Invited Review Article

Hydrogen Sulfide and Protein Persulfidation in Plant Stress Signaling

Mingjian Zhou[1,2,3], Yanjie Xie[4], Frank Van Breusegem[1,2#], Jingjing Huang[1,2*#]

[1]Department of Plant Biotechnology and Bioinformatics, Ghent University, Technologiepark 71, 9052 Ghent, Belgium

[2]VIB Center for Plant Systems Biology, 9052 Ghent, Belgium

[3]Laboratory Center of Life Sciences, College of Life Sciences, Nanjing Agricultural University, Nanjing 210095, PR China

[4]Co-Innovation Center for Sustainable Forestry in Southern China, State Key Laboratory of Tree Genetics and Breeding, Key Laboratory of State Forestry and Grassland Administration on Subtropical Forest Biodiversity Conservation, College of Life Sciences, Nanjing Forestry University, Nanjing 210037, China

[#] Senior authors

* To whom correspondence should be addressed: Jingjing Huang (jingjing.huang@ugent.be)

E-mail addresses and ORCID numbers:

Mingjian Zhou: mingjian.zhou@ugent.be (0000-0002-0899-0120)

Yanjie Xie: yjxie@njfu.edu.cn (0000-0002-3503-1267)

Frank Van Breusegem: frbre@psb.ugent.be (0000-0002-3147-0860)

Jingjing Huang: jingjing.huang@ugent.be (0000-0002-4963-3162)

Including 3 tables and 5 figures, 1 supplementary dataset

Word Counts: 6894

Highlight

Hydrogen sulfide acts as an important signaling molecule through protein persulfidation in plant resilience to various stresses.

Abstract

Hydrogen sulfide (H_2S) is increasingly recognized as a crucial signaling molecule in plants, playing key roles in regulating physiological processes and enhancing stress tolerance. This review provides an updated summary of H_2S signaling in plant stress responses, discussing its uptake from external environmental sources, its endogenous biosynthesis, and its broader functions in stress adaptation. We summarize the impact of H_2S on plants under various stress conditions and review the mechanisms through which it mediates signaling functions, with a particular focus on H_2S-mediated protein persulfidation. Additionally, we provide an overview of the current understanding of protein persulfidation in regulating physiological processes and stress responses in plants, offering both a general discussion of its effects under different stress conditions and specific examples to highlight its significance. Finally, we review recent proteomic studies on protein persulfidation in plants, comparing the identified persulfidated proteins across studies and highlighting shared biological processes and pathways. This review aims to consolidate the current understanding of H_2S signaling and its roles mediated by protein persulfidation in plants, while also offering insights to inspire future research in this rapidly evolving field.

Keywords

Cysteine thiol, hydrogen sulfide, persulfidation, plant stress response, proteomics, redox signaling

Introduction

Sulfur is a vital element for life, playing a crucial role in numerous biological processes, making it essential for the health and functioning of living organisms. It plays a unique role in reduction-oxidation (redox) biology due to its ability to exist in multiple oxidation states, from -2 to +6, in both inorganic and organic compounds (Figure 1A). In inorganic compounds, sulfur commonly exists as sulfate (SO_4^{2-}), sulfide (S^{2-}), sulfite (SO_3^{2-}), elemental sulfur (S_8), thiosulfate ($S_2O_3^{2-}$), hydrogen sulfide (H_2S), and more. These sulfur-containing compounds play crucial roles in natural processes such as the sulfur cycle and biological metabolism. They are also vital in various industrial applications, such as sulfate-reduction bioreactors, which are used to precipitate heavy metals from wastewater (Janssen *et al.*, 2001; Kopriva *et al.*, 2024). In organic compounds, sulfur is incorporated into various biomolecules, including for the synthesis of essential sulfur-containing compounds such as methionine (Met), cysteine (Cys), and glutathione (GSH) (Nozaki *et al.*, 2005).

Among all sulfur-containing inorganic compounds, H_2S is a colorless gas with a characteristic odor of rotten eggs and is associated with the lowest oxidation states of sulfur at -2 (Figure 1A). Therefore, H_2S can be considered as a reducing agent, donating electron to other sulfur atom to regulate their oxidation states. H_2S is moderately soluble in water and dissociates into bisulfide ion (HS^-) and a proton (H^+) in aqueous solutions with a pK_{a1} of 6.9 (Pomeroy, 1941), and further dissociates into sulfide ion (S^{2-}) and another proton at a pK_{a2} ranging between 12 and 17 (Meyer *et al.*, 1983) (Figure 1B). At physiological pH, around 7.4, both H_2S and HS^- are present, with HS^- being predominant in aqueous solutions (Hughes *et al.*, 2009), indicating that at the cellular level, the major functional form of H_2S is likely HS^-. While H_2S can freely diffuse across cellular membranes as a gasotransmitter, HS^- requires ion channels to move between subcellular organelles or cells (Kabil and Banerjee, 2010). This suggests that H_2S and HS^- may regulate cellular functions in distinct ways.

H_2S has been historically regarded as a harmful gas primarily resulting from industrial activities due to its toxicity (Beauchamp *et al.*, 1984). High concentrations of H_2S in plants can have detrimental effects, including inhibition of respiration and photosynthesis, disruption of energy metabolism, degradation of chlorophyll, and impairment of growth and development. In extreme cases, these effects can ultimately lead to plant death (Maas *et al.*, 1988; Oliva and

Steubing, 1976). In 1996, Abe and Kimura discovered that physiological concentrations (50-160 μM) of H_2S act as an endogenous neuromodulator in the mouse brain, potentially regulating neural development, learning, and memory (Abe and Kimura, 1996). This discovery sparked further research, leading to H_2S being proposed as the third gas transmitter following nitric oxide (NO) and carbon monoxide (Kimura, 2002). Recent evidence indicate that H_2S plays beneficial regulatory roles in plants, involved in various processes throughout the plant lifecycle, from seed germination to cell senescence (Fang *et al.*, 2022; Ma *et al.*, 2021; Mukherjee and Corpas, 2023; Zhang *et al.*, 2024e). H_2S interacts with signaling molecules like NO and plant hormones, enhancing plants' resistance to environmental stresses (Du *et al.*, 2017; Huang *et al.*, 2021; Shen *et al.*, 2020; Zboinska *et al.*, 2023). The primary mechanism by which H_2S exerts its cellular signaling functions is through protein persulfidation, a post-translational modification (PTM) that involves the covalent attachment of a sulfur atom to the sulfhydryl (thiol) group of protein Cys residues, modulating protein function, stability, and activity (Aroca *et al.*, 2021b; Corpas *et al.*, 2021; Huang and Xie, 2023). This modification is now recognized as a central process in H_2S-mediated signaling pathways.

This review provides an updated overview of H_2S signaling in plant stress responses, focusing on environmental acquisition and endogenous H_2S production. We summarize H_2S effects under various stress conditions and discuss the signaling mechanisms in plants, emphasizing protein persulfidation. We discuss specific studies as examples to update current knowledge on protein persulfidation in different physiological processes and stress responses. Furthermore, we summarize recent proteomics studies investigating protein persulfidation and compare the identified persulfidated proteins from selected studies, highlighting common biological processes. This review consolidates our current understanding of H_2S signaling and its functions mediated by protein persulfidation in plants, while identifying emerging trends and future directions.

How do plants acquire H_2S?

Plants acquire H_2S from external environmental sources and synthesize H_2S through specific biosynthesis systems. Each source plays a distinct role in maintaining sulfur homeostasis and in enabling H_2S to function as a signaling molecule, particularly under stress conditions.

Environmental sources

H_2S is a naturally occurring atmosphere gas, prevalent in regions with volcanic activity, geothermal vents, and wetlands. It is released through microbial activity, geological processes and industrial activities such as petroleum refining, paper manufacturing, and natural gas processing (Figure 2; De Kok *et al.*, 2009; Hose *et al.*, 2000; Stern, 2005; Watts, 2000). These industrial processes, particularly the disposal of factory waste, contribute significantly to H_2S emissions, largely due to the handling and processing of sulfur-containing materials (Yang and Allen, 2012). Moreover, the decomposition of organic materials containing sulfur can release H_2S under specific conditions. Sulfate-reducing bacteria facilitate this process by breaking down sulfur compounds such as SO_4^{2-}, SO_3^{2-}, S_8, and $S_2O_3^{2-}$ in anaerobic environments like wetlands, marshes, and waterlogged areas. This process is enhanced by elevated temperatures, lower pH levels, and higher availability of sulfur compounds (Deng *et al.*, 2018; Narayan *et al.*, 2023). These combined sources of H_2S are often accompanied by the production of SO_2 and carbonyl sulfide (COS) (Figure 2; Levinn *et al.*, 2019; Svoronos and Bruno, 2002). Plants absorb gaseous SO_2, H_2S and COS through leaf stomata. COS is quickly broken down by carbonic anhydrase via hydrolysis, producing H_2S, while SO_2 is converted into SO_3^{2-} that further be reduced to sulfide and indirectly contributes to H_2S production (Figure 2; Bloem *et al.*, 2011; Brychkova *et al.*, 2007; Sanderson *et al.*, 2006). Absorbed H_2S is transported to various plant tissues, where it acts as a signaling molecule in stress responses, contributes to the synthesis of Cys, Met, and GSH, and triggers protein persulfidation (Capaldi *et al.*, 2015; Huang and Xie, 2023; Zhang *et al.*, 2021). H_2S uptake by plants follows saturation kinetics controlled by mesophyll conductance, but are limited by mesophyll conductance at saturating levels (Ausma and De Kok, 2019). Plant responses to H_2S exposure vary based on concentration and species sensitivity. Low levels (0.03 µl L^{-1}) typical of industrial or agricultural areas inhibit growth in susceptible plants like spinach but stimulates growth in others such as lettuce, alfalfa, and sugar beet (De Kok, 1989; 1990; Thompson and Kats, 1978). Higher concentrations (0.3 µl L^{-1}) common near volcanoes damage some plants like lettuce, sugar beet, and grape vine while leaving others unaffected. Certain species, like maiden silvergrass (*Miscanthus sinensis*), tolerate H_2S levels up to 20 µl L^{-1}, reflecting their adaptation to volcanic environments (Mudd, 1979; Ausma and De Kok, 2019). Researchers often use H_2S donors, particularly sodium

hydrosulfide (NaHS), to study H_2S effects on various plant species, including wheat, rice, Arabidopsis, and apple (Du *et al.*, 2022; Gautam *et al.*, 2022; Shen *et al.*, 2021; Singh *et al.*, 2022). The applications and effects of H_2S donors in research and agriculture have been comprehensively reviewed (Huang and Xie, 2023).

Biosynthesis of H_2S in plants

H_2S is produced endogenously in plants through a series of enzymatic reactions, primarily involving sulfur assimilation and Cys degradation. The SO_4^{2-} pathway is the main strategy for sulfur assimilation. Plants absorb SO_4^{2-} from the soil via specific root sulfur transporters, distribute it through xylem vessels, and assimilate it throughout the plant (Gigolashvili and Kopriva, 2014) (Figure 2). In plant cells, adenosine triphosphate (ATP) activates the SO_4^{2-} transporter, SO_4^{2-} is then reduced by ATP sulfurylase to SO_3^{2-}, which is further reduced to H_2S by chloroplastic sulfite reductase (SiR) (Sim *et al.*, 2019; Takahashi *et al.*, 2011). H_2S subsequently reacts with O-acetylserine (OAS) by OAS (thiol)lyase (OAS-TL) to generate Cys. Although OAS-TLs can theoretically catalyze the reverse reaction, evidence suggests this is unlikely under physiological conditions (Huang and Xie, 2023). In addition to SiR, there are two types of H_2S biosynthesis enzymes: cysteine desulfhydrases (CDes) and β-cyanoalanine synthase (CAS) (Figure 2).

CDes catalyze Cys using pyridoxal 5'-phosphate (PLP) as cofactor to produce H_2S, pyruvate and NH_4^+, including L-cysteine desulfhydrase (LCD), bifunctional cystathionine gamma-lyase/cysteine synthase (DES1), and D-cysteine desulfhydrase 1 and 2 (DCD1 and DCD2) (De La O-Sánchez *et al.*, 2023). LCD and DES1 use L-cysteine as substrate and are mainly localized in cytosol, while a recent study has identified nuclear-localized LCDs in tomato, which play important roles in fruit ripening (De La O-Sánchez *et al.*, 2023; Hu *et al.*, 2020; Romero *et al.*, 2013). DCDs utilize D-cysteine as substrate, with Arabidopsis DCD1 localized in mitochondria, while its rice (*Oryza sativa*) homolog is found in chloroplasts (Moseler *et al.*, 2024; Riemenschneider *et al.*, 2005; Zhou *et al*., 2020b). The physiological relevance of D-cysteine remains puzzling, mainly because D-amino acids were long considered non-functional in plants (Kolukisaoglu, 2020). However, increasing evidence shows that DCDs play critical roles in H_2S-mediated plant stress tolerance (Shi *et al.*, 2015; Xiang *et al.*, 2023; Zhang *et al.*, 2020b; Zhao *et al* 2023). CAS catalyzes the conversion of Cys and cyanide into H_2S and β-cyanoalanin

using PLP as cofactor (García *et al.*, 2010; Yamaguchi *et al.*, 2000). In Arabidopsis, three genes encode CAS, including *CYS-C1*, *CYS-D1*, and *CYS-D2* (García *et al.*, 2010; Hatzfeld *et al.*, 2000; Jost *et al.*, 2000). CYS-C1 is the most abundant CAS enzyme, localized in mitochondria and contributing the majority of CAS activity in root and leaf tissue. The isoforms CYS-D1 and CYS-D2 are localized in the cytosol and are much less abundant (Watanabe *et al.*, 2008). Notably, the nitrogenase Fe-S cluster protein (NifS), a cysteine desulfurase that uses L-cysteine as a substrate to generate alanine and elemental sulfur (Mihara *et al.*, 1997; Zheng *et al.*, 1993), is often incorrectly described as an H_2S-producing enzyme. However, there is no evidence supporting this claim, and researchers should approach this assertion cautiously. The functions of NifS homologs in plants, including mitochondrial NifS-like 1 (NFS1) and plastidal NFS2, have been recently reviewed by Caubrière et al. (2023).

Among the various H_2S-producing enzymes in plants, DES1 is typically considered the main contributor to H_2S production due to its ubiquitous activity across different physiological processes (Laureano-Marín *et al.*, 2014; Vojtovič *et al.*, 2021). Biochemical characterization of *des1* mutants revealed a 20% to 25% reduction in total desulfhydrase activity and a 50% decrease in H_2S content compared to wild-type Arabidopsis plants (Álvarez *et al.*, 2010; Jin *et al.*, 2013). Consequently, *des1* mutant has often used as a model system to investigate the H_2S function in plants (Aroca *et al.*, 2015; Shen *et al.*, 2020; Zhou *et al.*, 2021). However, it has recently demonstrated that Arabidopsis cystathionine β-lyase (CBL) exhibited robust LCD activity (Wang *et al.*, 2022). Under normal growth conditions, H_2S concentration and production rate in *cbl* mutant plants were found to be 50% lower than those in wild-type plants, providing new insights into H_2S biosynthesis. The contribution of various pathways to total H_2S content may vary by plant species, tissue type, developmental stage, and environmental conditions (Heeg *et al.*, 2008; Takahashi *et al.*, 2011; Yamaguchi *et al.*, 2000). Detailed quantitative comparisons of theses pathways are scarce, making this an evolving area of research. As sulfide serves as a precursor for Cys biosynthesis, and Cys can be degraded to produce H_2S by CDes, a metabolic feedback loop is created. The levels of H_2S and Cys are tightly regulated and play crucial roles in sulfur metabolism, redox homeostasis, and cellular signaling in plants (Birke *et al.*, 2015; Gotor *et al.*, 2019; Romero *et al.*, 2013). Recently, Fang *et al.* (2023) showed that either H_2S or Cys treatment elevated each other's levels in Arabidopsis.

However, transcriptomic analysis revealed significant differences in gene responses to these treatments, indicating that while closely connected, H_2S and Cys play distinct roles in different metabolic pathways.

Significant variations in H_2S levels have been observed across plants species. For instance, H_2S concentrations in Arabidopsis and faba bean *(Vicia faba)* leaves were ranged between 1 to 5 $\mu mol \cdot L^{-1}$ measured by a microsulfide ion electrode (García-Mata and Lamattina, 2010; Ma *et al.*, 2018). *Allium* species exhibited relatively high H_2S levels, with leeks containing 1 μmol $H_2S \cdot g^{-1}$ fresh weight (FW) and garlic cloves measuring 0.54 μmol $H_2S \cdot g^{-1}$ FW (Muñoz-Vargas *et al.*, 2022). In contrast, broccoli showed lower H_2S concentrations in the $nmol \cdot g^{-1}$ FW range, while pepper fruits displayed H_2S levels in the $pmol \cdot g^{-1}$ FW range (Muñoz-Vargas *et al.*, 2022). Jin *et al.* (2018) quantified H_2S levels across 17 plant species, including Arabidopsis, tobacco, tomato, wheat, cucumber, cabbage, green bean, millet, moss, and others. Using traditional methylene blue method, H_2S concentrations ranged from 0.010 to 0.199 μmol g^{-1} FW; more sensitive electrode measurement yielded values between 0.177 and 0.708 μmol g^{-1} FW. The two detection methods did not reveal any consistent patterns in H_2S concentrations among species. The researchers further conducted tissue-specific analyses of H_2S content in siliques, seeds, stems, cauline leaves, rosette leaves, and roots of 8-week-old Arabidopsis plants and investigated temporal changes in H_2S levels in rosette leaves at different developmental stages. Both detection methods consistently demonstrated that flowers and roots accumulated the highest H_2S concentrations and younger plants (2 weeks old) exhibited significantly higher H_2S content than more mature plants (4 weeks and older) (Jin *et al.*, 2018). Overall, these findings highlight the diversity of H_2S levels across plant species influenced by intrinsic and environmental factors.

What does H_2S do for plants under various stress conditions?

Environmental stresses such as drought, salinity, extreme temperatures, heavy metals, and nutrient deficiencies profoundly impact crop productivity, promoting plants to evolve complex regulatory pathways for enhanced resilience (Akpinar *et al.*, 2012; Sha *et al.*, 2024; Suzuki *et al.*, 2014; Zhang *et al.*, 2024d; Zhu, 2016). The following sections will discuss the regulatory functions of H_2S in defense mechanisms against these abiotic challenges (Table 1).

Drought stress

Drought stress primarily induces stomatal closure, leading to decreased transpiration (Zhang *et al.*, 2020). The impact of H_2S on stomatal movement has been well-documented (Huang and Xie, 2023; Liu and Xue, 2021; Ren *et al.*, 2022). Additionally, H_2S triggers non-stomatal factors like increased photosynthetic pigmentation and Rubisco activity, as well as nitrogen metabolism, contributing to improved drought resilience (Kaya and Shabala, 2023; Zhang *et al.*, 2023c; Zhao *et al.*, 2024). Furthermore, recent research indicates that both exogenous H_2S application and endogenous H_2S induced by drought stress act upstream of NO, enhancing the activity of the ascorbate-glutathione (AsA-GSH) cycle, also known as the Foyer-Halliwell-Asada cycle (Noctor and Foyer, 1998). This crucial antioxidant system detoxifies reactive oxygen species (ROS), particularly hydrogen peroxide (H_2O_2), which accumulates under stress. H_2S enhanced this cycle by modulating the activities and transcript levels of key enzymes: ascorbate peroxidase (APX), glutathione reductase (GR), and dehydroascorbate reductase (Shan *et al.*, 2011; 2020). By promoting redox reactions catalyzed by these enzymes, H_2S helps maintain cellular redox homeostasis and mitigate oxidative stress during drought. Substantial evidence also supports the positive effects of combining H_2S with other chemicals on plant growth and development during drought stress. For example, in wheat, the combined application of H_2S and silicon improved drought and pathogen resistance, leading to better photosynthetic efficiency and plant growth (Naz *et al.*, 2021). Similarly, the combination of H_2S and salicylic acid (SA) increased antioxidant enzyme activity, reduced oxidative stress as indicated by lower levels of H_2O_2, malondialdehyde content, and electrolyte leakage, ultimately improving crop yield under drought conditions (Akin and Kaya, 2024). Moreover, H_2S synergized with melatonin to regulate the Na^+/H^+ antiport system and nitrogen metabolism, further enhancing plant resilience to both drought and salt stress (Khan *et al.*, 2024a).

Salt stress

In Arabidopsis, H_2S alleviated salt stress by interacting with the auxin signaling pathway, crucial for root growth regulation under stress conditions. H_2S influenced the gene expression of *PIN2*, a key auxin transporter gene, promoting salt tolerance and modulating root growth in response to salinity (Yang *et al.*, 2023). In wheat, exogenous H_2S application positively modulated the AsA-GSH system, enhanced osmolyte accumulation, and improved nutrient

uptake, collectively contributing to improved growth and yield under salt stress (Kumari *et al.*, 2023). Similarly, in brinjal and tomato seedlings, H_2S maintained Na^+/K^+ homeostasis and regulated nitrogen metabolism, highlighting its role in promoting salt tolerance through ion balance and metabolic adjustments (Raju and Prasad, 2023). In cucumber, foliar spraying of H_2S improved photosynthesis, activated the AsA-GSH cycle, and upregulated genes involved in ion balance and other signaling pathways, including the Salt Overly Sensitive and Mitogen-Activated Protein Kinase (MAPK) pathways. MAPK cascades controlled cellular ROS levels, enhanced antioxidant enzyme activities, and regulated salt-responsive gene expression, aiding plant adaptation to high salinity (Luo *et al.*, 2023). The combined application of H_2S with selenium in strawberries demonstrated synergistic effects under saline conditions, evidenced by strengthened antioxidant activity, increased leaf chlorophyll and carotenoid content, enhanced nutrient absorption, and significantly improved growth and yield (Pourebrahimi *et al.*, 2023). In maize, the combined treatment of H_2S and SA resulted in enhanced antioxidant activity, better ionic balance, and improved photosynthetic pigments, leading to increased salt tolerance (Shoukat *et al.*, 2023). A recent study demonstrated that overexpression of *CAS* genes enhances salt stress tolerance in Arabidopsis. It revealed that both cyanide and H_2S levels increased during stress; however, the increase was significantly lower in *CAS* overexpression plants compared to wild-type plants after three days of salt stress treatment. Since CAS catalyzes the conversion of Cys and cyanide into H_2S and β-cyanoalanine, these findings suggest that salt stress tolerance may be closely linked to cyanide metabolism and sulfide assimilation pathways (Xu *et al.*, 2023). Additionally, H_2S mitigated salinity stress in sunflowers by enhancing photosynthesis, Rubisco activity, and chloroplast integrity through upregulation of the Rubisco small subunit gene *HaRBCS* during salt stress (Younis and Mansour, 2024). Moreover, H_2S enhanced salt tolerance in tomato seedlings by upregulating the expression of strigolactones (SLs) synthesis-related gene *SlD27,* essential for maintaining adequate SL production and regulating various physiological processes in plants (Kapulnik and Koltai, 2014; Yang *et al.*, 2024a). Overall, these findings indicate that H_2S enhances salt stress tolerance by participating in various biological processes and cellular pathways.

Temperature stress

In heat-stressed wheat and rice seedlings, H_2S helped maintain redox homeostasis, reduced lipid

peroxidation, and protected photosynthesis (Gautam *et al.*, 2022; Havva *et al.*, 2022). H_2S also mitigated cold stress in peach and tomato. In cold-stored peaches, H_2S acted downstream of methyl jasmonate to reduce chilling injury by downregulating *polyphenol oxidase* gene expression, modulating energy metabolism, and enhancing antioxidant defenses (Yu *et al.*, 2024). In tomato, H_2S improved cold tolerance by maintaining plasma membrane stability, regulating energy metabolism, and delaying cell wall degradation, thus alleviating cold storage damage (Li *et al.*, 2024a). Furthermore, H_2S was shown to interact with MAPK signaling pathway in tomato during cold stress, particularly MPK4, which upregulated cold-responsive genes and enhanced the plant's ability to withstand low temperatures (Wu *et al.*, 2024). Overall, H_2S serves as a versatile signaling molecule that enhances plant resilience to both high and low temperature stresses through its regulatory effects on antioxidant systems, energy metabolism, and signaling pathways.

Heavy metal stress

Substantial evidence indicates that H_2S plays a significant role in plant responses to heavy metals such as cadmium (Cd), chromium (Cr), nickel (Ni), and copper (Cu) (Bu *et al.*, 2024; Javad *et al.*, 2022; Singh *et al.*, 2022; Valivand and Amooaghaie, 2021; Yusuf *et al.*, 2023; Zulfiqar *et al.*, 2024) (Table 2). For instance, foliar application of NaHS has been shown to improve Cd-stress tolerance in soybean plants. This improvement was evidenced by reduced ion leakage, improved relative water content, increased photosynthetic pigments in leaves, and enhanced shoot length and fresh weight during Cd stress (Oloumi *et al.*, 2024). The study also demonstrated that H_2S alleviates Cd toxicity by boosting glutathione metabolism, enhancing the activity of glutathione reductase, and increasing the expression of GSH synthase family genes, including *chloroplastic-like GSH synthase* (*GS288*), *chloroplastic GSH synthase* (*GS473*), and *homoglutathione synthase* (*HGS*). Additionally, Bu *et al.* (2024) reported that exogenous application of NaHS alleviates germination delay and root growth inhibition caused by Cr stress in alfalfa (*Medicago sativa L.*). This effect was likely due to the regulation of intracellular ROS homeostasis, as NaHS treatment significantly reduced H_2O_2 and O_2^- levels while notably enhancing the activities of antioxidant enzymes including catalase (CAT), APX, and GR. In hydroponically grown zucchini (*Cucurbita pepo L.*) seedlings, NaHS treatment was shown to enhance Ni-stress tolerance by restoring mineral balance, lowering proline levels,

increasing sugar levels, and inducing antioxidant metabolites, including flavonoids and phenolic compounds (Valivand and Amooaghaie, 2021). H_2S regulation of proline accumulation was also observed in cucumber (*Cucumis sativus*) under Cu stress. NaHS treatment increased proline content under Cu stress by activating the activities of proline synthesis enzymes, including pyrroline-5-carboxylate synthetase and proline dehydrogenase (Yusuf *et al.*, 2023). More interestingly, a recent study demonstrated that an innovative strategy combining growth-promoting rhizobacteria (*Azospirillum brasilense*) with NaHS treatment could significantly enhance Cd resistance in pak choi (*Brassica chinensis L.*). This co-application increased shoot biomass, boosted the activities of antioxidant enzymes, including peroxidase (POD) and superoxide dismutase (SOD), and reduced Cd translocation (Cui *et al.*, 2023). Collectively, these studies demonstrate the pivotal role of H_2S in improving plant tolerance to heavy metal stress, offering promising strategies for enhancing crop productivity and food safety in heavy metal-contaminated soils.

How does H_2S enhance plant stress resilience?

H_2S plays diverse roles in plant stress responses through specific molecular mechanisms that enhance resilience to environmental challenges. H_2S confers stress tolerance primarily by boosting the plant's antioxidant system, which mitigate oxidative damage caused by stress. Additionally, H_2S regulates protein function through persulfidation (Figure 3). The following sections will explore the critical roles of H_2S in plant stress tolerance.

H_2S enhance antioxidant system upon stress responses

During environmental changes, plant cells produce ROS, including superoxide anion ($O_2^{\cdot -}$), H_2O_2, hydroxyl radical (·OH), and singlet oxygen ($^1O_2$) (Figure 3A; Tripathy and Oelmuller, 2012). Overproduction of ROS leads to oxidative stress, damaging cellular components (Apel and Hirt, 2004; Foyer and Noctor, 2005). Plants manage ROS levels through tightly regulated antioxidant mechanisms, including GSH, ascorbate (AsA), and various antioxidant enzymes (Foyer and Noctor, 2011; Waszczak *et al.*, 2018). H_2S is recognized as a potential antioxidant that scavenges ROS, directly neutralizing them through chemical reactions and converting O_2^- into less harmful products such as ·OH and H_2O_2 (Figure 3A). H_2S can further neutralize H_2O_2 and OH^- by transforming them into water and elemental sulfur, thus reducing oxidative stress

(Bergstedt *et al.*, 2022). However, the likelihood of H_2S acting as a direct scavenger of oxidants in biological systems is limited by its low tissue concentrations (Filipovic *et al.*, 2018). H_2S enhances the activity and gene expression of various antioxidant enzymes, including SOD, CAT, APX, POD, and glutathione-S-transferase (GST) under different stress conditions (Table1; Figure 3A; Chen *et al.*, 2021; Dawood *et al.*, 2022; Kaya and Uğurlar, 2024; Li *et al.*, 2024b; Lin *et al.*, 2022; Shalaby *et al.*, 2023; Song *et al.*, 2024; Wu *et al.*, 2024; Younis and Mansour, 2023) For instance, exogenous application of NaHS enhanced SOD and APX activities in rice (*Oryza sativa L.*) leaves under both non-stress and high-temperature conditions, promoting thermotolerance (Gautam *et al.*, 2022). In *Reaumuria soongorica,* a typical perennial salt-yielding halophytic shrub with strong adaptability to saline and desert soils, NaHS treatment increased SOD, CAT, and POD activities in seedlings under salt stress (Liu *et al.*, 2023). In sunflower, priming with 0.5 mM NaHS significantly enhanced in *HaGST* transcription, with increases of 22.1% in shoots and 18.2% in roots compared to NaCl treatment alone, alleviating salinity-induced adverse effects (Younis and Mansour, 2023). Furthermore, transcriptomic analyses in cold-sensitive chili pepper plants identified 416 differentially expressed genes (DEGs) in response to cold stress following NaHS treatment. These DEGs were enriched in pathways associated with AsA-GSH metabolism, starch-sucrose metabolism, and plant hormone signal transduction (Song *et al.*, 2024).

H_2S plays a crucial role in maintaining the levels of redox metabolites, including GSH, Cys, and AsA. For example, exposure of Arabidopsis plants to H_2S gas or treatment of tobacco suspension cells with NaHS significantly increased Cys and GSH levels (Birke *et al.*, 2015; Li *et al.*, 2015). Exogenous application of NaHS mitigated Cd^{2+} toxicity by promoting GSH accumulation in Arabidopsis roots (Jia *et al.*, 2016), while foliar application of NaHS alleviated Cd toxicity in soybean by increasing both Cys and GSH content and upregulating several GSH synthase genes (Oloumi *et al.*, 2024). H_2S also restored the redox status of AsA and GSH. For example, H_2S enhanced the transcription of AsA and GSH biosynthetic enzyme genes in strawberry seedlings under heat stress (Christou *et al.*, 2014). A recent study by Guo *et al.* (2023) examined the beneficial effects of H_2S during Cd stress in Chinese willow *(Salix matsudana* Koidz), an allotetraploid tree species known for its high stress resistance. Exogenous application of NaHS reduced oxidative stress and restored the plant growth parameters during

Cd stress through increasing the activity and gene expression levels of antioxidative enzymes, including SOD, CAT, APX, and GR while facilitating the maintenance of GSH and AsA contents (Guo *et al.*, 2023). Overall, H_2S plays significant roles in enhancing antioxidant system in plants under environmental stresses. While its direct antioxidant effects through its reaction with ROS are somewhat limited biologically, its more significant role lies in the indirect regulation of antioxidant enzymes and metabolites. Future research should focus on investigating *in situ* H_2S concentration within plant cells during stress conditions and determining whether H_2S regulates key antioxidant enzymes at the gene expression level or through protein activity modulation or PTMs. This will be crucial for advancing our understanding of H_2S-mediated stress responses in plants.

H_2S-mediated protein persulfidation regulating plant stress responses

Due to the wide range of oxidation states of sulfur, the Cys thiol group is highly susceptible to oxidation, leading to various oxidative PTMs (Huang *et al.*, 2018; Huang and Xie, 2023). Initially, the thiol group can react with H_2O_2 to form sulfenic acid (R-SOH), which can undergo further oxidation to form sulfinic acid (R-SO_2H) and irreversibly to sulfonic acid (R-SO_3H) (Figure 3B; Huang *et al.*, 2024). Sulfinic acid can be reduced back to R-SOH, a process requiring both ATP and the enzyme sulfiredoxin (SRX) (Biteau *et al.*, 2003; Liu *et al.*, 2006). The sulfiredoxin based R-SO_2H reduction was initially thought to be specific only to certain peroxiredoxins (Prxs). However, a study in mammalian systems has revealed a broader range of substrates for this process (Akter *et al.*, 2018). In plants, the known substrates of sulfiredoxin remain limited to plastid 2-Cys Prxs and mitochondrial PrxIIF (Huang *et al.*, 2024). Sulfenic acid can also react with H_2S to form a persulfide (R-SSH), a modification process known as persulfidation (Figure 3B; Cuevasanta *et al.*, 2015; Huang and Xie, 2023). Under excessive oxidative conditions, protein R-SSH can be further oxidized to form higher-order species such as S-sulfocysteines (R-SSO_nH) (Bianco *et al.*, 2016), which can be enzymatically reduced back to the thiol form by redox-active enzymes like thioredoxin (TRX) and glutaredoxin (GRX). While these reducing reactions by redoxins have been confirmed in mammalian systems, they have so far only been verified *in vitro* for plant proteins (Figure 3B; Dóka *et al.*, 2016; 2020; Filipovic, 2015; Ju *et al.*, 2016; Moseler *et al.*, 2021; Wedmann *et al.*, 2016). H_2S reacts specifically with oxidized thiols, particularly -SOH groups and disulfides, to form -SSH, rather

than with reduced thiols (Cuevasanta *et al.*, 2015, Huang and Xie, 2023). The kinetics of H_2S reactions with low-molecular-weight albumin disulfides and -SOH groups have been determined. The rate constant for the formation of persulfides from H_2S and -SOH is 270 $M^{-1}S^{-1}$, which is significantly higher than the rate constant for reactions with disulfides (0.6 $M^{-1}S^{-1}$). This suggests that the formation of protein -SSH primarily occurs through the reaction of H_2S with -SOH groups. However, in some specific cases, such as with human sulfide quinone oxidoreductase, exceptionally high reaction rates between protein disulfides and H_2S have been observed (Cuevasanta *et al.*, 2017). In addition to these non-enzymatic processes, persulfidation can be enzymatically catalyzed by specific proteins. Two key enzymes involved in this process are 3-mercaptopyruvate sulfurtransferases (MST) and cysteinyl-tRNA synthetases (CARS). MST acts as a protein persulfidase, engaging in direct protein-to-protein transpersulfidation reactions (Moseler *et al.*, 2021; Pedre *et al.*, 2023). CARS has been identified as a principal Cys persulfide synthase, playing a crucial role in the endogenous production of both low molecular weight polysulfides and polysulfidated proteins (Akaike *et al.*, 2017). This aspect of enzymatic persulfidation has been extensively discussed in a recent review article (Moseler *et al.*, 2024). Protein persulfidation directly regulate various biological processes by affecting diverse types of plant proteins, including transcription factors (TFs), metabolic enzymes, antioxidant enzymes, protein kinases, as well as other stress-regulatory proteins (Figure 4, Table 2). In the following sections, we will explore specific examples of how persulfidation regulates these protein functions in detail.

Transcription factors

H_2S-induced persulfidation regulates various TFs in plants. In abscisic acid (ABA) signaling, persulfidation of TF ABA insensitive 4 (ABI4) at Cys250 has been shown to enhance ABI4's ability to regulate target genes like *MAPK kinase kinase 18* (*MAPKKK18)* and *DES1,* activating downstream signaling pathways and affecting seed germination and seedling growth. This positions ABI4 as a key hub linking ABA signaling with the MAPK cascade, underscoring the role of persulfidation in fine-tuning ABA signal transduction. It has also been demonstrated that ABI4 persulfidation boosted its stability by slowing 26S proteasome-mediated degradation during Arabidopsis seeds germination, highlighting its crucial role in H_2S-induced delay of seed germination (Zhou *et al.*, 2021; 2022; Figure 4A, Table 2). Additionally, a recent study in

391 tomato (*Solanum lycopersicum L.*) revealed that persulfidation enhanced TF WRKY71's
392 binding affinity to the *CAS1* promoter, inhibiting *CAS1* transcription (Sun *et al.*, 2023).
393 Moreover, persulfidation at Cys396 of tomato SlWRKY6 attenuated expression of ripening-
394 related genes *SlSGR1* and *SlSAG12* (Zhang *et al.*, 2024c). Both studies contribute to the
395 understanding of H_2S-delayed tomato fruit ripening. In pear (*Pyrus communis L*), persulfidation
396 of PyMYB10 was shown to enhance its interaction with PyMYB6 while weakening its
397 interaction with PyMYB73, downregulating anthocyanin biosynthesis-related genes. This
398 inhibits anthocyanin synthesis, contributing to H_2S-delayed color change during fruit ripening
399 (Yao *et al.*, 2023).
400 *Metabolic enzymes*
401 In Arabidopsis, cytosolic glyceraldehyde-3-phosphate dehydrogenases (GAPDHs), including
402 GapC1 and GapC2, undergo S-nitrosylation and S-glutathionylation at Cys150, which
403 reversibly inhibit their activities (Bedhomme *et al.*, 2012; Zaffagnini *et al.*, 2013). Initial
404 proteomics analysis also identified persulfidation of both cytosolic and chloroplastic GAPDHs
405 in Arabidopsis, with H_2S enhancing GapC1 activity through persulfidation (Aroca *et al.*, 2015).
406 Further research demonstrated that persulfidation at Cys160 of GapC1 not only augmented its
407 activity but also promoted its cytosol-to-nucleus translocation (Aroca *et al.*, 2017b). The
408 enzyme DES1, crucial in sulfur metabolism and H_2S production in plants, was found to undergo
409 persulfidation at Cys44 and Cys205, rapidly activating the enzyme and elevating intracellular
410 H_2S levels to regulate ABA-triggered stomatal closure (Shen *et al.* 2020). Recent studies have
411 expanded our understanding of persulfidation in other metabolic enzymes. For example,
412 persulfidation of cytosolic glucose-6-phosphate dehydrogenases (G6PDs) in Arabidopsis and
413 tomato at conserved Cys residues. Using cross-linking coupled mass spectrometry (MS),
414 structural modeling, and biochemical characterization, it was demonstrated that persulfidation
415 of Arabidopsis G6PD6 altered its homotetrameric structure, increased its affinity for $NADP^+$,
416 and promoted its oligomerization, consequently leading to enhanced activity, particularly under
417 salt stress conditions (Wang *et al.*, 2023, Figure 4B, Table 2). Additionally, persulfidation of
418 succinate dehydrogenase (SDH), known as Complex II of the electron transport chain, was
419 found to enhance its enzymatic function, resulting increased ATP synthesis, which is essential
420 for guard cell dynamics and stomatal closure (Zhang *et al.*, 2024b).

Antioxidant enzymes

Several studies have reported the persulfidation of antioxidant enzymes. Initial proteomic analysis identified persulfidation of cytosolic APX1 in Arabidopsis, with H_2S enhancing its activity (Aroca *et al.*, 2015). In tomato, specific persulfidation sites were identified during NaHS-mitigated copper oxide nanoparticle stress, including Cys234 on APX1, Cys61 on POD5, and Cys234 on CAT1. While H_2S enhanced the activities of APX1 and POD5, it decreased CAT1 activity (Li *et al.*, 2020b, Figure 4C, Table 2). These findings indicate that persulfidation differentially regulates antioxidant enzymatic activity during plant defenses.

Protein kinase

H_2S has been shown to positively regulate the ABA signaling through persulfidation of the protein kinase Open Stomata1/Snf1 related Protein Kinase 2.6 (SnRK2.6). Persulfidation sites identified at Cys131 and Cys137 of SnRK2.6 were found to enhance its activity and promoted its interaction with the downstream TF ABA-responsive element-binding factor 2 (ABF2) (Chen *et al.*, 2020). A subsequent study demonstrated that persulfidation altered SnRK2.6 protein structure, enhancing its phosphate transfer efficiency at Ser175, resulting in increased phosphorylation of SnRK2.6. This not only enhanced its binding affinity to ABF2 but also regulated its own persulfidation (Chen *et al.*, 2021). The interplay between persulfidation and phosphorylation of SnRK2.6 synergistically modulates ABA signaling and enhances plant responses to drought stress. Additionally, persulfidation of MPK4 was shown to boost its kinase activity and contribute to alleviating cold stress in Arabidopsis. Persulfidation was observed even when each of the eight Cys residues was individually mutated in separate MPK4 variants. This suggests that MPK4 persulfidation likely occurs on multiple Cys sites simultaneously, rather than being dependent on a single specific Cys residue (Du *et al.*, 2021). The precise mechanisms underlying this modification require further investigation.

Other Stress-regulatory proteins

Respiratory Burst Oxidase Homolog D (RBOHD) plays a crucial role in generating ROS in plant cells. Persulfidation at Cys825 and Cys890 of Arabidopsis RBOHD was found to enhance its activity, promoting a burst of ROS and leading to ABA-triggered stomatal closure (Shen *et al.*, 2020). Persulfidation also plays an important role in autophagy, a macromolecular degradation pathway linked to endoplasmic reticulum (ER) stress, activated in response to

various stresses. Laureano-Marín *et al.* (2020) demonstrated that ABA-induced persulfidation specifically targeted autophagy-associated (ATG) Cys proteases ATG4a at Cys170, inhibiting its proteolytic activity and negatively regulating autophagy. Additionally, Aroca *et al.* (2021a) showed that persulfidation of ATG18a at Cys103 enhanced its binding ability to specific phospholipids, inhibiting autophagosome maturation under ER stress. Beyond its role in autophagy, persulfidation has been implicated in other cellular processes. In tomato, persulfidation of BOI-related E3 ubiquitin-protein ligase 3 (BRG3) at Cys206 and Cys212 was found to reduce its ubiquitination activity and decrease its interaction with TF WRKY71. This led to increased WRKY71 levels and enhanced binding to the *CAS1* promoter, inhibiting *CAS1* expression and ultimately delayed fruit ripening (Sun *et al.*, 2023, Figure 4D, Table 2). Furthermore, persulfidation of Arabidopsis plasma membrane H^+-ATPase (PMA1) was shown to enhance its binding to General Regulatory Factor 4, activating PMA1 to maintain K^+/Na^+ homeostasis, thereby improving salt tolerance (Ma *et al.*, 2023). Overall, H_2S-mediated persulfidation is involved in various biological processes by modulating the functions of different protein classes.

How can proteomics studies advance understanding of protein persulfidation in plants?

Recent advancements in redox proteomics have greatly advanced our understanding of redox regulation mediated by protein Cys PTMs (Willems *et al.*, 2021). Various proteomic techniques have been developed for detecting protein persulfidation, including Tag-switch and Dimedone-switch methods, which have been recently comprehensively reviewed (Huang and Xie, 2023). These proteomic studies have successfully identified persulfidated proteins under different physiological and stress conditions in plants (Aroca *et al.*, 2017a; García-Calderon *et al.*, 2023; Jurado-Flores *et al.*, 2021;2023b; Laureano-Marín *et al.*, 2020; Matamoros *et al.*, 2024; Muñoz-Vargas *et al.*, 2024; Zhang *et al.*, 2024a). In this section, we focus on discussing MS-based proteomic studies that investigate protein persulfidation in plants and present a comparative analysis of selected studies to provide insights into the similarities in the processes or pathways involving persulfidation-sensitive proteins (Table 3, Figure 5).

Overview of proteomics studies on protein persulfidation

In Arabidopsis, proteomic studies have shown that persulfidation affects a wide range of

biological processes, including the tricarboxylic acid (TCA) cycle, the Calvin cycle, and carbon metabolism (Table 3). A notable study identified 2,015 and 2,130 persulfidated proteins from the leaves of wild-type and *des1* mutant Arabidopsis plants, respectively (Aroca *et al.*, 2017a). This finding highlights the broad impact of protein persulfidation on plant physiology. Later, a comparative and quantitative proteomic analysis was used to detect persulfidated proteins in Arabidopsis leaves treated with ABA. The analysis revealed that ATG4a exhibited the highest difference in persulfidation levels between untreated and ABA-treated samples (Laureano-Marín et al., 2020). Further research revealed over 5,200 persulfidated proteins in Arabidopsis roots, with 1,674 proteins showing significant alterations during nitrogen starvation, which are primarily involved in pathways like protein degradation and autophagy (Jurado-Flores *et al.*, 2021). A subsequent label-free quantitative proteomic analysis examined protein persulfidation levels when plants grown under nonphotorespiratory conditions were transferred to air and found that 1,082 out of 1,096 (98.7%) proteins were more persulfidated under suppressed photorespiration (García-Calderon *et al.*, 2023). Environmental factors like light and carbon availability have also been shown to influence persulfidation through proteomic analysis. In Arabidopsis leaves, proteins related to ER folding were highly persulfidated in darkness, while proteins involved in degradation were more affected in light, reflecting context-dependent regulation *(*Jurado-Flores *et al., 2023b)*. Additionally, studies in legumes have shown that persulfidation is essential for nitrogen-fixing nodule development. In beans, H_2S treatment enhanced redox balance within nodules, crucial for nitrogen fixation, while aging nodules exhibited reduced persulfidation and disrupted redox homeostasis (Matamoros *et al.*, 2024). Furthermore, the first persulfidation proteome in rice identified 3,443 proteins involved in key biological processes including sulfide signaling, redox homeostasis, energy pathways, and water transport (Zhang *et al.*, 2024a). The study also revealed that water transport activity was regulated by sulfide, correlating with increased persulfidation of aquaporins. This differs from current knowledge in Arabidopsis, suggesting persulfidation plays distinct roles in different plant species.

Comparative analysis of proteomic identified persulfidated protein in Arabidopsis

We conducted a comparative analysis of the identified persulfidated proteins from four proteomic studies, where protein persulfidation was analyzed in wild-type Arabidopsis leaves

under non-stress, drought stress, ABA or high CO_2 treatment conditions (Table 3, Aroca *et al.*, 2017a; García-Calderon *et al.*, 2023; Jurado-Flores *et al.*, 2023a; Laureano-Marín *et al.*, 2020). Firstly, we utilized 2015 persulfidated proteins identified from 30-day-old plants under non-stress condition (Aroca *et al.*, 2017a). For the second dataset, we included 1157 persulfidated proteins identified from both untreated and ABA-treated plants (Laureano-Marín *et al.*, 2020). Additionally, we incorporated a list of 2568 persulfidated proteins identified and quantified under both nonstress and drought-stress conditions (Jurado-Flores *et al.*, 2023a). Lastly, we included a list of 1452 proteins identified under both normal conditions and those grown under high CO_2 conditions (García-Calderon *et al.*, 2023). Our analysis revealed that 600 proteins were consistently identified across all four studies (Figure 5A, Supplementary Dataset S1). We consider these proteins to be particularly prone to persulfidation regardless of growth conditions, highlighting their likely central roles in persulfidation-mediated regulation. We further conducted Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses to identify general patterns among them.

KEGG pathway analysis showed that these 600 common proteins are primarily involved in metabolic pathways (309 proteins), biosynthesis of secondary metabolites (197 proteins), and carbon metabolism (103 proteins) (Figure 5B). GO analysis provided further insights into the biological roles of these persulfidated proteins (Figure 5C). In the biological processes' category, many proteins were involved in response to cold (46 proteins), protein folding (29 proteins), and the TCA cycle (24 proteins), highlighting persulfidation's contribution to both stress adaptation and fundamental energy metabolism. For molecular function category, the proteins were primarily involved in protein binding (166 proteins), ATP binding (134 proteins), and mRNA binding (93 proteins), indicating the role of persulfidation in regulating protein interactions and energy-related processes. While these findings highlight some commonality among persulfidation-sensitive proteins in certain biological processes, particularly in stress response related proteins and energy metabolism, they also highlight the broad involvement of persulfidation in critical cellular functions. Despite many proteomics studies on protein persulfidation have been conducted in plants, precise information on which Cys sites undergo persulfidation remains largely uncharted. Additionally, we lack knowledge about the stoichiometry of persulfidation levels under stress conditions due to the absence of specialized

quantitative redox proteomics approaches. The adoption of advanced proteomic profiling strategies for the identification and quantification of the full repertoire of persulfidated Cys sites in plants presents a promising future direction.

Conclusions and perspectives

This review highlights the important signaling roles of H_2S and protein persulfidation in plant stress biology. H_2S regulates the plant antioxidant system primarily through indirect mechanisms affecting key enzymes and metabolites, rather than direct reactions with ROS. However, the specific molecular pathways through which H_2S modulates these antioxidant systems remain unclear. Future research should determine whether these effects occur at the transcriptional level, through protein activity modulation, or via PTMs like persulfidation. Additionally, understanding *in situ* H_2S concentrations and dynamics within plant cells during stress will be key to advancing our understanding of H_2S-mediated stress responses.

Persulfidation has emerged as a key PTM in H_2S signaling. While progress has been made, the extend of its contribution to plant stress resilience remain unknown. Proteomic techniques offer powerful tools to advance our understanding of protein persulfidation in H_2S signaling. Identifying persulfidated proteins has provided valuable insights into the regulatory networks govering plant responses to various environmental stresses and signaling pathways, such as drought, ABA signaling, nutrient starvation. However, advanced proteomic techniques are needed to map the persulfidated Cys sites and quantify persulfidation levels more accurately, which is essential for understanding how persulfidation influences plant stress responses and resilience.

Supplementary data

Supplementary Dataset S1. List of 600 common persulfidated proteins identified in Arabidopsis across four proteomic studies

Acknowledgements

The analysis and visualization of proteomics data were supported by https://www.bioinformatics.com.cn/. The Venn diagram was generated using https://bioinformatics.psb.ugent.be/webtools/Venn/, and Figures 1-4 were created with

BioRender.com. We would like to acknowledge the assistance of ChatGPT for language editing during the preparation of this manuscript.

Author contribution

M.Z. and J.H. wrote the manuscript, M.Z., J.H., and F.V.B. revised the manuscript, Y.X provided suggestions.

Conflict of interest

The authors declare no conflict of interest.

Funding

This work received support from the Research Foundation-Flanders, including a senior postdoctoral fellowship (grant number 1227020N to J. H.) and a research project (grant number G007723N to F. V. B). Additional support was provided by the China Scholarship Council (grant number 202206850038) and Ghent University (grant number BOF24/CDV/126) to M. Z., and the National Natural Science Foundation of China (grant number 42370299 to Y. X.).

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Tables:

Table 1: H_2S-induced plant abiotic stress tolerance

Stress	Species	Condition	H_2S treatment	Functional effects	References
Drought	Wheat (*Triticum aestivum*)	10% PEG	0.3 mM NaHS	Enhance the activity of the AsA-GSH cycle, maintaining cellular redox homeostasis and mitigate oxidative stress during drought	(Shan *et al.*, 2011;2020)
	Arabidopsis	10 μM ABA	100 μM NaHS	Function in the regulation of HY1 retrograde signaling, involving in ABA-induced stomatal closure	(Zhang *et al.*, 2020a)
	Rice (*Oryza sativa* L.)	Withhold watering	100 μM NaHS	Reestablish redox homeostasis and activate ABA biosynthesis and signaling, promoting drought tolerance	(Zhou *et al.*, 2020a)
	Safflower (*Carthamus tinctorius* L.)	50/70% water	0.5 mM NaHS	Regulating secondary metabolism, oxidative defense, and elemental uptake, showing greater improvement in plant growth	(Amir *et al.*, 2021)
	Wheat (*Triticum aestivum* L.)	Withholding water	0.3 mM NaHS	Together with silicon, increase the plant growth and enhance the drought stress tolerance and *Puccinia triticina* resistance	(Naz *et al.*, 2021)
	Soybean (*Glycine max*)	20-60% water	100 μM NaHS	Upregulate enzyme activities and gene expression level of antioxidants, as well as osmotic protective substance content and related gene expression levels, strengthening the plant antioxidant capacity.	(Lin *et al.*, 2022)
	Pepper (*Capsicum annuum* L.)	40% water	0.2 mM NaHS	Upregulate of nitrogen metabolism and improve drought stress tolerance	(Kaya and Shabala, 2023)
	Chinese cabbage (*B. rapa* L. ssp. *pekinensis*)	Withhold watering	100 μM NaHS	Increase photosynthetic pigmentation and Rubisco activity, then improve drought resistance	(Zhang *et al.*, 2023c)

	Wheat (Triticum aestivum L.)	50% deficit irrigation	0.3 mM NaHS	Together with salicylic acid, decrease H_2O_2, MDA, and reduce electrolyte leakage, improving drought resistance	(Akin and Kaya, 2024)
	Pepper (*Capsicum annuum* L.)	50% water	0.2 mM NaHS	Interact with GSH, further reduce NOX, SOD, and H_2O_2 values and increased GPX activity, accelerate S-nitrosylation, thereby improve the drought tolerance.	(Kaya and Uğurlar, 2024)
	Tomato (*Solanum lycopersicum* L.)	40% water	Only use 1 mM HT as H_2S scavenger	Regulate the Na^+/H^+ antiport system and nitrogen metabolism, enhancing plant resilience to both drought stress	(Khan *et al.*, 2024b)
	Foxtail Millet (*Setaria italica* L.)	Withhold watering	100 μM NaHS	Modulate carbon and nitrogen metabolism and increases drought tolerance	(Zhao *et al.*, 2024)
Salt	*Malus hupehensis*	150 mM alkaline salt	0.5 mM NaHS	Regulate Na^+/K^+ homeostasis and oxidative stress, mitigating the alkaline salt stress	(Li *et al.*, 2020a)
	Eggplant *(Indam Supriya)*	20 mM NaCl	40 μM NaHS	Repair membrane damage, maintain the redox homeostasis of cell and bringing the seedlings back to the healthy state	(Raju and Prasad, 2021)
	Bean (*Phaseolus vulgaris* L.)	50-70 mM NaCl	100 μM NaHS	Reduce H_2O_2, MDA, proline, sucrose content, enzyme activity, and ABA content, mitigating the negative impacts of salinity	(Ekinci *et al.*, 2023)
	Wheat (*Triticum aestivum* L.)	100 mM NaCl	0.2 mM NaHS	Decrease leaf Na^+ content, alter leaf water status, leaf K^+ and involved enzymes in AsA-GSH, and NO levels, alleviating salt stress	(Kaya *et al.*, 2023b)
	Wheat (*Triticum aestivum* L.)	100 mM NaCl	100 μM NaHS	Modulate AsA-GSH system, osmolytes production, nutrient content to cope up with salt-induced toxicity	(Kumari *et al.*, 2023)
	R. soongorica	300 mM NaCl	25 μM NaHS	Reduce O_2^-, proline and MDA, increase the activity of antioxidant enzymes and the content of osmoregulators, improving salt tolerance	(Liu *et al.*, 2023)

Cucumber (*Cucumis sativus* L.)	50 mM NaCl	200 μM NaHS	Upregulate SOS-related gene expression to maintain Na^+/K^+ balance, and increase the expression of MAPK-related genes to enhance salt tolerance	(Luo *et al.*, 2023)
Strawberry (*Fragaria×ananassa*)	40 mM NaCl	200 μM NaHS	Together with selenium, strengthened the antioxidant system and adjust the balance of nutrient absorption, thereby reducing the adverse effects of salinity stress	(Pourebrahimi *et al.*, 2023)
Tomato (*Solanum lycopersicum*)	20 mM NaCl	40 μM NaHS	Maintain the Na^+/K^+ homeostasis and the C/N ratio, conferring plant salt stress tolerance	(Raju and Prasad, 2023)
Broccoli (*Brassica oleracea*)	9.68 $dS.m^{-1}$ saline soil 7.12 $dS.m^{-1}$ saline irrigation water	0.1/0.2 mM NaHS	Regulate antioxidant enzyme activity, increase photosynthetic pigments and nutrient content, maintain cell membrane stability, reduce H_2O_2 and MDA accumulation	(Shalaby *et al.*, 2023)
Maize (*Gold cultivar*)	100 mM NaCl	0.5 mM NaHS	Regulate ionic balance, biochemical attributes, photosynthetic pigments and key antioxidants, mitigating salinity stress	(Shoukat *et al.*, 2023)
Coriander (*Coriandrum sativum* L.)	1% NaCl	50 μM NaHS	Increase morphological parameters, pigment and osmolyte contents, facilitating salt stress resistance	(Thapar Kapoor *et al.*, 2023)
Arabidopsis	50-200 mM NaCl	Overexpress CAS genes	Maintain relatively high levels of antioxidant enzyme activity, enhancing salt stress tolerance	(Xu *et al.*, 2023)
Arabidopsis	100 mM NaCl	100 μM NaHS	Regulate PIN2 expression to modulate auxin signaling and thereby alleviate salt stress.	(Yang *et al.*, 2023)
Tomato (*Solanum lycopersicum* L.)	75/150 mM NaCl	100 μM NaHS	Arrange mineral element and hormone content, increase photosynthetic activity, transpiration rate, stomatal conductivity and intercellular CO_2 concentration, improving salt tolerance	(Yildirim *et al.*, 2023)
Cyclocarya paliurus	0.4% NaCl	0.5 mM NaHS	Maintain photosynthetic capacity and reduce leaf biomass loss, promote NO synthesis and reduce oxidative damage	(Zhang *et al.*, 2023b)

	Millet (*Setaria italica* L.)	100 mM NaCl	200 μM NaHS	Regulate osmotic adjustment substances reduce electrolyte permeability, activate the expression of salt-resistant genes to alleviate salt stress	(Zhang *et al.*, 2023d)
	Tomato (*Solanum lycopersicum* L.)	150 mM NaCl	25 μM NaHS	Enhance the expression of Strigolactones synthesis-related genes, increases endogenous SL levels, thereby improving salt tolerance.	(Yang *et al.*, 2024a)
	Sunflower (*Helianthus annuus* L.)	150 mM NaCl	0.5 mM NaHS	Modulate ion hemostasis, cellular redox balance, and gene expression, enhancing salinity tolerance	(Younis and Mansour, 2024)
Tempera-ture	Wheat (*Triticum aestivum* L.)	40 ºC	0.1 mM NaHS	Regulate of carbohydrate metabolism, protecting heat stress-induced photosynthetic inhibition	(Iqbal *et al.*, 2021)
	Rice (*Oryza sativa* L.)	40 ºC	200 μM NaHS	Activate antioxidant enzymes, detoxicate ROS, accumulate osmolytes, combating the negative effects of heat stress	(Gautam *et al.*, 2022)
	Wheat (*Triticum aestivum* L.)	42 ºC	300 μM NaHS	Increase SOD, CAT and guaiacol peroxidase activity in roots, improving heat resistance	(Havva *et al.*, 2022)
	Cucumber (*Cucumis sativus* L.)	5 ºC	1.0 mM NaHS	Relieve the oxidative damage and photoinhibition, improving chilling tolerance	(Wu *et al.*, 2022)
	Alfalfa (*Medicago sativa* L.)	4 ºC	500 μM NaHS	Enhance photosynthetic capacity, regulate the antioxidant defense system, enhancing low-temperature tolerance	(Gao *et al.*, 2023)
	Pepper (*Capsicum annuum* L.)	5 ºC	1.0 mm NaHS	Enhance membrane lipids antioxidant capacity, protect the integrity of the membrane system	(Song *et al.*, 2023)
	Tomoto (*Solanum lycopersicum*)	5 ºC	1-1.5 mM NaHS	Regulate energy and cell wall metabolism alleviate schilling injury	(Li *et al.*, 2024a)
	Tomato (*Ailsa Craig*)	5 ºC	1.0 mM NaHS	Alleviate membrane lipid peroxidation and oxidative damage	(Wu *et al.*, 2024)
	Peaches (*Prunus persica L.*)	5 ºC	5 mM NaHS	Downregulate polyphenol oxidase gene expression and enhancing antioxidant defenses	(Yu *et al.*, 2024)

Heavy metal	Zucchini (*Cucurbita pepo* L.)	50 mg/L $Ni(NO_3)_2$	100 μM NaHS	Restore mineral balance, lower the accumulation of proline, elevate sugar level, and induce the secretion of flavonoids and phenolic compounds, thereby enhance Ni tolerance	(Valivand and Amooaghaie, 2021)
	Trigonella foenum-graecum	1-2 mM $CdCl_2$	100-200 μM NaHS	Induced biosynthesis of spermidine and putrescine, reduced H_2O_2 production and electrolyte leakage, alleviating cadmium stress	(Javad *et al.*, 2022)
	Pak choi (*Brassica chinensis L.*)	6.24 mg kg^{-1} $CdCl_2$	0.3 mM NaHS	Increase shoot biomass, boost the activities of POD and SOD, reduced Cd translocation, enhancing Cd resistance	(Cui *et al.*, 2023)
	Salix matsudana	150 mg/kg Cd	50 mg kg^{-1} NaHS	Regulate glutathione metabolism, alleviating cadmium-induced oxidative stress	(Guo *et al.*, 2023)
	Tomoto *(Solanum lycopersicum* L.*)*	50 μM Cr	0.1 mM NaHS	Modulate the ascorbate-glutathione cycle, chromium sequestration, subcellular allocation of chromium	(Kaya *et al.*, 2023a)
	Rice (*Oryza sativa* L.)	26.52 mg/L Cr (III)	100 μM NaHS	Manipulating the enzymatic and non-enzymatic antioxidants in alleviating oxidative stress	(Lin *et al.*, 2023)
	Mungbean *(Vigna radiata* L.*)*	90-120 μM Cr	100 μM H_2S	Modulate chromium, nutrients and reactive oxygen species accumulation to alleviate chromium toxicity	(Singh *et al.*, 2023)
	Spinacia oleracea	50 ppm Cd	100 μM NaHS	Manage the structure of chloroplast and photosynthetic efficiency to alleviate Cd toxicity	(Yadav *et al.*, 2023)
	Cucumber (*Cucumis sativus*)	25-100 μM $CuSO_4 \cdot 5H_2O$	100 μM NaHS	Enhance photosynthetic performance, boost antioxidant system, promote proline accumulation, reduce lipid peroxidation, H_2O_2 content and electrolyte leakage, collectively alleviate copper-induced toxicity	(Yusuf *et al.*, 2023)
	Miscanthus acchariflorus	50 μM Cd	50 μM NaHS	Reduce the oxidative damage to the organism to mitigate Cd stress	(Zhang *et al.*, 2023a)
	Alfalfa *(Medicago sativa* L.)	0-4 mM Cr (III)	0.02-0.1 mM NaHS	Regulate intracellular ROS homeostasis and redox balance	(Bu *et al.*, 2024)

	Soybean (*Glycine max*)	50 µM Cd	50 µM NaHS	Reduce ion leakage, improve relative water content, and increase photosynthetic pigments to cope with Cd stress	(Oloumi *et al.*, 2024)
	Maize (*Zea mays* L.)	100 µM $K_2Cr_2O_7$	50 µM NaHS	Increase pectin methylesterase activity, improve antioxidant enzyme activity, improve Cr tolerance	(Yang *et al.*, 2024b)
	Stock (*Matthiola incana* L.)	50 mg L^{-1} Cd	1.5 mM NaHS	Together with melatonin, enhance cadmium-induced oxidative stress resistance	(Zulfiqar *et al.*, 2024)

ABA, Abscisic Acid; AsA-GSH, Ascorbate-Glutathione; C, Carbon; CAT, Catalase; Cd, Cadmium; CO_2, Carbon Dioxide; Cr, chromium; Cu, Copper; H_2O_2, Hydrogen Peroxide; HT, Hypotaurine; HY1, Long Hypocoty1; K^+, Potassium Ion; MAPK, Mitogen-Activated Protein Kinase; MDA, Malondialdehyde; N, Nitrogen; Na^+, Sodium Ion; NaHS, Sodium Hydrosulfide; NaCl, Sodium Chloride; Ni, Nickel; NO, Nitric Oxide; NOX, NADPH Oxidase; O_2^-, Superoxide Anion; PEG, Polyethylene Glycol; PIN2, PIN-Formed 2; ROS, Reactive Oxygen Species; SOD, Superoxide Dismutase.

Table 2. Persulfidation regulates protein function through diverse pathways

Protein type	Species	Protein	Detection material	Treatment	Functional effects	Reference
Transcription factor	*Arabidopsis*	ABI4	Recombinant protein and 7-day old seedlings	10 μM ABA	Enhance the transactivation activity of *MAPKKK18*, control ABA responses, involving in H_2S-induced stomatal closure	(Zhou *et al.*, 2021)
				NA	Maintain ABI4 protein stability, playing a crucial role in H_2S-induced delay of seed germination	(Zhou *et al.*, 2022)
	Tomato (*S. lycopersicum*)	WRKY71	Recombinant protein	NA	Enhance binding ability to the *CAS1* promoter, participating H_2S-induced delay in fruit ripening	(Sun *et al.*, 2023)
	Pear (*Pyrus* L.)	MYB10	Recombinant protein	NA	Enhance the interaction with PyMYB6 and reduce the interaction with PyMYB73 to inhibit anthocyanin synthesis, involving in H_2S-induced delay in color change during fruit ripening	(Yao *et al.*, 2023)
	Tomato (*S. lycopersicum*)	WRKY6	Recombinant protein	NA	Inhibit the transcriptional activity, attenuate the expression of *SlSGR1* and *SlSAG12*, contributing to H_2S-induced delay in fruit ripening	(Zhang *et al.*, 2024c)
Metabolic enzyme	*Arabidopsis*	GAPC1	Recombinant protein	NA	Enhance activity and promote translocation from the cytosol to the nucleus.	(Aroca *et al.*, 2017b)
	Arabidopsis	DES1	Recombinant protein and 4-week-old leaves	10 μM ABA	Increase enzymatic activity to regulate guard cell redox homeostasis and ABA signaling, contributing to H_2S-induced stomatal closure	(Shen *et al.*, 2020)
	Arabidopsis	G6PD6	Recombinant protein and 5-day old seedlings	150 mM NaCl	Modify protein tetrameric structure to enhance enzymatic activity, protecting protein from overoxidation, contributing to H_2S-induced salt resistance	(Wang *et al.*, 2023)
	Tomato *(S. lycopersicum)*	G6PDC				
	Arabidopsis	SDH1-1	Recombinant protein and 4-week-old leaves	NA	Increase SDH activity and induce stomatal closure, contributing to H_2S-induced abiotic/biotic stress responses	(Zhang *et al.*, 2024b)
Antioxidant enzyme	*Arabidopsis*	APX1	Recombinant protein	NA	Increase enzymatic activity	(Aroca *et al.*, 2015)
	Tomato (*S. lycopersicum*)	APX1 POD5	Recombinant protein	20-100 mg/L copper oxide	Increase APX1, POD5 enzymatic activity and decrease CAT1 enzymatic activity, involving in H_2S-induced	(Li *et al.*, 2020b)

		CAT1		nanoparticles	resistance to phytotoxic effect	
Protein kinase	*Arabidopsis*	SnRK2.6	Recombinant protein and 3-week-old leaves	10 μM ABA	Enhance kinase activity and promotes interaction with ABF2, thereby contributing to the role of H_2S in facilitating ABA signaling and ABA-induced stomatal closure	(Chen *et al.*, 2020)
					Induced structural change, increase kinase activity playing an important role in H_2S-induced stomatal closure	(Chen *et al.*, 2021)
	Arabidopsis	MPK4	Recombinant protein	4 ºC	Increase MPK4 activity, contributing to H_2S-induced cold resistance	(Du *et al.*, 2021)
Other stress-regulatory proteins	*Arabidopsis*	RBOHD	Recombinant protein and 4-week-old leaves	10 μM ABA	Increase enzymatic activity to regulate guard cell redox homeostasis and ABA signaling, contributing to H_2S-induced stomatal closure	(Shen *et al.*, 2020)
	Arabidopsis	ATG4	Recombinant protein	50 μM ABA	Inhibit ATG4 proteolytic activity and negatively regulate H_2S-mediated autophagy	(Laureano-Marín *et al.*, 2020)
	Arabidopsis	ATG18A	Recombinant protein	5 μg/mL Tunicamycin	The persulfidation of ATG18a enhances its binding capacity to the specific phospholipid PtdIns(3)P, which subsequently reduces its activity in autophagy, leading to a negative regulation of the autophagic process	(Aroca *et al.*, 2021a)
	Arabidopsis	PMA1	Recombinant protein	100 mM NaCl	Promoted the binding of PMA1 to GRF4, contributing to H_2S-induced salt resistance	(Ma *et al.*, 2023)
	Tomato (*S. lycopersicum*)	BRG3	Recombinant protein	NA	Reduce its ubiquitination activity and weakening its interaction with the WRKY71 transcript, the binding activity of persulfidated WRKY71 to the CAS1 promoter is subsequently enhanced, leading to H_2S-induced delay in fruit ripening	(Sun *et al.*, 2023)

ABA, Abscisic Acid; ABI4, Abscisic Acid Insensitive 4; APX1, Ascorbate Peroxidase 1; ATG18A, Autophagy Related Gene 18a; BRG3, BOI-Related E3 Ubiquitin-Protein Ligase 3; CAS1, Cyanoalanine Synthase1; CAT1, Catalase 1;DES1, Cysteine Desulfhydrase 1; ER, Endoplasmic Reticulum; G6PD, Glucose-

6-phosphate Dehydrogenase; GAPDH, Glyceraldehyde 3-Phosphate Dehydrogenase; GRF4, General Regulatory Factor 4; H_2O_2, Hydrogen Peroxide; JA, Jasmonic Acid; MPK4, Mitogen-Activated Protein Kinases 4; MYB10, Myeloblastosis Transcription Factors 10; NA: Not Applicable; NaCl, Sodium Chloride; PMA1, Plasma Membrane H^+-ATPase; POD5, Peroxidase 5; PtdIns(3)P, Phosphatidylinositol 3-Phosphate; RBOHD, Respiratory Burst Oxidase Homolog Protein D; SDH1-1, Succinate Dehydrogenase 1-1; SlSAG12, Senescence-Associated Gene 12; SlSGR1, Staygreen 1; SnRK2.6, SNF1-Related Proteinkinase 2.6; V-ATPase, Vacuolar H^+-Translocating ATPase; WRKY6, WRKY Transcription Factors 6; WRKY71, WRKY Transcription Factor 71.

Table 3. Study on persulfidation proteomics across different conditions and species

Species	Genetic background	Tissue	Methodology	Condition	Number of persulfidated proteins	Reference
Arabidopsis (*Arabidopsis thaliana*)	WT	Leaf	Tag-switch	Normal	2015	(Aroca *et al.*, 2017a)
	des1				2130	
	WT	Leaf	Tag-switch	With and without ABA	1157	(Laureano-Marín *et al.*, 2020)
	WT	Leaf	Dimedone-switch	Drought	2568	(Jurado-Flores *et al.*, 2023a)
	WT	Leaf	Dimedone-switch	High CO_2	1452	(García-Calderon *et al.*, 2023)
	WT	Leaf	Tag-switch	Light	4599	(Jurado-Flores *et al.*, 2023b)
				Dark		
				Carbon Deprivation		
	WT	Root	Tag-switch	Nitrogen Starvation	5214	(Jurado-Flores *et al.*, 2021)
Pepper (*Capsicum annuum* L.)	WT	Fruit	Dimedone-switch	Different stages of ripening	891	(Muñoz-Vargas *et al.*, 2024)
Bean (*Phaseolus vulgaris*)	WT	Nodule	Tag-switch	Normal	967	(Matamoros *et al.*, 2024)
Rice (*Oryza sativa* L.)	WT	Leaf	Dimedone-switch	Drought	3324	(Zhang *et al.*, 2024a)

ABA, Abscisic Acid; CO_2, Carbon Dioxide; DES1, Cysteine Desulfhydrase 1; WT, Wild Type.

Figures

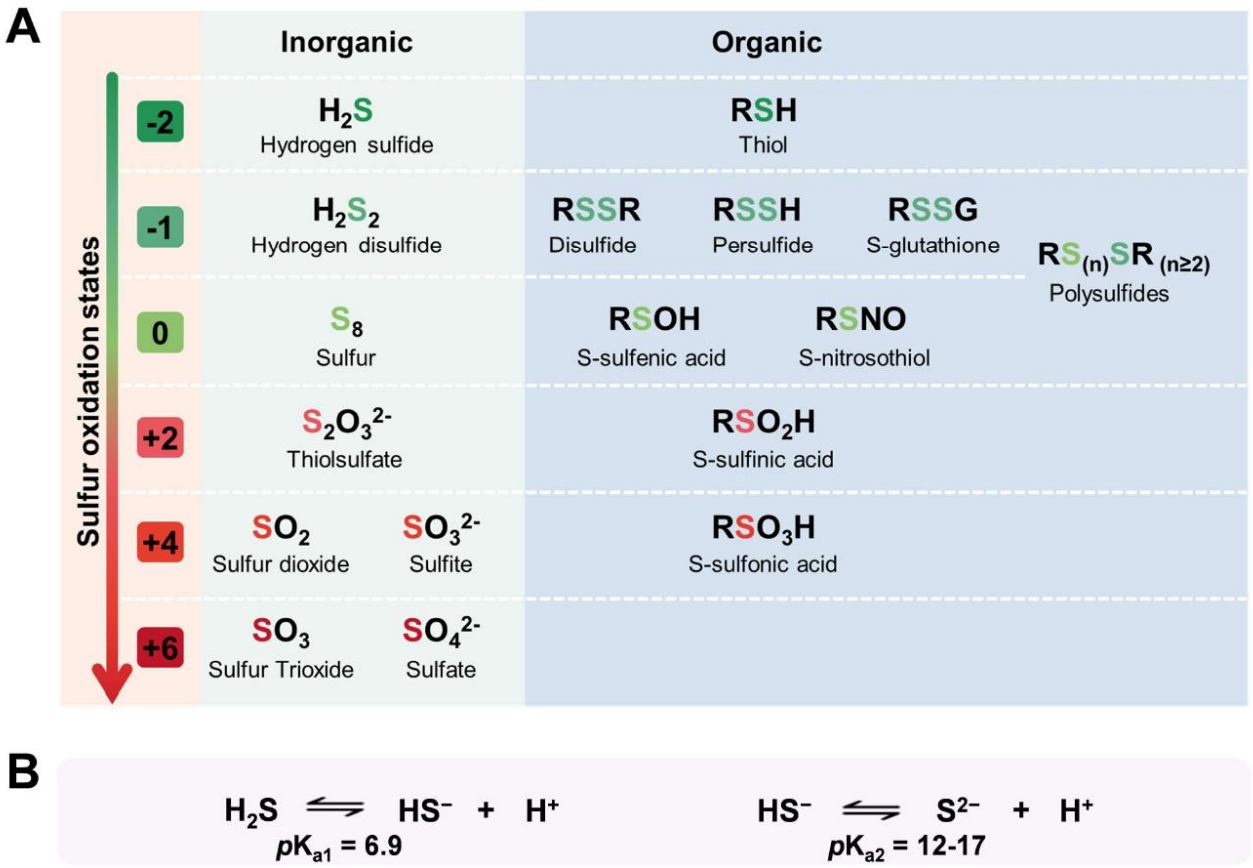

Figure 1. Oxidation states of sulfur in biologically relevant compounds and the dissociation of H_2S. (A) The oxidation states of sulfur in various organic and inorganic compounds vary from -2 to +6. R represents the rest of the molecule, indicating the variable side chain in protein structures. (B) The hydrolysis process of hydrogen sulfide. H^+, Hydrogen Proton; HS^-, Bisulfide Ion; *p*Ka, Dissociation Constant; S^{2-}, Sulfide Ion.

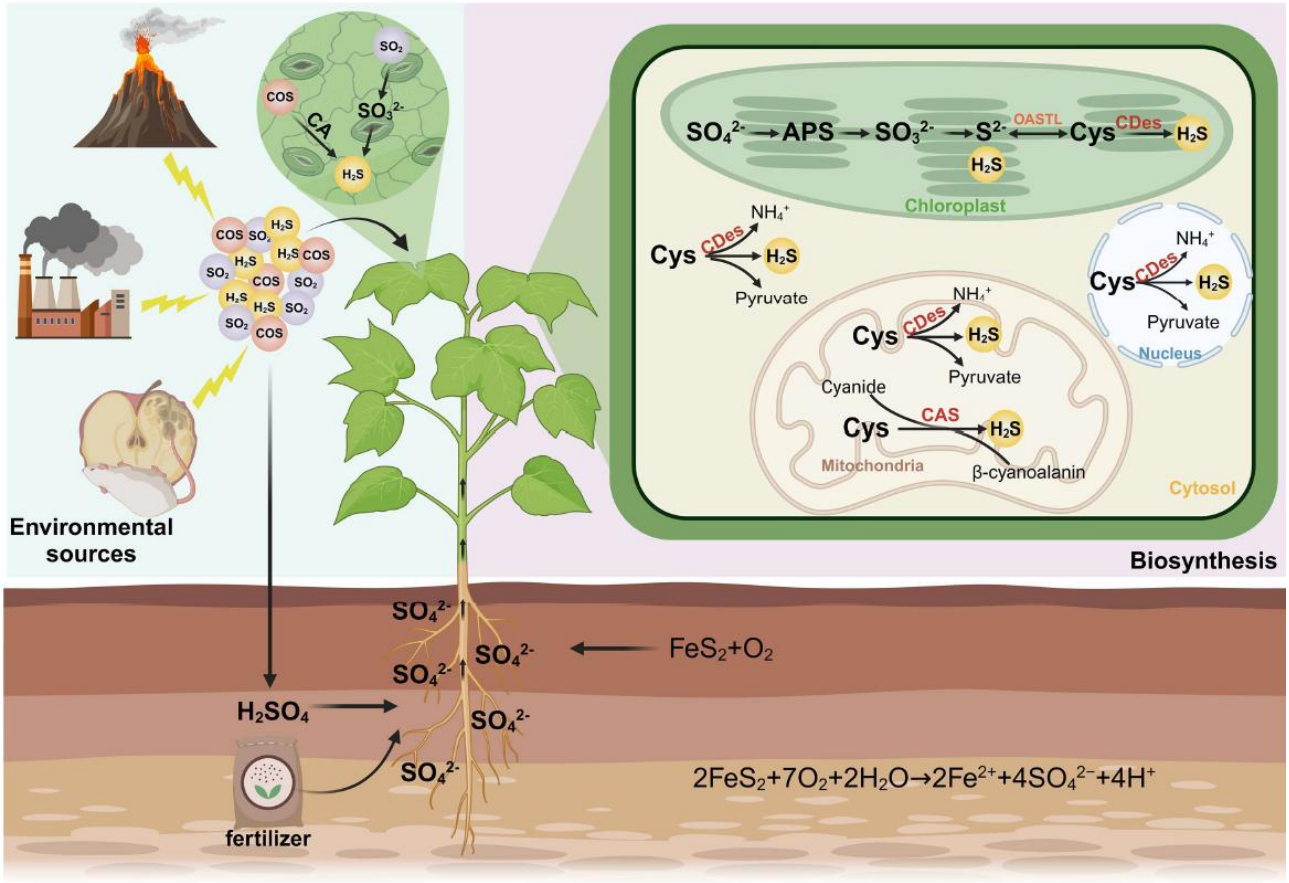

Figure 2. Absorption and transformation of different source of H_2S in plants. Volcanic eruptions, industrial activities, and the decomposition of animals and plants release substantial quantities of H_2S, SO_2, COS into the environment. Plants absorb these sulfur-containing compounds through stomata, making them available for assimilation. Simultaneously, SO_4^{2-} from diverse sources in the soil is taken up by plant root and incorporated into the sulfur metabolic pathways. Within plant cells, multiple H_2S synthases are present in various organelles, catalyzing the production of H_2S from sulfur-containing precursors. These interconnected processes collectively maintain the H_2S cycle and homeostasis within the plant, ensuring the efficient utilization and recycling of sulfur resources. APS, Adenosine 5'-Phosphate Sulfate; CA, Carbonic Anhydrase; CAS, Cyanoalanine Synthase; CDes, Cysteine Desulfhydrase; H_2S, Hydrogen Sulfide; OAS, O-Acetylserine; OASTL, *O*-Acetylserine Thiolyase; SO_2, Sulfur Dioxide; SO_3^{2-}, Sulfite; SO_4^{2-}, Sulfate.

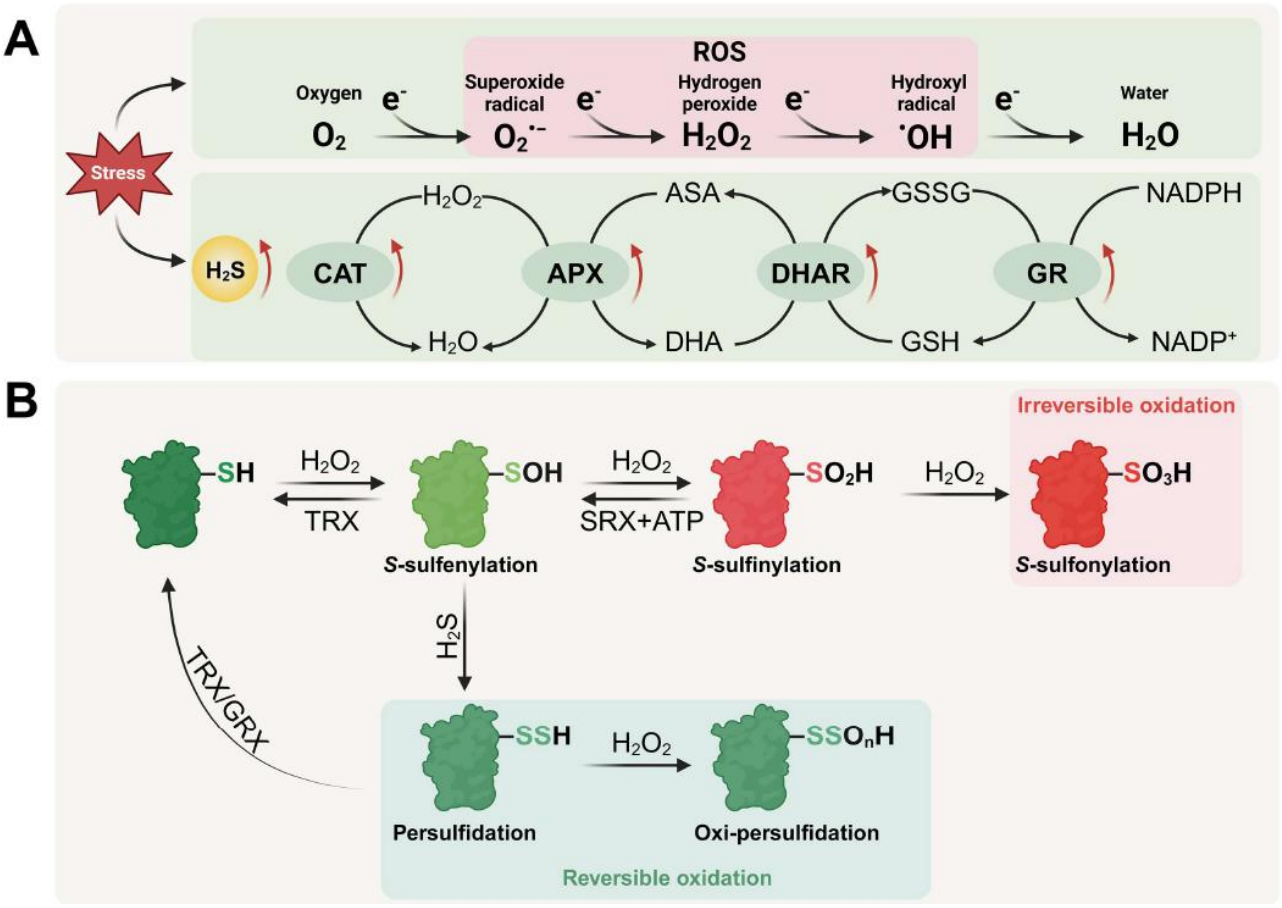

Figure 3. The molecular mechanism of H_2S signaling in plants. (A) Under stress conditions, ROS are generated through the sequential one-electron reduction of oxygen. Simultaneously, stresses also trigger an increase in H_2S production. H_2S, in turn, enhances the activity of various antioxidant enzymes and regulates the ascorbate-glutathione (AsA-GSH) cycle, ultimately alleviating stress-induced oxidative damage. (B) Schematic representation of oxidative post-translational modifications occurring on cysteine residues. Cysteine residues are oxidized by H_2O_2 to form R-SOH. R-SOH can undergo further oxidation to R-SO_2H and irreversibly to sulfonic acid (R-SO_3H). R-SO_2H are reduced back to R-SOH in the presence of ATP and SRX. However, in plants, this reaction has only been observed for 2-Cys Prx and PrxIIF. R-SOH react with H_2S to form R-SSH, which may be further oxidized to form R-SSO_nH. Both R-SSH and R-SSO_nH can be enzymatically reduced back to the thiol form by redox-active enzymes, TRX and GRX. APX, Ascorbate Peroxidase; AsA, Ascorbic Acid; ATP, Adenosine Triphosphate; CAT, Catalase; 2-Cys Prx, Chloroplastic 2-Cys Peroxiredoxin; DHA, Dehydroascorbic Acid; DHAR, Dehydroascorbate Reductase; GR, Glutathione Reductase; GRX, Glutaredoxin; GSH, Glutathione; GSSG, Oxidized Glutathione; H_2O_2, Hydrogen Peroxide; H_2S, Hydrogen Sulfide; NADPH, Nicotinamide Adenine Dinucleotide Phosphate; PrxIIF, Mitochondrial Peroxiredoxin. ROS, Reactive Oxygen Species; SRX, Sulfiredoxin; TRX, Thioredoxin.

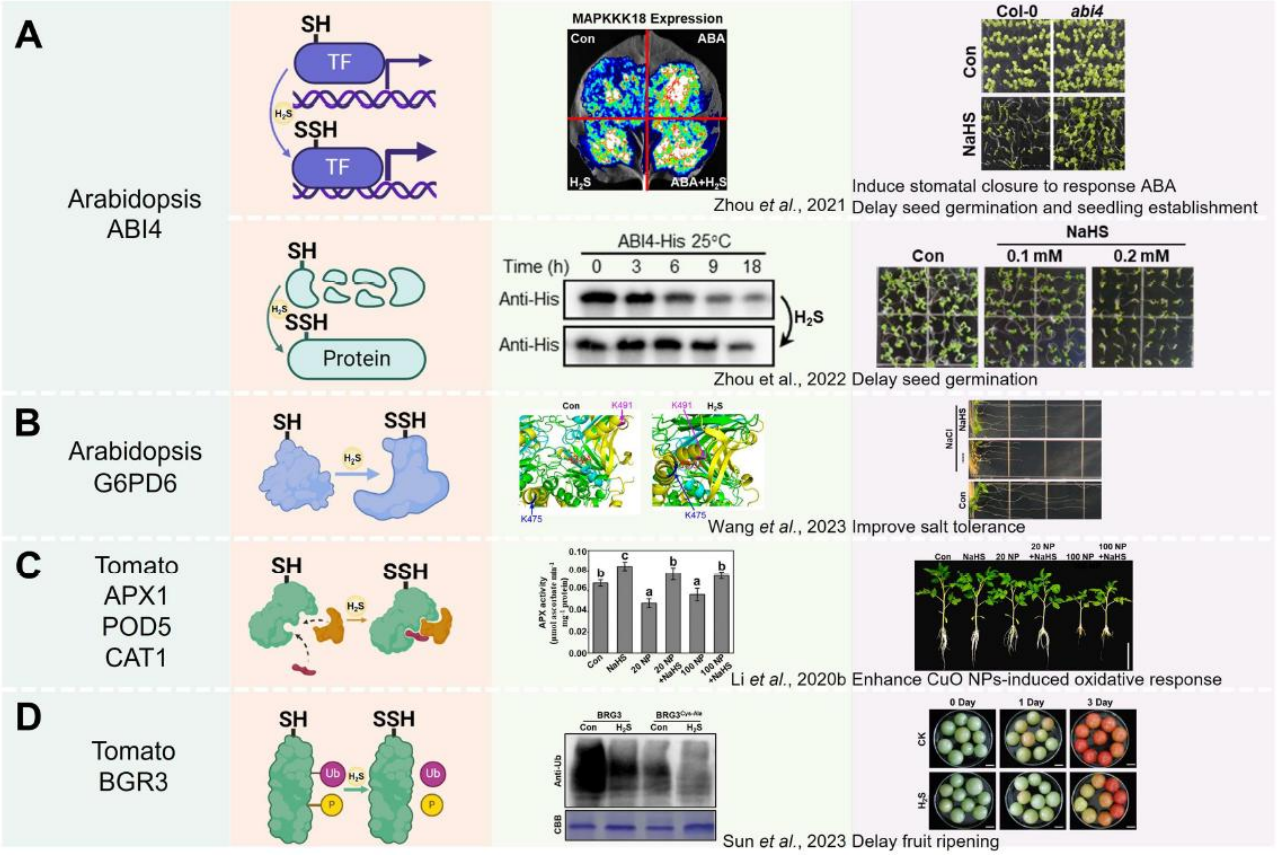

Figure 4. (A) Persulfidation modulates the binding of transcription factors to downstream gene promoters, thereby regulating gene expression. Persulfidation of ABI4 enhances the expression of MAPKKK18, activating downstream MAPK signaling and modulating ABA responses in Arabidopsis. The persulfidation of ABI4 slows down the degradation rate during seed germination in Arabidopsis, thereby delaying seed germination. (B) Persulfidation alters protein structure. The persulfidation of G6PDs changes the protein structure, enhancing salt tolerance in Arabidopsis. (C) H_2S enhanced the activities of APX1 and POD5 but decreased CAT1 activity through persulfidation in tomato, enhancing CuO NPs-induced oxidative response. (D) Persulfidation influences other post-translational modifications. Persulfidation of BRG3 and WRKY71 reduces the ubiquitination level of WRKY71, ultimately delaying the ripening in tomato. ABA, Abscisic Acid; ABI4, Abscisic Acid Insensitive 4, NaHS, Sodium Hydrosulfide; APX1, Ascorbate Peroxidase 1; BRG3, BOI-Related E3 Ubiquitin-Protein Ligase 3; CAT1, Catalase 1; CBB, Coomassie Brilliant Blue Protein Stain; G6PD6, Glucose-6-phosphate Dehydrogenase 6; H_2S, Hydrogen Sulfide; MAPKKK18, Mitogen-Activated Protein Kinase Kinase Kinase 18; POD5, Peroxidase 5; WRKY71, WRKY Transcription Factor 71.

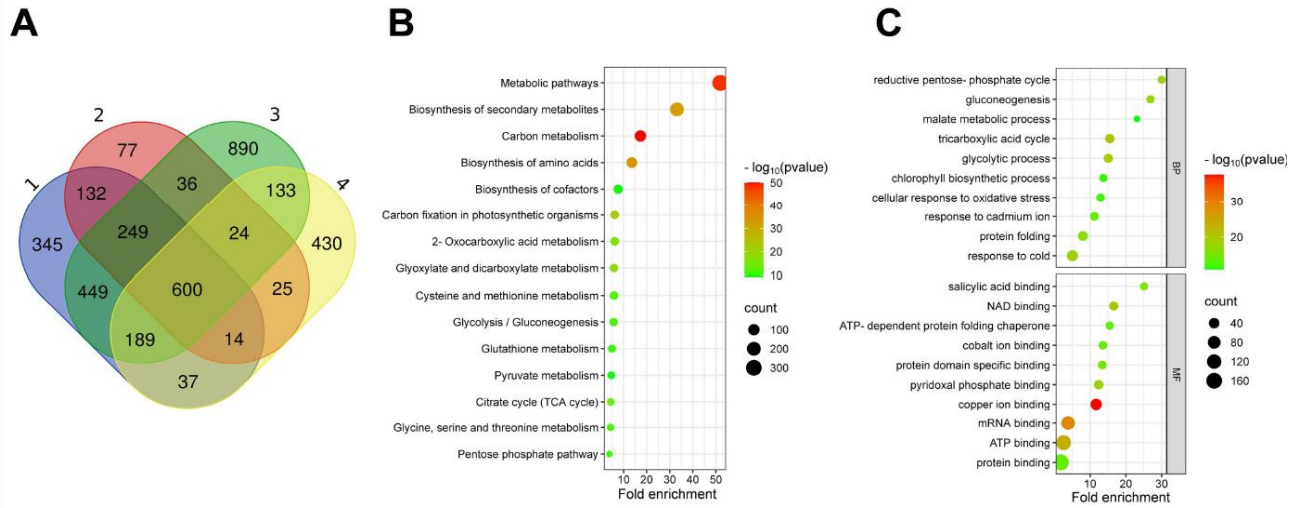

Figure 5. Comparison of persulfidated proteins identified in Arabidopsis. (A) Comparative analysis of identified persulfidated proteins from four proteomic studies using a Venn diagram (Aroca *et al*., 2017a; Laureano-Marín *et al*., 2020; Jurado-Flores *et al*., 2023a; García-Calderon *et al.,* 2023). (B) KEGG pathway enrichment analysis of 600 common proteins among the four proteomic studies. (C) GO analysis for Biological Processes and Molecular Functions of 600 common proteins among the four proteomics studies. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genome.