA; jasmonic acid, JA and salicylic acid, SA) were comparable (range from 3.7% to 8.9%). Under normal growth conditions (WT vs. WT + H_2S), ABA generally exhibited an insensitivity to exogenous H_2S, whereas the other seven hormone classes, particularly auxin, demonstrated strong responses to exogenous H_2S. Interestingly, under drought-stress conditions, the responses of ABA and auxin to exogenous H_2S differed significantly, with ABA replacing auxin as the highly active member in the H_2S-signaling response (Table 2). Consequently, this study

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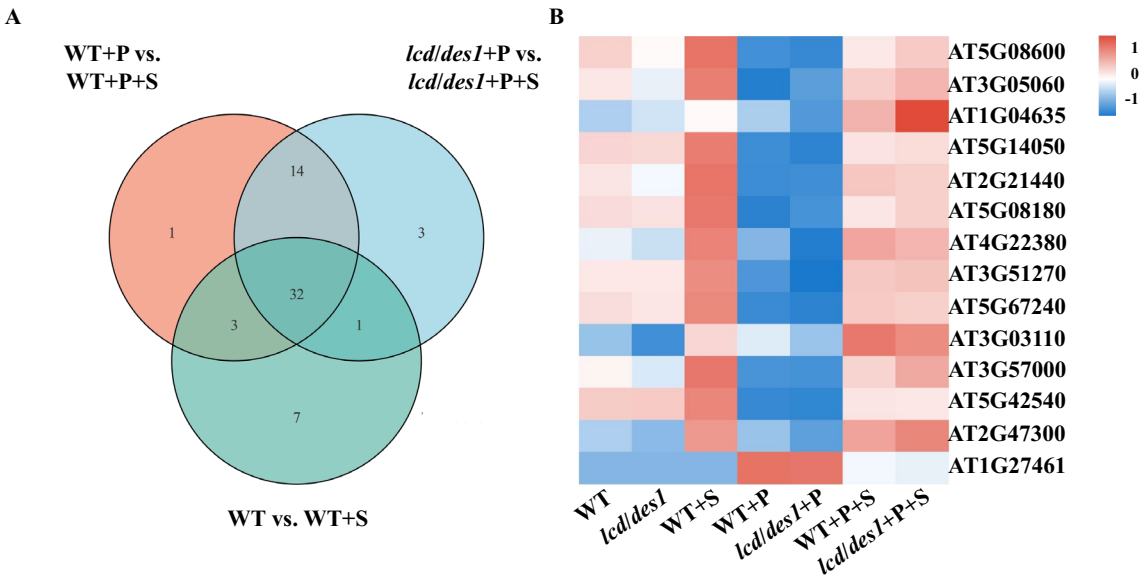


Fig. 5 Visualization of DEGs associated with ribosome biogenesis regulated by H_2S signaling. **A** Venn analyses of DEGs associated with ribosome biogenesis in three comparative groups; **B** 14 DEGs identified from the Venn analyses were further subjected to hierarchical clustering analysis. P, PEG; S, H_2S

specifically focused on the DEGs involved in the response of 'drought stress-H_2S' within the ABA hormone signaling pathway (highlighted in yellow in Fig. 6).

Transcription factors effectively respond to H_2S signaling to aid *Arabidopsis* in combating drought stress

Transcription factors (TFs), which are regulatory proteins located upstream of genes, play crucial roles in plant growth, development, and environmental stress responses. They have significant regulatory functions in *Arabidopsis* during drought stress (Sakuraba et al. 2015; Zhao et al. 2016; Chen et al. 2017). To examine the changes in TF expression resulting from H_2S-mediated alleviation of drought stress, subsequent studies focused on three comparison groups: T1 vs. T3 (WT vs. WT + H_2S), T4 vs. T6 (WT + PEG vs. WT + PEG + H_2S), T5 vs. T7 (*lcd/des1* + PEG vs. *lcd/des1* + PEG + H_2S). Among these groups, 213, 226, and 174 TFs exhibited differential expression, respectively (Table S5). The hierarchical clustering analysis revealed that H_2S primarily down-regulated the expression of the majority of TFs, with only a small subset showing up-regulation under normal growth conditions (Fig. 7A). However, under drought-stress conditions, H_2S either up-regulated or down-regulated the expression of TFs, thereby aiding *Arabidopsis* in combating drought stress (Fig. 7B). This study examined the enrichment of the top 10 TF families across three comparison groups, revealing that H_2S had the greatest impact on the number of *basic Helix-Loop-Helix* (*bHLH*) genes within the gene family, regardless of control (CK) or drought conditions. Furthermore, regardless of differences in genetic background, the top five gene families affected by H_2S under drought conditions were *bHLH, NAM-ATAF*$_{1/2}$-*CUC*$_{1/2}$ (*NAC*), *myeloblastosis* (*MYB*), *MYB_related*, and *ethylene-responsive factor* (*ERF*) (Fig. 7C–E).

H_2S enhanced drought resistance in *Arabidopsis* seedlings by mediating the antioxidant system

Drought stress has the potential to disrupt the delicate intracellular balance between ROS production and elimination. The efficient regulation of ROS homeostasis by the antioxidant system is essential for plants to effectively adapt to drought stress. To comprehensively investigate the role of H_2S in modulating the antioxidant system for drought-stress mitigation, the expression patterns of genes encoding vital antioxidant enzymes under the regulation of H_2S were analyzed. Drought stress led to the down-regulation of three *SOD* genes, whereas H_2S treatments significantly up-regulated one of these genes. The up-regulation of genes encoding *APX* and *CAT* in response to drought was more pronounced compared with the down-regulation of genes. In addition, exogenous H_2S treatments did not affect the expression levels of these genes. Furthermore, drought stress resulted in the up-regulation of genes that encode glutathione peroxidase (GPX) and glutathione reductase (GR), whereas H_2S treatments specifically up-regulated *GPX* and had no significant impacts on *GR* expression (Fig. 8A). Subsequently, the MDA and H_2O_2

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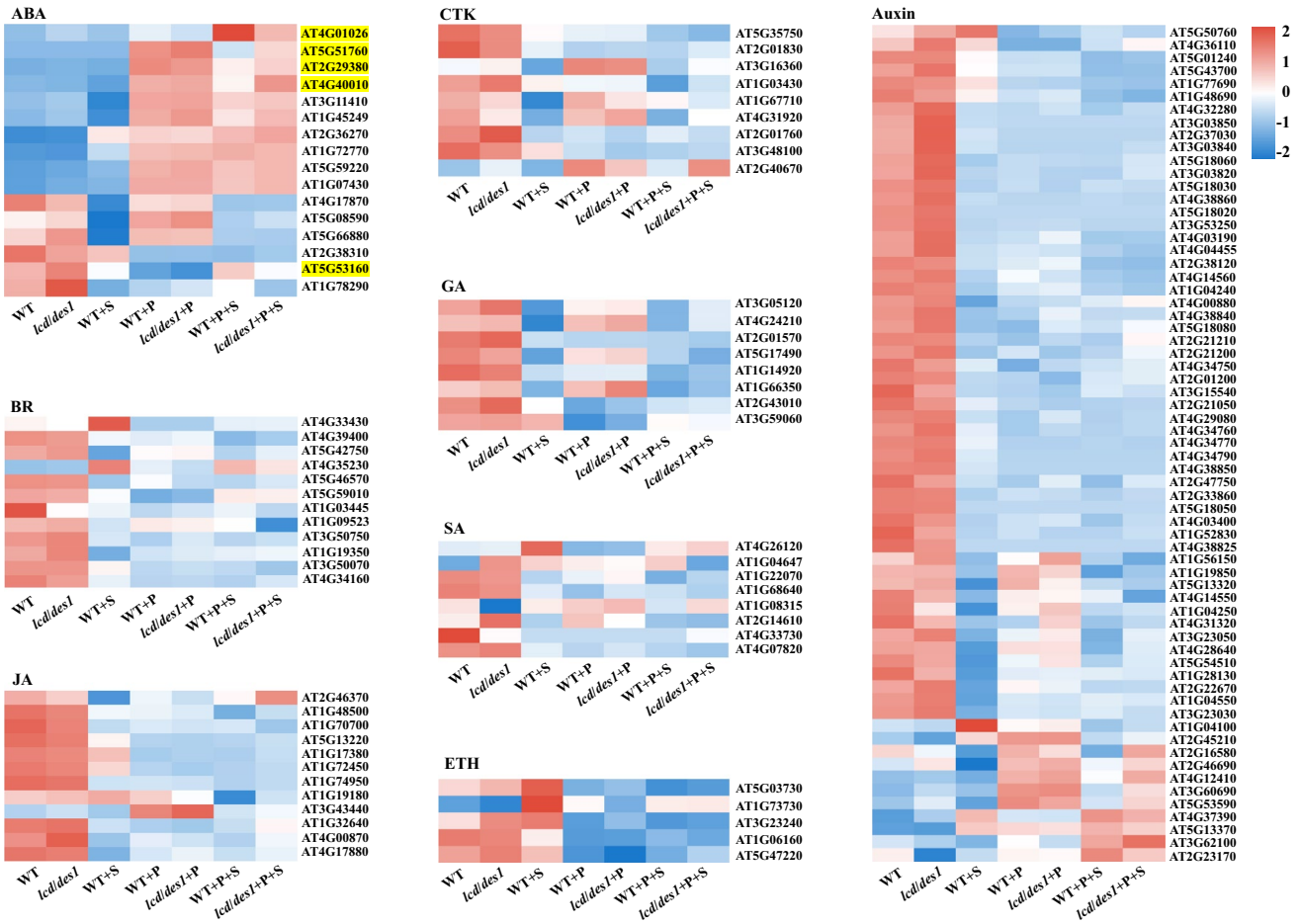


Fig. 6 Hierarchical clustering analysis of phytohormone-related genes with significant expression differences. A hierarchical clustering analysis was performed on genes associated with phytohormones that showed significant expression variations. The analysis involved the relative expressions of these genes under seven conditions: WT, *lcd/des1*, WT + S, WT + P, *lcd/des1* + P, WT + P + S, and *lcd/des1* + P + S, where P = PEG and S = H_2S. The annotations of DEGs by The *Arabidopsis* Information Resource are presented on the right side of the heatmap. Genes are represented with different colors, and the color gradient indicates the relative levels of expression, ranging from low (blue) to high (red). The abbreviations below correspond to the respective phytohormones: ABA, abscisic acid; BR, brassinosteroid; JA, jasmonic acid; CTK, cytokinine; GA, gibberellin; SA, salicylic acid; ETH, ethylene

levels were measured in the various treatment groups to assess their impacts. There were substantial elevations in the MDA and H_2O_2 levels in both WT and *lcd/des1* plants. However, *lcd/des1* plants exhibited a significant decrease compared with WT plants. Moreover, the application of H_2S effectively decreased the levels of these compounds in both genotypes (Fig. 8B, C). Plants experience damage due to drought stress, which triggers survival mechanism involving antioxidant enzyme systems, which include SOD, CAT, APX, and POD. The study's findings align with transcriptomic data, indicating that drought stress increased the activities of antioxidative enzymes, including SOD, CAT, APX, and POD. Exogenous H_2S treatments increased SOD and POD enzyme activities, while having no significant impact on CAT and APX enzyme activities (Fig. 8D–G). Consequently, it can be inferred that H_2S mainly enhances SOD and POD activities, leading to decreased ROS levels and ultimately improving drought resistance in plants.

Validation of RNA-Seq results by qRT-PCR.

Twelve DEGs were randomly chosen for qRT-PCR validation to assess the reliability of the transcriptome dataset. As shown in Fig. 9, the 12 selected DEGs belonged to six categories: photosynthetic carbon assimilation (*ribulose 1,5-bisphosphate carboxylase small subunit 1A/1B*, *RBCS1A/RBCS1B*), photosynthetic pigment capture (*light harvesting chlorophyll A/B, LHCA/LHCB*), mitogen-activated protein kinase (*mitogen-activated protein kinase 4*,

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Table 2 Modifications in the responses of ABA-related DEGs to H2S signal regulation

Gene ID	Gene name	T1 vs. T3		T4 vs. T6		T5 vs. T7	
		Fold change	Regulate	Fold change	Regulate	Fold change	Regulate
AT4G01026	*PYL7*	–	–	3.985	up	2.024	up
AT5G51760	*AHG1*	–	–	0.071	down	0.261	down
AT2G29380	*HAI3*	–	–	0.14	down	0.336	down
AT4G40010	*SRK2F*	–	–	0.339	down	–	–
AT3G11410	*PP2CA*	0.403	down	–	–	–	–
AT1G45249	*ABF2*	–	–	0.498	down	–	–
AT2G36270	*ABI5*	2.938	up	–	–	–	–
AT1G72770	*HAB1*	3.405	up	–	–	–	–
AT5G59220	*SAG113*	2.738	up	–	–	–	–
AT1G07430	*AIP1*	5.23	up	–	–	–	–
AT4G17870	*PYR1*	0.25	down	–	–	–	–
AT5G08590	*SRK2G*	0.35	down	0.418	down	0.411	down
AT5G66880	*SRK2I*	0.432	down	–	–	–	–
AT2G38310	*PYL4*	0.277	down	–	–	–	–
AT5G53160	*PYL8*	–	–	2.232	up	–	–
AT1G78290	*SRK2C*	0.393	down	–	–	–	–

p-adjusted < 0.001; T1 vs. T3 (WT vs. WT + H_2S); T4 vs. T6 (WT + PEG vs. WT + PEG + H_2S); T5 vs. T7 (*lcd/des1* + PEG vs. *lcd/des1* + PEG + H_2S)

MPK4; *mitogen-activated protein kinase kinase kinase 1*, *MEKK1*), drought-resistant TF (*dehydration-responsive element-binding protein 2A-1*, *DREB2A-1; responsive to desiccation 29A*, *RD29A*), ion channel (*two-pore channel 1*, *TPC1*; *calcium exchanger 1, CAX1*), and others (*homeodomain glabrous 2, HDG2*; *senescence associated gene 13, SAG13*). Their expression patterns were basically consistent with the transcriptome data (Fig. 9; Table S6).

Discussion

This study utilized RNA-Seq technology to investigate the differential expression of *A. thaliana* genes in response to H_2S regulation during drought stress. The results indicated a significant association between genes responsive to H_2S regulation under drought stress and various processes, such as ribosome biogenesis, plant hormone signaling transduction, TFs, and antioxidant systems. These findings provide valuable insights into the physiological role of H_2S in enhancing drought resistance.

H_2S enhanced plant drought tolerance by regulating the transcription levels of proteins associated with ribosome biogenesis

Ribosome biogenesis plays a crucial role in the evolution of life itself, as it is solely responsible for protein production in all cells. The intricate process encompasses ribosomal DNA transcription, pre-ribosomal RNA processing, RNA modification, and ribosomal RNA assembly with ribosomal proteins and assembly factors (Guo et al. 2023). Each of these steps depends on the precise regulation of numerous ribosomal proteins and ribosome biogenesis factors. Any mutation in these components can potentially result in severe developmental disorders (Weis et al. 2015). Therefore, ribosome biogenesis also serves as a pivotal factor in stress responses across species.

In a previous study, a comprehensive analysis was conducted using the GO and KEGG databases to identify 154 DEGs influenced by H_2S during ribosome biogenesis. Through a Venn analysis (Fig. 5A), the 154 identified DEGs were classified into three broad categories. The second category encompassed 14 DEGs that exhibited specific responsiveness to H_2S regulation, particularly under drought stress. Subsequently, a hierarchical clustering analysis was performed on these DEGs. Notably, among the identified DEGs, *AT1G27461* showed a unique pattern of down-regulation in response to H_2S, whereas the remaining 13 DEGs displayed up-regulation.

AT1G27461 is a DRG that encodes a 39.5-kDa nucleoprotein. Its expression is up-regulated under abiotic-stress conditions, playing a crucial role in enhancing drought tolerance mechanisms in *A. thaliana*. Its expression has been observed in dehydrated tissues, especially in leaf tissues, as a response to drought stress (Moon et al. 2016). The findings, depicted in Fig. 5B, corroborate previous reports indicating that drought stress up-regulates *AT1G27461* expression, whereas exogenous H_2S administration leads to significant down-regulation. This suggests that H_2S effectively

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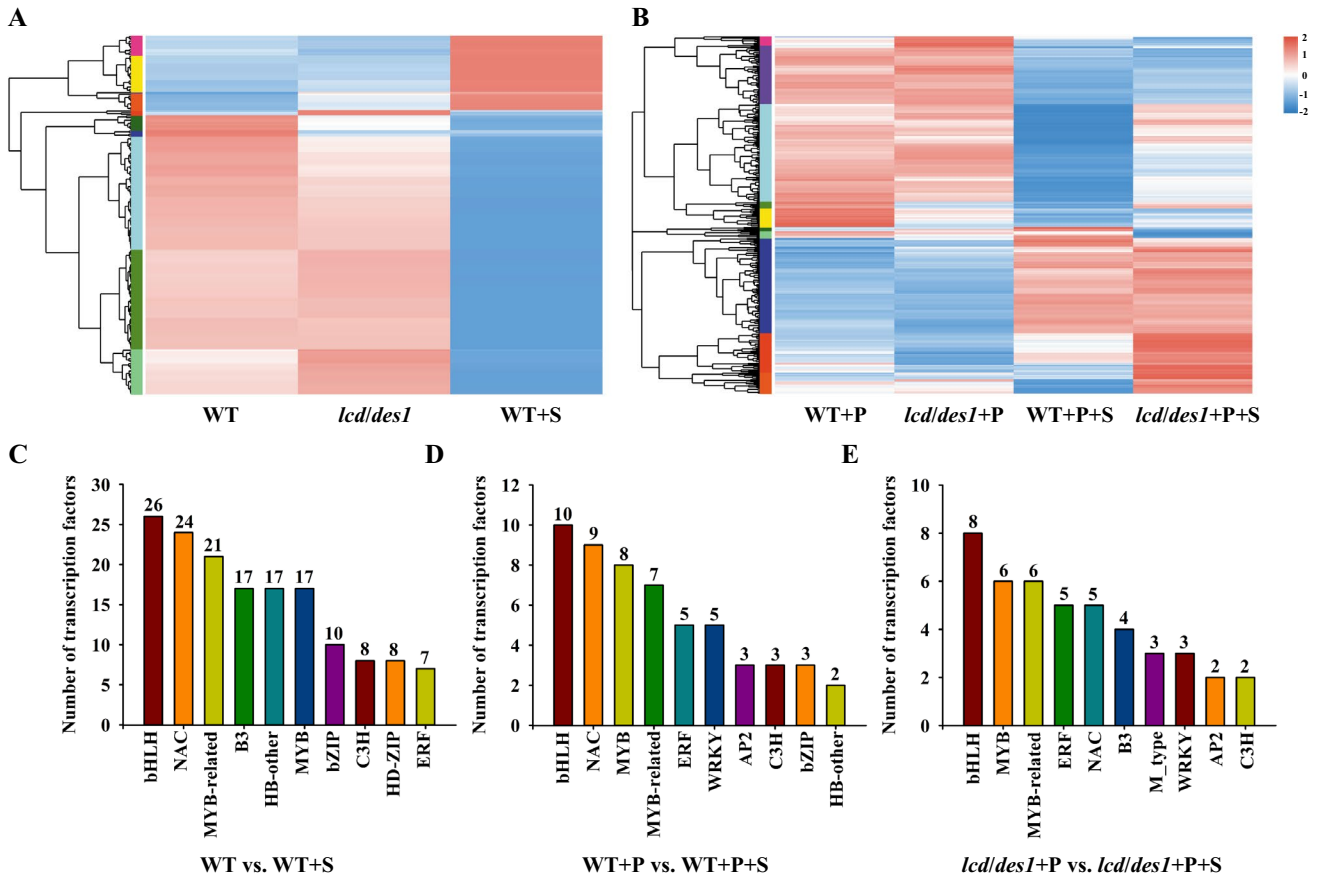


Fig. 7 Statistical analysis of TFs regulated by H_2S signaling in response to drought stress. **A**, **B** Heatmap diagrams display the relative expression levels of differentially expressed TFs in the following groups: WT, *lcd/des1*, and WT + S **A**; and in WT + P, *lcd/des1* + P, WT + P + S, and *lcd/des1* + P + S **B**; **C**–**E** Comparisons of differentially expressed TFs were conducted between WT vs. WT + S **C** WT + P vs. WT + P + S **D** and *lcd/des1* + P vs. *lcd/des1* + P + S **E** P, PEG; S, H_2S

suppresses the transmission of stress signals, thereby mitigating the up-regulation of *AT1G27461* expression triggered by drought stress.

Furthermore, the expression patterns of the 13 other candidate genes were completely opposite to those of *AT1G27461*. The proteins encoded by these DEGs, including ribonuclease *AT1G04635* (Wang et al. 2012), ribonucleoprotein-encoding *AT5G14050* (Sunkar and Zhu 2004), RNA degradation enzyme-encoding *AT5G67240* (Ramachandran and Chen 2008), and export protein-encoding *AT3G03110* (Blanvillain et al. 2008), were positively regulated by H_2S. Their shared function is to facilitate the normal ribosome biogenesis progress. Therefore, any impact on these proteins could lead to abnormal ribosomal function, potentially compromising the precise supply of proteins required for plant growth and development. This study demonstrated a positive correlation between the expression patterns of the 13 DEGs and H_2S under drought stress, indicating a protective role of H_2S in maintaining protein stability or enhancing the activities encoded by these DEGs.

H_2S primarily participates in the dual-negative regulatory signal pathway of ABA to activate drought responses in plants

Phytohormones, such as ABA, auxin, BR, CTK, ETH, GA, JA, SA, and strigolactone, are signaling compounds that participate in various biological processes to regulate plant growth, development, and stress responses (Waadt et al. 2022). Drought stress is a severe environmental factor.

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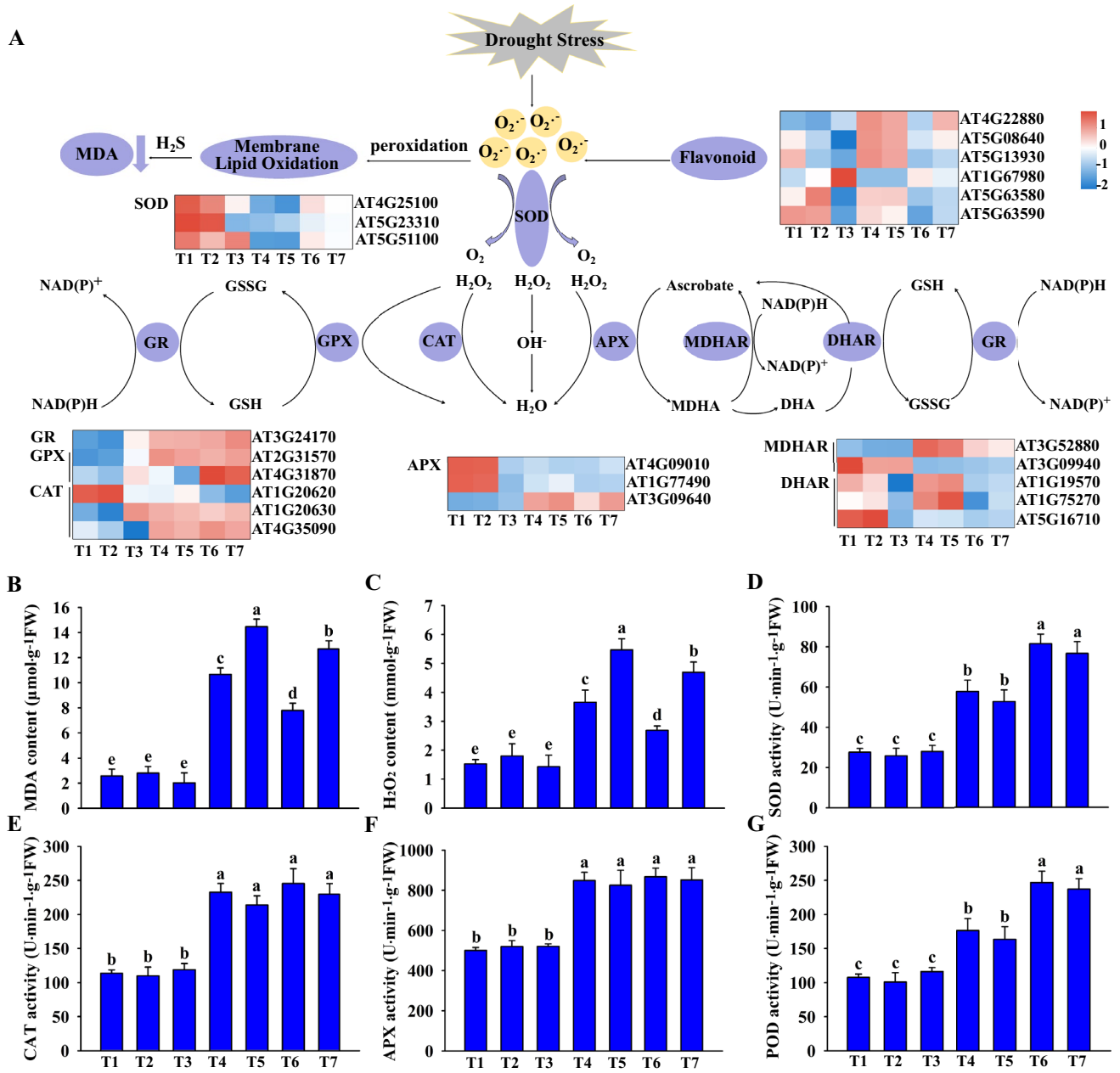


Fig. 8 Influence of H_2S signaling on the antioxidant performance of *Arabidopsis* seedlings under drought stress. T1–7: T1, WT; T2, *lcd/des1*; T3, WT + S; T4, WT + P; T5, *lcd/des1* + P; T6, WT + P + S; T7, *lcd/des1* + P + S; P, PEG; S, H_2S. **A** Distribution of DEGs responding actively to the regulation of H_2S signaling in the ROS metabolic pathway; **B**–**G** The contents of MDA (B) and H_2O_2 **C**, as well as the enzyme activities of the major antioxidant enzymes SOD **D**, CAT **E**, APX **F**, and POD **G**, were determined in *Arabidopsis* seedlings under drought stress and/or different H_2S levels

ABA, known as an 'adversity hormone', serves as the primary signal for plants to cope with drought stress. The ABA signaling pathway has a dual-negative regulatory system, PYR/PYL/RCAR-|PP2C-|SnRK2s, which consists of three key components: pyrabactin resistance (PYR), pyrabactin resistance-like (PYL), and the regulatory component of ABA receptors (RCAR), type 2C protein phosphatases (PP2Cs) (negative regulator), and sucrose non-fermenting-1 (SNF-1)-related protein kinases 2 (SnRK2s) (positive regulator) (Umezawa et al. 2010; Cutler et al. 2010; Chen et al. 2020c). Under drought stress, the level of ABA in plants increases. ABA binds to its receptor to form the ABA-PYR/PYL/RCARs complex, which combines with PP2Cs and inhibits their phosphatase activity. As a result, SnRK2s are phosphorylated and become active, initiating downstream responsive elements that positively regulate ABA signaling

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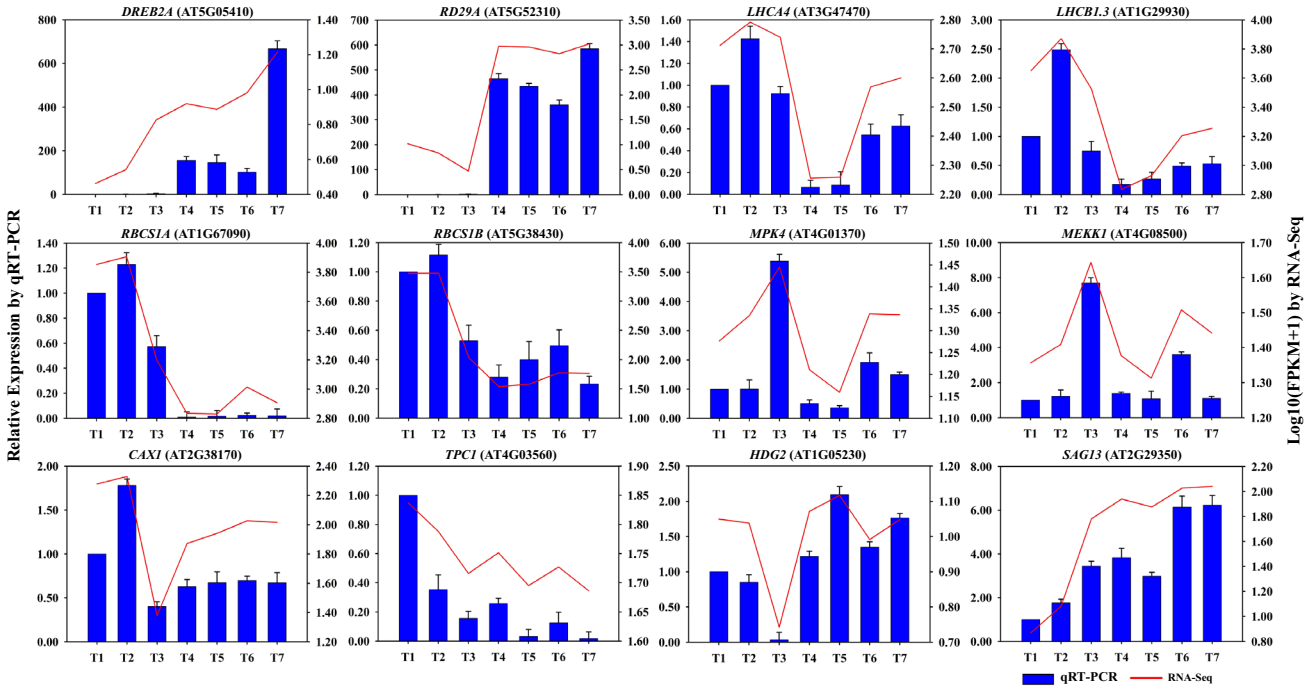


Fig. 9 Transcriptional expression levels of 12 candidate genes detected by qRT-PCR and RNA-Seq. Blue bars represent the relative expression level detected by qRT-PCR using the $2^{-\Delta\Delta CT}$ method, with an associated standard error bar ($n=3$). Red lines indicate transcript abundance changes of RNA-Seq data calculated using the Log10(FPKM + 1) method. T1–7 on the *X*-axis represent different treatment groups. T1–7: T1, WT; T2, *lcd/des1*; T3, WT + S; T4, WT + P; T5, *lcd/des1* + P; T6, WT + P + S; T7, *lcd/des1* + P + S; P, PEG; S, H_2S

responses. These responses include the regulation of stomatal aperture, channel activity in guard cells, and the expression of some ABA-responsive genes (Hsu et al. 2021; Fujita et al. 2011; Muhammad Aslam 2022).

This study identified 135 DEGs associated with eight distinct classes of phytohormone signals. All these genes are regulated by exogenous H_2S, as detailed in Table S4. A subset of these DEGs, specifically responsive to H_2S regulation under drought stress, exhibits a strong correlation with ABA (Fig. 6). Consequently, future research will primarily focus on five actively expressed DEGs (highlighted in yellow) that play crucial roles in three key components of the ABA dual-negative regulatory signaling pathway (PYR/PYL/RCAR- |PP2C- |SnRK2s). This finding aligns with previous research reported by Liu et al. (2023). Among the identified DEGs, *AT4G01026* and *AT5G53160* encode PYL receptors (Huang et al. 2023; Lim et al. 2013), whereas *AT5G51760* and *AT2G29380* encode PP2C phosphatases (Nishimura et al. 2007). Additionally, *AT4G40010* encodes SnRK2 kinase (Table 2). In the presence of drought stress, the application of exogenous H_2S induced the expression levels of *PYL7* (*AT4G01026*) and *PYL8* (*AT5G53160*), suggesting that exogenous H_2S effectively activates the ABA-signaling pathway by promoting the binding of ABA to its receptors. These findings align with previous reports (Jin et al. 2013; Lim et al. 2013; Li et al. 2017; Ma et al. 2016). Conversely, the transcriptional expression levels of *ABA-hypersensitive germination 1* (*AT5G51760*) and the *highly ABA-induced PP2C protein 3 (AT2G29380*) were supressed by H_2S. Both encode PP2C phosphatase and serve as direct targets of the ABA-PYR/PYL/RCAR complex. The transcriptional expression of these genes was down-regulated in the presence of H_2S. This suppression is likely to decrease the activity of PP2C, as reported by Li (2017). In conclusion, under drought-stress conditions, exogenous H_2S indirectly enhances the activation of SnRK2 by positively regulating the transcriptional expression of ABA receptors and synergistically inhibits the transcriptional expression of PP2C. Activated SnRK2 can enhance plant drought resistance in two ways. First, it regulates the expression of downstream resistance genes by phosphorylating TFs, specifically ABA-responsive element binding protein and related binding factors (Jin et al. 2013; Li et al. 2017). Second, it activates slow anion channel-associated 1 or inhibits Kv channel in *A. thaliana* 1 through phosphorylation, leading to a decrease in K^+ ion concentrations in guard cells, which results in a reduction in cell turgor pressure and ultimately promotes stomatal closure (Du et al. 2019). However, unexpectedly, this study found that the transcription levels of three SnRK2-like DEGs (*SRK2F*, *SRK2C*, and *SRK2I*) were all suppressed by

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exogenous H_2S. In particular, *SRK2F* (*AT4G40010*) showed down-regulated expression in response to exogenous H_2S under drought stress, which contradicts the requirement for SnRK2 enzyme activation during emergency conditions. However, previous reports indicate that SnRK2.6 mutants affect stomatal closure in response to ABA. SnRK2.6 activity is stimulated by ABA, but gene expression is not regulated by ABA (Mustilli et al. 2002; Yoshida et al. 2002). Similarly, the impact of exogenous H_2S on the SRK2F enzyme activity may not necessarily correlate positively with its transcriptional expression level under drought stress. Additionally, Chen et al. (2020b) have demonstrated that H_2S can stimulate the activation of SnRK2.6 through persulfidation at Cys131 and Cys137. Therefore, it is reasonable to infer that when exposed to drought stress, exogenous H_2S can activate SRK2F at a post-translational level through persulfidation, thereby facilitating the transduction of ABA signaling.

H_2S regulates TFs in response to environmental stimuli and physiological signals

TFs have long been recognized as crucial regulators in response to various abiotic stresses. They engage with cis-regulatory elements located in the promoter regions of stress-related genes, thereby controlling gene expression and enhancing stress tolerance. In *Arabidopsis*, approximately 1,500 TFs have been implicated in regulating the expression of stress-related genes (Lata and Prasad 2011). Several TFs, such as MYB, WRKY, bHLH, and NAC, have been identified as pivotal regulators of plant drought tolerance. Drought stress can be influenced by certain *MYB* genes, which regulate lateral root growth. To illustrate, *AtMYB77* has been identified as a positive regulator of lateral root growth in response to drought stress through auxin signaling (Shin et al. 2007). Additionally, Chen et al. (2017) discovered that WRKY46, WRKY54, and WRKY70 are involved in BR-regulated plant growth and also play roles in drought response. The *wrky46 wrky54 wrky70* triple mutant displays impairments in BR-regulated growth but exhibits enhanced tolerance to drought stress. Furthermore, Liu et al. (2014) conducted experiments revealing that *bHLH122* is up-regulated under drought-stress conditions, and transgenic plants that overexpress *bHLH122* demonstrate increased resistance to drought stress. The *bhlh122* mutant exhibits a greater sensitivity to drought stress compared with WT plants. NAC TFs, which are specific to plants, play vital roles in responses to abiotic stress. *AtNAC016* plays a role in responses to drought stress. The *nac016* mutant shows enhanced drought tolerance, whereas the *NAC*- overexpressing plants exhibit reduced drought tolerance (Sakuraba et al. 2015). H_2S, an essential gas signaling molecule, can mitigate drought stress by modulating TFs. The induction of H_2S leads to an up-regulation of the *lateral organ boundaries-domain 16* gene, leading to a significant increase in lateral roots and enhanced stress resistance in plants (Mathur et al. 2022). The RNA-Seq analysis conducted by Li et al. (2017) identified MYB, bHLH, basic leucine zipper, and NAC as the top four enriched TFs in response to drought stress. Furthermore, they observed a significant impact of H_2S treatment on the induction or inhibition of these TFs. This study revealed that the bHLH family had the largest number of enriched TFs, followed by the NAC, MYB, and MYB_related4, and ERF TF families during H_2S treatment (Fig. 7D, E). TFs play essential roles in various developmental processes, including the regulation of stomatal movement, lateral root development, auxin signal transduction, and leaf senescence (Shin et al. 2007; Xie et al. 2000; Oh et al. 2011; Guo and Gan 2006; Kim et al. 2009). Additionally, the findings highlighted a significant number of TFs responded to physiological concentrations of H_2S under normal conditions (Fig. 7C). Consequently, H_2S may regulate gene expression by influencing TFs, ultimately impacting plant growth and development. These results underscore the ability of H_2S to respond to environmental and physiological signals via the regulation of TFs.

H_2S enhanced drought resistance in *Arabidopsis* seedlings by mediating the antioxidant system

Stress induces ROS generation and lipid peroxidation, leading to injury in *Arabidopsis* plants. In response to drought stress, the concentrations of H_2O_2 and MDA substantially increase in the plants. The addition of exogenous H_2S alleviated membrane impairments and reduced H_2O_2 levels (Fig. 8B, C). Antioxidant enzymes, such as SOD, APX, CAT, and POD, play vital roles in effectively eliminating ROS, thereby diminishing oxidative damage in plants (Ashraf 2009). Here, the activities of antioxidant enzymes SOD, CAT, APX, and POD increased under drought conditions, facilitating the conversion of ROS to H_2O. Additionally, the application of H_2S further enhanced the activities of SOD and POD enzymes, while having no impact on CAT and APX. These findings indicated that exogenous H_2S may regulate the synthesis and scavenging of H_2O_2 during drought stress (Fig. 8D–G). Furthermore, the expression of the SOD-encoding genes decreased during drought stress, while SOD enzyme activity increased. This suggests that the SOD-encoding genes may respond quickly to drought stress, resulting in a return of the SOD expression levels to normal, or even lower, at the time of detection. In addition to an efficient antioxidant enzyme system, non-enzymatic antioxidants levels also play roles in maintaining the dynamic ROS balance in plants. A transcriptome analysis revealed that H_2S treatments did not affect the expressions of *GR*, *dehydroascorbate reductase*, and *monodehydroascorbate*

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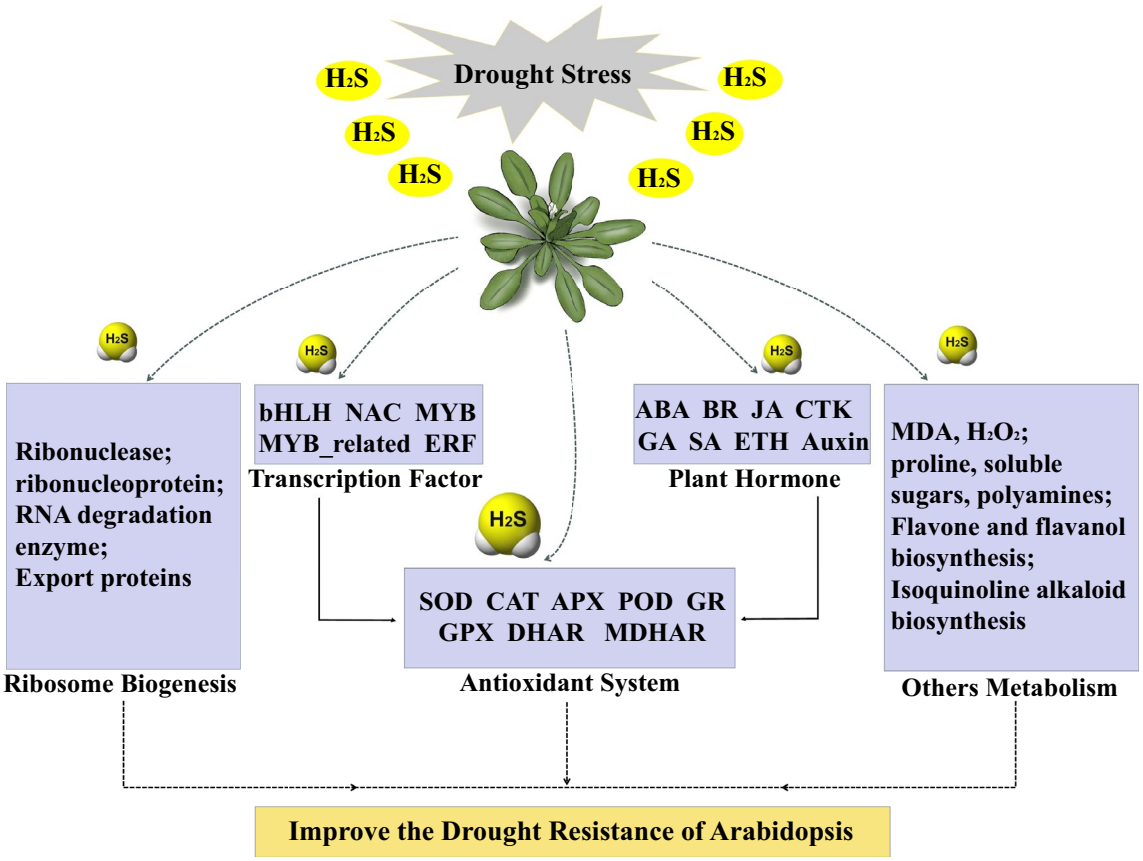


Fig. 10 A proposed model for *Arabidopsis* drought-stress responses based on the RNA-Seq analysis. The regulatory model of *Arabidopsis* seedlings under drought stress includes ribosome biogenesis, transcription factors, antioxidant systems, plant hormones, and other metabolisms. Dotted lines indicate indirect activation. Solid lines represent activation

reductase, but up-regulated the expression of *GPX*. Thus, H_2S may enhance its enzymatic activity by up-regulating the expression of *GPX*, thereby increasing the ability to eliminate ROS and participation in the ascorbate–glutathione (AsA–GSH) cycle, ultimately aiding *Arabidopsis* drought-stress resistance.

H_2S can enhance plant stress tolerance by regulating multiple factors, including TFs, plant hormones, and antioxidant systems (Li et al. 2017; Zhou et al. 2020; Shan et al. 2018). TFs play vital roles in enhancing plant resistance to drought by influencing the expressions of genes encoding antioxidant enzymes and their corresponding enzyme activity levels. Specifically, the TF ZmMYB-CC10 has been identified as binding to the promoter region of *ZmAPX4*, leading to increases in APX expression and activity. This mechanism ultimately reduces the levels of H_2O_2, thereby enhancing drought tolerance (Zhang et al. 2022c). Furthermore, the overexpression of the *BpMYB123* gene in plants can significantly enhance the activities of POD and SOD enzymes, while simultaneously reducing the levels of H_2O_2, superoxide anion, electrolyte leakage, and MDA under drought-stress conditions. These effects ultimately lead to the enhancement of drought resistance in plants (Lv et al. 2021). Additionally, the transcriptome analysis in this study revealed that H_2S can either up- or down-regulate the expressions of numerous TFs. Thus, H_2S may influence the antioxidant system by modulating TF expression, thereby enhancing drought resistance in plants. These findings provide a basis for understanding the regulatory mechanism of the H_2S-TF-ROS signaling cascade in enhancing stress tolerance. ABA and H_2O_2 are vital signaling molecules in plants that enable them to adapt to drought stress, and they exhibit reciprocal regulation. Under drought-stress conditions, ABA synthesis is activated, triggering the production of ROS and subsequently boosting the activities of antioxidant enzymes to eliminate ROS. Additionally, the application of ABA biosynthesis inhibitors can reduce H_2O_2 levels and decrease antioxidant enzyme activity (Xing et al. 2016). CAT, an H_2O_2 scavenger, can inhibit the promotion of ABA-induced adventitious roots and the increase in POD activity. This suggests that endogenous H_2O_2 is involved in ABA-induced adventitious root growth (Li et al. 2018). Furthermore, ABA and H_2O_2 synergistically enhance wheat resistance to drought stress (Wang et al. 2020). Transcriptome

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sequencing results indicate that H_2S primarily enhances plant drought resistance by modulating the ABA signal transduction pathway during drought stress. Thus, H_2S regulates the antioxidant system by impacting the ABA signal cascade pathway, thereby improving drought resistance in plants. This provides a foundation for understanding the regulatory mechanism behind the H_2S-ABA-ROS signaling cascade's involvement in enhancing stress tolerance.

Conclusions

This study utilized *A. thaliana* WT and the *lcd/des1* double mutant as materials to investigate the DEGs involved in responses to H_2S regulation under drought stress using RNA-Seq technology. A total of 3,043 DEGs were identified (1,949 from WT, 1,094 from *lcd/des1*), with 675 overlapping. These DEGs were then subjected to GO and KEGG pathway enrichment analyses. The DEGs associated with H_2S-regulated responses to drought stress mainly involved 'ribosome biogenesis', 'hormone signaling', 'antioxidant system', and 'drought-responsive TFs'. Based on the aforementioned analyses, a preliminary model for drought resistance mediated by H_2S signaling has been constructed (Fig. 10). This model provides comprehensive insights into the underlying mechanisms of drought-stress response facilitated by H_2S signaling, thereby identifying potential targets for improving drought resistance in crops. Furthermore, it serves as a valuable reference for developing novel strategies aimed at enhancing plant drought resistance.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10725-024-01197-z.

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Author contributions XFH participated in experimental design, conducted the majority of experiments and data analyses, and wrote the manuscript. HYC contributed to data analyses and manuscript drafting. ZQW and XYJ were responsible for preparing experimental materials, collecting samples, extracting RNA, and performing qRT-PCR experiments. ZPJ and YXP assisted in experimental design and manuscript revision. All the authors reviewed and approved the final version of the manuscript.

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Data availability All the relevant data can be found in the manuscript and supplementary materials.

Declarations

Conflict of interest The authors declare no conflicts of interest.

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