hydrogen

MDPI

*Review*

# Hydrogen Sulfide: A Robust Combatant against Abiotic Stresses in Plants

**Kanika Khanna [1,*], Nandni Sharma [2], Sandeep Kour [2], Mohd. Ali [2], Puja Ohri [2,*] and Renu Bhardwaj [1,*]**

[1] Plant Stress Biology, Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar 143005, India
[2] Department of Zoology, Guru Nanak Dev University, Amritsar 143005, India; sharmanandni1303@gmail.com (N.S.); koursandeep0309@gmail.com (S.K.); xyaansari774@gmail.com (M.A.)
* Correspondence: kanika.27590@gmail.com (K.K.); ohri11puja@gmail.com (P.O.); renubhardwaj82@gmail.com (R.B.)

**Abstract:** Hydrogen sulfide ($H_2S$) is predominantly considered as a gaseous transmitter or signaling molecule in plants. It has been known as a crucial player during various plant cellular and physiological processes and has been gaining unprecedented attention from researchers since decades. They regulate growth and plethora of plant developmental processes such as germination, senescence, defense, and maturation in plants. Owing to its gaseous state, they are effectively diffused towards different parts of the cell to counterbalance the antioxidant pools as well as providing sulfur to cells. $H_2S$ participates actively during abiotic stresses and enhances plant tolerance towards adverse conditions by regulation of the antioxidative defense system, oxidative stress signaling, metal transport, $Na^+/K^+$ homeostasis, etc. They also maintain $H_2S$-Cys-cycle during abiotic stressed conditions followed by post-translational modifications of cysteine residues. Besides their role during abiotic stresses, crosstalk of $H_2S$ with other biomolecules such as NO and phytohormones (abscisic acid, salicylic acid, melatonin, ethylene, etc.) have also been explored in plant signaling. These processes also mediate protein post-translational modifications of cysteine residues. We have mainly highlighted all these biological functions along with proposing novel relevant issues that are required to be addressed further in the near future. Moreover, we have also proposed the possible mechanisms of $H_2S$ actions in mediating redox-dependent mechanisms in plant physiology.

**Keywords:** abiotic stress; hydrogen sulfide; oxidative stress signaling; antioxidants; metal uptake; $Na^+/K^+$ homeostasis; protein persulfidation

check for updates

**Citation:** Khanna, K.; Sharma, N.; Kour, S.; Ali, M.; Ohri, P.; Bhardwaj, R. Hydrogen Sulfide: A Robust Combatant against Abiotic Stresses in Plants. *Hydrogen* **2021**, *2*, 319–342. https://doi.org/10.3390/hydrogen2030017

Academic Editor: Thomas Klassen

Received: 22 June 2021
Accepted: 4 August 2021
Published: 11 August 2021



## 1. Introduction

Abiotic stresses mainly comprise of numerous stresses such as heavy metal, drought, light, flooding, freezing, salinity, and many more abrupt environmental conditions. Plants, due to their non-sessile nature, are exposed to these fluctuations at immediate pace [1]. Therefore, plants are abruptly affected by these abiotic factors in terms of growth and metabolism. The instant reaction in plants occurs in the form of generation of reactive oxygen species (ROS), in the form of superoxide radicals, singlet oxygen species, malondialdehyde (MDA), hydrogen peroxide ($H_2O_2$), and various other free radicals that are notably increased during stressed conditions [2]. ROS generation in plants mostly takes place in different sites such as chloroplasts, peroxisomes, mitochondria, and apoplasts, thereby affecting their normal functioning during abiotic stressed conditions [2]. For instance, the generation of singlet oxygen species in chloroplasts alters gene programming of nuclear genes causing chlorosis and cell programmed death to take place [3]. Interestingly, plants possess a range of tolerance mechanisms to cope with abiotic stresses in the form of antioxidant enzymes namely, catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), glutathione reductase (GR), glutathione-S-transferase (GST), etc., which improve the ROS-scavenging and reduce the stress levels in plants [4]. Apart from this, a series of physiological and biochemical mechanisms are possessed by the plants for improving their stress tolerance.

Owing to numerous geothermal events and anoxic atmosphere, $H_2S$ is present in the environment in abundance and has been thought to be involved in origin of life [5]. For example, sulfur-comprising compounds such as amino acids (cysteine and methionine) can be formed in $H_2S$ enriched environment [6]. From the past hundred years, $H_2S$ is known to be a colorless poisonous gas with unpleasant odour, similar to rotten eggs, known to affect different kingdoms of life [7]. It alters cellular metabolism, and mitochondrial activity by negatively inhibiting cytochrome c oxidases [8]. Since the past decades, the novel function of $H_2S$ has been known to act as a signaling molecule in regulating different biological and physiological plant processes. Specifically with enhanced understanding about various gasotransmitters, identification of $H_2S$ as a novel transmitter was revealed and it was accepted as a biologically active molecule [9]. Evidences in regard to the role of $H_2S$ as signaling molecules in plants have been illustrated. $H_2S$ play a vital role in various processes of plants such as growth, adventitious root branching, development, seed germination, senescence, stress responses, etc. [10]. A large body of literature determined the protective role of $H_2S$ to signal plant acclimation and resistance mechanism against abiotic stresses such as heavy metals, drought, salinity, freezing, flooding, heat, and osmotic stress [7,8,10]. To illustrate, $H_2S$ enhanced Cr-stress tolerance in barley by stimulating photosynthetic attributes and lowering its absorption in the soil [11]. It also enhanced chlorophyll and protein content in plants subjected to salt stress along with inhibiting ROS accumulation, contributing towards salt resistance in rice [12]. It is noteworthy that $H_2S$ donors when applied exogenously induce the endogenous $H_2S$ levels. For instance, endogenous levels of $H_2S$ were also triggered in *Arabidopsis* exposed to drought, most likely due to higher expression levels of L-desulfhydrase and D-desulfhydrase enzymes [13]. $H_2S$ donors such as NaHS stimulate internal $H_2S$ levels in maize [14].

Meanwhile, it is quite surprising that the role of $H_2S$ is often linked with ROS in plants during stresses conditions. The mechanism of action of $H_2S$ is related to oxidative stress and both antagonistic and synergistic studies with $H_2S$ and ROS have been reported in plants for regulating plant stress responses towards adverse environmental conditions [8]. Several mechanisms have been proposed by which $H_2S$ interact with oxidative stresses, yet their inter-relationships are still required to be elucidated [15]. Given that the vital role of $H_2S$ in plant processes, many researchers are focused to understand the role of $H_2S$ across cell membranes. It has been studied that $H_2S$ gets transported in membranes via the diffusion process without any carrier protein or facilitator [16]. By this nature, $H_2S$ act as signaling molecule with its efficacy to participate in different physiological and metabolic processes for plant protection. However, $H_2S$ is an ideal gas in plants but lesser used due to its intricacies in maintenance of concentrations during experimentation. Subsequently, the compounds that generate $H_2S$ in water, light, thiols and related enzymes are mostly applied in functional studies [17]. The release rate of donors is quite complex to be determined therefore, and optimal concentrations are also difficult to maintain. Taking into account diverse roles of $H_2S$ in plants and their applications in plants, we have shed light on their beneficial aspects, biochemical and functional roles in plants during abiotic stresses. We have also summarized the $H_2S$ and its role during oxidative stresses, antioxidant defense mechanisms, metal transport, and ion homeostasis. Moreover, the $H_2S$-mediated mechanisms in plants in terms of post-translational modification of cysteine residues and protein persulfidation have also been elucidated.

## 2. Multifunctional Capacity of $H_2S$

$H_2S$ can be produced from natural sources such as volcanic eruptions and anaerobic bacterial reduction of sulfur as well as anthropogenic sources such as petroleum extractions, coal mines, natural gas, and biogas processing industries [18,19]. It has low threshold, and humans, having highly developed olfaction, can discern as low as 1 μM of $Na_2S$ in solution [20]. $H_2S$ was first described as a poisonous gas in 1713, and, ever since, many papers have reported its toxicity in nearly all kingdoms of life [21]. The maximum permissible concentration of this toxic gas for a daily 8 h exposure is 20 ppm, whereas inhalation of higher concentrations can cause serious health issues and may prove to be lethal [5]. Though cytotoxicity of $H_2S$ has been noticed at higher concentrations, at low concentrations, it acts as a gaseous signaling molecule and is recognized as the third endogenous gasotransmitor in plants after nitric oxide (NO) and carbon monoxide (CO) [22,23]. $H_2S$ has the ability to interact with thiol (-SH) groups that are present in peptides such as reduced glutathione (GSH), and also with proteins that alter their functions. This sort of interaction, converting cysteine thiols (-SH) into persulfide (-SSH) groups is called persulfidation [15]. Protein persulfidation, an oxidative posttranslational modification of cysteine residues, represents a mechanism of signaling by $H_2S$. It is also entailed in biosynthetic pathways that need sulphur transfer, for instance, iron-sulphur clusters, biotin, thiamine, lipoic acid, molybdopterin, and sulphur-containing bases in RNA [5]. These posttranslational modifications of cysteine residues can act as a protective mechanism under oxidative stress conditions.

$H_2S$ has a complex biochemistry. It is a weak acid and in aqueous solution, can be dissociated into hydrosulfide ($HS^-$) and sulfide ($S^{2-}$) anions with dissociation constants ($pKa_1$ and $pKa_2$) of 6.9 and >12, respectively (Chen et al., 2020a). In aqueous environment, equilibrium also depends on temperature, so the following reactions occurs at 20 °C [15]:

$$H_2S + H_2O \rightleftharpoons HS^- + H_3O^+ \text{ (pKa = 6.88)}$$

$$HS^- + H_2O \rightleftharpoons S^{2-} + H_3O^+ \text{ (pKa = 14.15)}$$

Therefore, in biological samples having a physiological pH of around 7 and at 37 °C, $HS^-$ and $H_2S$ are the major forms whereas $S^{2-}$ is present in negligible concentration [5]. Biological activity of $H_2S$ in cellular compartments depends upon its ability to concentrate in and permeate through the lipid bilayer. $H_2S$ is hydrophobic and twice as soluble in the lipid bilayer as in water, so it can diffuse through biological membranes and they foist significant resistance, which slows down diffusion of $H_2S$ leading to its accumulation at the site of formation [24]. In contrast to water molecules, aquaporins or other protein facilitators are not required for the transportation of $H_2S$ across the lipid bilayer [25].

$H_2S$ plays a vital role in biological activities occurring in mammalian and plant tissues (Figure 1). So, it is crucial to measure the endogenous levels of this molecule. Several techniques have been established to determine $H_2S$ levels in biological samples. These include methylene blue colorimetric assays, fluorescent probes, polarographic sensors, ion-selective electrodes (ISEs), liquid chromatography-mass spectrometry (LC-MS/MS), gas chromatography, and HPLC coupled with UV, fluorescence, or electrochemical detection [15,20]. To monitor the levels of $H_2S$ in environmental samples, various sensors such as chemical sensors, optical sensors, colorimetric sensors, and more recently paper-based devices are being utilized [19]. In plant cells, $H_2S$ can be generated through enzymatic as well as non-enzymatic routes and the significance of its metabolism depends upon sub-cellular compartment, plant parts, optimal environmental and stressful conditions involved [15]. $H_2S$ signaling regulates stomatal movement, germination, growth and senescence [26]. $H_2S$, when applied exogenously, tends to attenuate the negative effects of different abiotic stresses. It was found to be a key factor in sequestering cadmium in *Populus euphratica* cells under cadmium stress [27]. $H_2S$ interaction with abscisic acid (ABA) helped in drought tolerance in *Arabidopsis* by mediating stomatal closure [28].

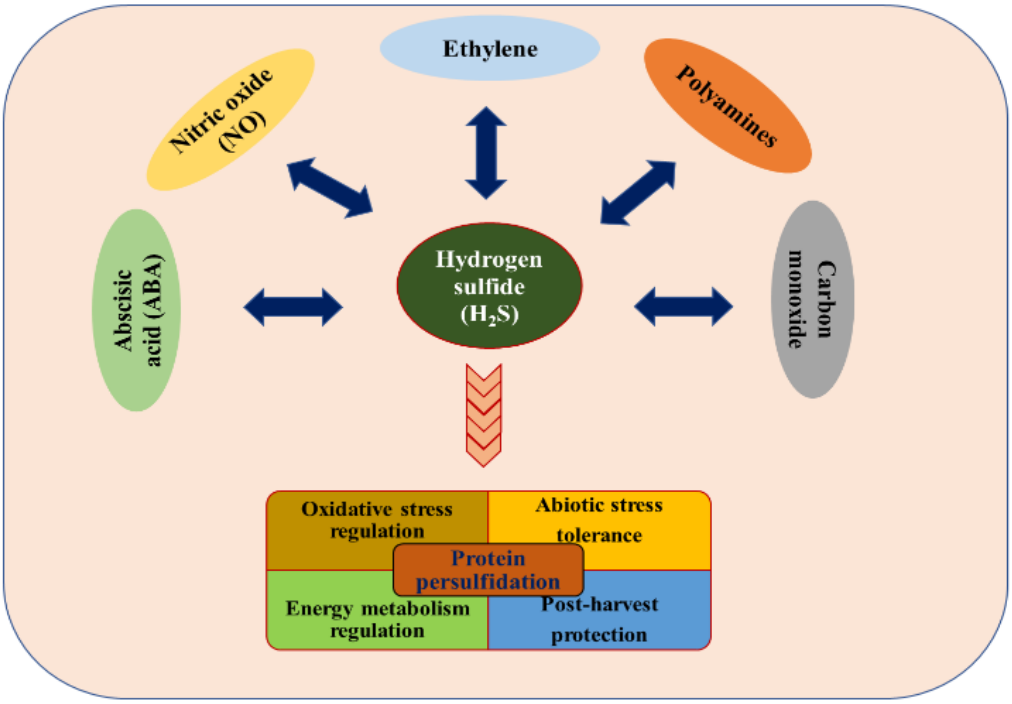


**Figure 1.** Role of hydrogen sulfide in mediating the different physiological processes in plants by undergoing interaction with plant hormones and other gasotransmitters.

## 3. Biosynthesis and Physiological Functions of $H_2S$ in Plants

Plants have the ability to synthesize and consume $H_2S$. Wilson and his coworkers, by using a sulfur-specific flame photometric detector, observed that the leaves of plants such ascorn (*Zea mays* L.), cucumber (*Cucumis sativus* L.), pumpkin (*Cucurbita pepo* L.), and soybean (*Glycine max* L.) emitted $H_2S$ at a rate of approximately 40 pmol/min [29]. This was the first report that detected the presence of $H_2S$ in plants and showed that $H_2S$ could be generated endogenously [30]. Furthermore, plants were found to continuously emit $H_2S$ following exposure to exogenous sulfate, sulfite, bisulfite, and L-cysteine [29,31]. This showed that plants get rid of excess inorganic sulfur by emitting $H_2S$ and that $H_2S$ production assists in homeostasis of sulfur assimilation [30,32].

In plant cells, $H_2S$ is found in different sub-cellular compartments (chloroplast, cytosol, and mitochondria) where enzymes linked to sulfur and cysteine metabolism have the potential to produce $H_2S$ (Figure 2). Various enzymes that are involved in $H_2S$ metabolism include L/D-cysteine desulfhydrase, sulfite reductase, cyanoalanine synthase, cysteine synthase, and O-acetylserine(thol)lyase isoforms [33]. When it comes to endogenous production of $H_2S$ in plants, chloroplast is an important player. During sulfate reduction pathway, sulfite reductase (SiR), which is present in chloroplast, catalyses the reduction of sulfite to sulfide [21]. As described above, $H_2S$ is a weak acid and can dissociate into $H^+$ and $HS^-$ ions in aqueous solution. $H_2S$ is mainly present in the form of $HS^-$ under neutral pH conditions but at higher pH this $HS^-$ can further dissociate to $H^+$ and $S^{2-}$ ions [34]. These anionic forms are unable to diffuse freely through the chloroplast membranes. In the chloroplast stroma, pH is increased from neutral to basic (pH 8) [35]. Hence, most of $H_2S$ is dissociated into its anionic form of $HS^-$ in the chloroplast. This form of sulfide cannot permeate through the chloroplast envelop and is transported by an unknown active transporter [36].

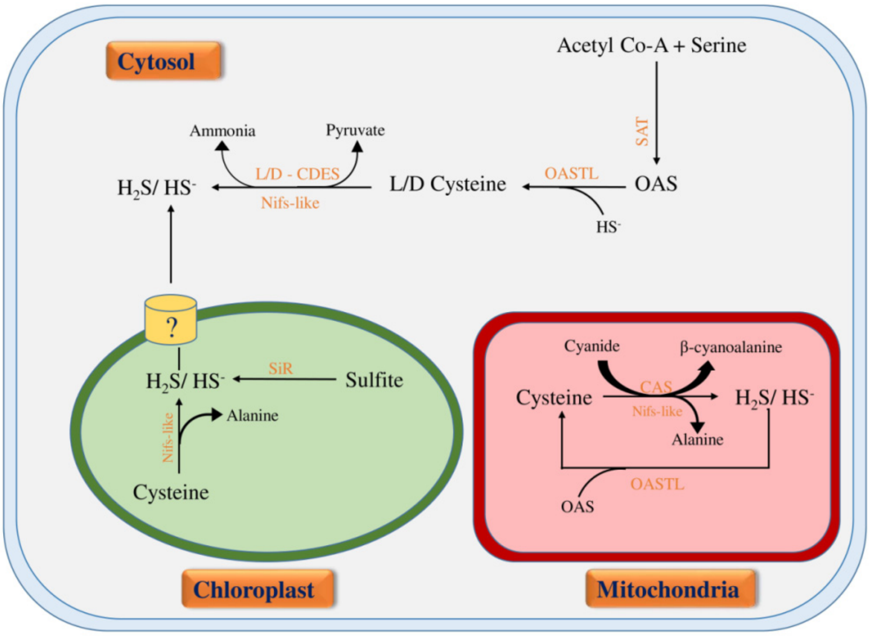


**Figure 2.** Biosynthesis of $H_2S$ in plants: In plants, biosynthesis of $H_2S$ occurs in cytosol, chloroplast, and mitochondria. In the cytosol, L/D cysteine is formed by the addition of sulfide into OAS (O-acetyl-ser) catalyzed by OASTL (O-acetylserine(thiol)lyase) enzyme. $H_2S$ is released from L/D cysteine in a reaction catalyzed by L/D-CDES (L/D cysteine desulfhydrases) along with the release of ammonia and pyruvate. In chloroplast, $H_2S$ is formed from sulfite by SiR (sulfite reductase) during photosynthetic sulfate reduction pathway. It is transported to cytosol from chloroplast by an unknown transporter. In mitochondria, $H_2S$ is formed from cysteine by the concomitant release of cyanide and β-cyanoalanine catalyzed by CAS (β-cyanoalanine synthase). Nifs-like proteins also release $H_2S$ by converting cysteine to alanine.

In the cytosol, $H_2S$ is metabolically generated from cysteine (Figure 2). $H_2S$ is a by-product of cysteine biosynthesis that is catalyzed by O-acetylserine(thiol)lyase (OASTL) enzymes [30,36]. Cysteine biosynthesis occurs in two steps: first an intermediary product O-acetyl-Ser (OAS) is formed from acetyl-CoA and serine by serine acetyltransferase (SAT), then cysteine is formed by the incorporation of sulfide into OAS catalyzed by OASTL [30]. $H_2S$ is released from cysteine by the action of L-cysteine desulfhydrase (L-CDES) enzyme (specific for L-cysteine) and D-cysteine desulfhydrase (D-CDES) enzyme (specific for D-cysteine) accompanied by the production of pyruvate and ammonia [21,36]. L-cysteine desulfurases such as Nifs-like proteins, which are also present in chloroplast and mitochondria, produce $H_2S$ inside the plant cells by catalyzing the conversion of cysteine to alanine and elemental sulfur or sulfide [36].

$H_2S$ can also be produced in mitochondria during cyanide detoxification (Figure 2). β-cyanoalanine synthase (CAS), a mitochondrial based enzyme, generates $H_2S$ by catalyzing the transmutation of cyanide to β-cyanoalanine at the expense of cysteine [36]. The $H_2S$ thus formed is further used by mitochondrial isoform of OASTL to synthesize cysteine, which in turn is used by CAS for the detoxification of cyanide, producing a cyclic pathway in mitochondria [37]. Apart from these sites, the presence of $H_2S$ is also reported in *Arabidopsis* peroxisomes however, whether it is endogenously generated or imported from other compartments is still unknown [38].

Endogenous generation of $H_2S$ has been observed to be induced in response to several abiotic stresses, and involves different molecules related to signaling pathways. Recently, Fang and his coworkers provided evidence that in response to chromium stress, a transcription factor TGA3 enhances $H_2S$ production in *Arabidopsis* by regulating *LCD* expression through calcium/calmodulin-2 dependent pathway [39]. Under stress conditions, sulfide levels have been shown to be increased by the activities of $H_2S$ producing desulfhydrases [40–42]. Involvement of nitric oxide (NO), ethylene, ABA and salicylic acid has been reported in the regulation of $H_2S$ production in plants [43,44]. Signaling by $H_2S$ also leads to stomatal closure by regulating the activity of core components of the guard cell network [45].

## 4. Beneficial Aspects of $H_2S$ in Plants under Abiotic Stressed Conditions

Experimental evidences in the present era depicted that exogenously applied $H_2S$ alleviated the negative effects of various abiotic stressors. Various studies indicating the positive impact of $H_2S$ in plants under abiotic stresses have been represented in Table 1. However, the concentration levels, time of exposure and different kinds of $H_2S$ donor to be used and adapted under diverse conditions vividly shows the external symptoms of recovery after the $H_2S$ treatment. Alongside, at a physiological and biochemical level, the nitro-oxidative stress markers assessed were in the form of proteins, lipid peroxidation, nitration, nitrosylation, and oxidation to modulate in the different manner [15]. Subsequently, it led to coordinated action of antioxidative toolbox in the form of enhanced activities of superoxide dismutase (SOD), ascorbate peroxidase (APOX), catalase (CAT), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR), guaiacol peroxidase (POD), and non-enzymatic antioxidants (ascorbic acid, glutathione, tocopherol, etc.). These results clearly indicated the positive linkage among $H_2S$ and ROS; for instance, ascorbate peroxidase and catalase are specific targets for persulfidation and are also altered by NO and post-translational modifications as well as S-nitrosylations and nitration, respectively [33]. Interestingly, it forms a substantial relation among $H_2S$, ROS and NO, specifically during stressful conditions.

**Table 1.** Role of $H_2S$-mediated abiotic stress tolerance in various plants.

| S.No | Abiotic Stress | Plants | $H_2S$ | Mechanism of Action | References |
|---|---|---|---|---|---|
| 1. | Cold | *Arabidopsis thaliana* | NaHS | Induced MPK4 kinase activity. | [46] |
| 2. | Osmotic stress | *Arabidopsis thaliana* | NaHS | Stomatal closure mediated by enhanced activities of phospholipase Dδ and $H_2S$. | [47] |
| 3. | Salt | *Kandelia obovata* | NaHS | Improved photosynthesis, quantum efficiency of photosystem II, membrane integrity, hormone biosynthesis, and proteins related to antioxidation, heat-shock proteins, chaperonins, nitrogen metabolism, glycolysis and ascorbate–glutathione (AsA–GSH) cycle. | [48] |
| 4. | Salinity | *Malus hupehensis* | NaHS | Declined oxidative damage and $Na^+$, and increased antioxidant enzyme activities, $K^+$ content to maintain the homeostasis, and modulated expression of *SOS1* and *SKOR* under salt stress. | [49] |
| 5. | Heavy metal (Cd) | *Vigna radiata* | Hypotaurine | Improved antioxidant enzymes and components of ascorbate-glutathione cycle, photosynthesis, and carbohydrate metabolism. | [50] |
| 6. | Salinity | *Cucumis sativus* | NaHS | Modulated expression of genes encoding photosynthesis, carbon metabolism, amino acids, and proteins (*Cysteine synthase 1*, *Glutathione S-transferase U25-like*, *Protein disulfide-isomerase*, and *Peroxidase 2*). | [51] |

**Table 1.** *Cont.*

| S.No | Abiotic Stress | Plants | $H_2S$ | Mechanism of Action | References |
|---|---|---|---|---|---|
| 7. | Low temperature | *Cucumis sativus* | NaHS | Enhanced antioxidative defense system with improved levels of cucurbitacin C. | [52] |
| 8. | High temperature | *Zea mays* | NaHS | Stimulated antioxidative defense actions, seed germination rate, and proline accumulation. | [53] |
| 9. | Heavy metal (Al) | *Oryza sativa* | NaHS | Enhanced root elongation, antioxidant activities with reduced oxidative stress markers, and Al content in root tips. | [54] |
| 10. | Heavy metal (Cr) | *Zea mays* | NaHS | Higher antioxidant activities (SOD, POD, CAT) with reduced Cr accumulation within plants. | [55] |
| 11. | Drought | *Triticum aestivum* | NaHS | Stimulated ABA synthesis and antioxidant enzyme activities (CAT, POD, SOD, GST) with reduced oxidative stress markers in roots as well as shoots. | [56] |
| 12. | Salinity | *Oryza sativa* | NaHS | Decrease the uptake of $Na^+$ and the $Na^+$:$K^+$ ratio. | [57] |

In this milieu, due to the collective reports of the beneficial aspects of $H_2S$ on plants during adverse conditions, it could be explored in the biotechnological applications for enrichment of soils and water sources in agri-ecosystems. Nanoparticles with the tendency to synthesize $H_2S$ during optimal conditions also require further explorations in the near future. In brief, the exogenous $H_2S$ forms a promising technique to palliate the adverse effects in plants subjected to abiotic stresses. Furthermore, it could be extrapolated for their other applicability such as in seed germination, root development, shoot development, branching patterns, climacteric as well as non-climacteric ripening of fruits, etc. Albeit, the fundamental research on gaining knowledge about $H_2S$ metabolism in plants (endogenous/exogenously applied) at a cellular and molecular level needs to be investigated. Moreover, $H_2S$-targets in cell and signaling cascade with other molecules namely, NO, $H_2O_2$, as well as phytohormones, is strongly recommended to be explored further.

### 4.1. *$H_2S$ and Oxidative Stress Signaling*

$H_2S$ interaction among ROS and oxidative modification to regulate oxidative stress is one of the foremost signaling mechanisms to take place. ROS oxidises protein and cysteine group to sulfenic acid by sulfenylation, and so called sulfenylated proteins are regulated via thioredoxin systems [58]. Further, the excessive ROS induces oxidation of sulfenic acid into sulfinic acid/sulfonic acid that may cause inactivation of proteins [59]. The persulfidation, antioxidants enzymatic and non-enzymatic, and peroxiredoxin also comprises cysteine residues that are vulnerable to oxidative modifications via ROS [60]. Apart from activating antioxidative responses, $H_2S$ also reacts with protein cysteine, sulfenic acid, to form persulfides. This is further reduced to thiols for recovering their cell functions [5] (Figure 3). Henceforth, this process operates as a protective pathway by averting hyper-oxidation of antioxidants and protein thiol group into thiol modification in the form of sulfinic and sulfonic acid. Consequently, it reduces ROS-dispensation ability during stressed conditions. A proteomic study reported in persulfidated or sulfenylated *Arabidopsis* revealed that nearly 645 proteins were susceptible to modifications [61].

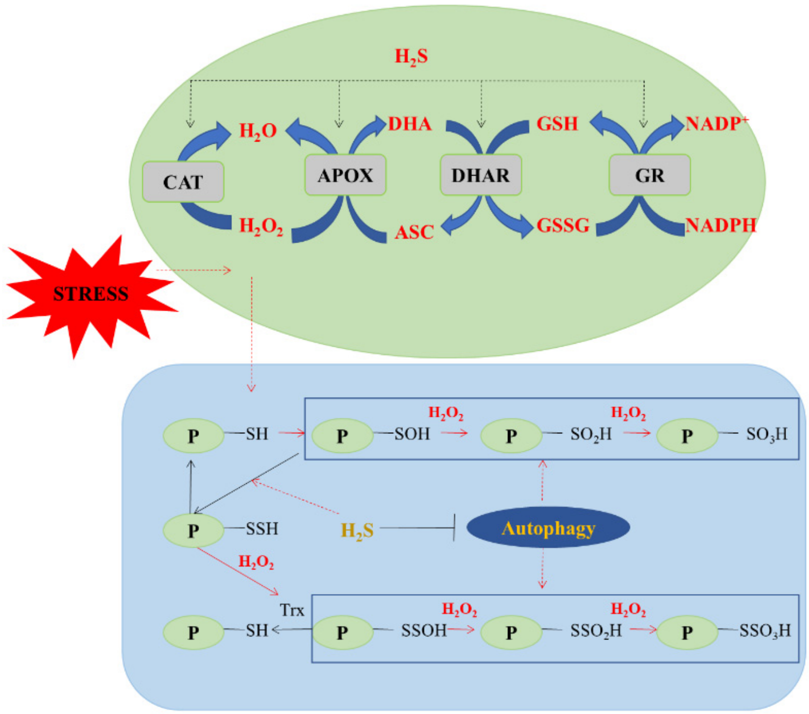


**Figure 3.** Diagrammatic description of inter-relationship between $H_2S$ and oxidative stress signaling. Abiotic stresses induce ROS and $H_2S$, which metabolizes the catalytic action of various antioxidant enzymes such as SOD, POD, CAT, APOX, DHAR, GST, GR, etc. and reduce their substrates ascorbate, glutathione and NADPH to lower the ROS generation through metabolizing $H_2O_2$. After the encounter of oxidative stress, protein cysteine thiols are also oxidized by $H_2O_2$ into sulfenic acid, which are further hyperoxidised into sulfinic acid and sulfonic acid. $H_2S$ and sulfenic acids further combine to form persulfidated proteins and most importantly they can also reduce back to thiols through thioredoxin enzyme that potentially reduce the disulfide bonds. Sidewise, persulfidated proteins in combination with ROS also give rise to perthiosulfinic and perthiosulfonic acids that might get reduced very easily by thioredoxins to generate thiols. $H_2S$ also regulate autophagy that could denature oxidized proteins and maintain stability of proteins. Dual role of $H_2S$ aids plants to operate their proteins efficiently against ROS-generated oxidative stress with consumption of energy through de novo synthesis of proteins for growth, development, and defense mechanisms.

Amid, KEGG pathway investigation along with domain enrichment display that overlapping of various proteins are specifically involved in different metabolic pathways namely, Kreb's cycle, Glycolysis, Calvin cycle, protein, and amino acid metabolism. Intriguingly, proteins act as putative candidature for $H_2S$-mediated signaling in plants under stress severity. Strikingly, $H_2S$ also regulates photosynthesis in several plants, photosynthetic bacterial species, and nitrogen metabolism in stressed plants [62]. Moreover, the persulfides are highly reactive towards ROS in contrast to thiols, thereby, they could be oxidised by ROS into perthiosulfenic acids, subsequently leading to the generation of perthiosulfinic and perthiosulfonic acid, respectively [5]. Contrastingly, both sulfinic and sulfonic acids are formed by irreversible reaction, yet a complete toolkit of reducing a system such as thioredoxins are restored in the form of oxidized cysteines that can convert them back into the reduced forms [36]. Henceforth, this implies to the mechanism by which $H_2S$-mediated oxidative stress and protein modification is protected from stresses.

In addition, autophagy is a process that gets activated during wide categories of stresses such as nitrogen, carbon, or any other nutrient deprivation and this process mainly disrupts the cytoplasmic bridges along with various organelles comprising of ROS-regulating and scavenging enzymes [63] (Figure 3). To elucidate, CAT enzyme localized within peroxisome is degraded by authophagy through $H_2O_2$ intervention [64]. Alongside, autophagy also denatures oxidised proteins in plants during stressed conditions. On the contrary, the basic autophagic process that degrade cytoplasm and its components for raw materials and energy related metabolic activities, the fine tuner process encompassing $H_2S$-mediated persulfidation forms a much more conventional, rational, and competent approach in terms of growth, development, and defense related processes in plants, specifically during stressed conditions. In the forging arguments, the previous studies conducted revealed the negative aspect of autophagic regulation by $H_2S$ in *Arabidopsis* [37]. Additionally, they also suspected that $H_2S$ when applied exogenously mitigated nitrogen deficiency and modulated the levels of anthocyanins along with suppressing autophagy and nitrogen deficiency in plants. Besides, $H_2S$-mediated responses also enhanced ROS levels, thereby showing the trio among ROS, $H_2S$, and autophagy during protein modifications in plants [65].

Nevertheless, a positive coordination among $H_2S$ and oxidative signaling has been found in plants exposed to stressed conditions (Figure 3). Apart from the antagonistic responses among $H_2S$ and ROS, they also participate in stress responses. If we visualize at thermodynamic state, $H_2S$ is unable to interact with protein cysteines to convert them into persulfides [5]. However, one descriptive mechanism in persulfide synthesis is $H_2S$ reacting with sulfenic acid. Henceforth, the oxidative stress generated at a specific intensity allows the $H_2S$-induced signaling pathway to get initiated via persulfidation in response to stresses. In line with this notion, the suitable mechanism of persulfide generation is through the reaction of $H_2S$ and sulfenic acid. Indeed, the oxidative stress markers were also observed in endoplasmic reticulum along with persulfidation in mammalian cells [66]. This is in concomitant with the sulfenic acid role during protein persulfidation. However, the plant guard cell differentiation also depicts the most suitable example to interpret the complexation among $H_2S$ and ROS. Guard cells regulate stomatal movements during harsh environmental conditions such as drought, flooding, freezing, etc. More recently, the studies have also affirmed that $H_2S$ and NADPH oxidases, RBOHD and RBOHF, which generate $H_2O_2$ near apoplast are a pre-requisite in the ABA-mediated stomatal regulation process in guard cells [67]. Enigmatically, various different proteins that play a crucial role in stomatal processes in their opening and closing are specific targets for persulfidation and sulfenylation [61]. These proteins basically involve Ca-dependent protein kinases (CPK3 and CPK6) along with mitogen-activated protein kinases (MPK3, MPK6, and MPK4), respectively [68,69]. These are specific targets in abilities to acquire the mechanisms underlying $H_2O_2$-mediated $H_2S$-based stomatal closure. In another case, $H_2O_2$ in combination with $H_2S$ is also mediated polyamine-induced UV-B radiation stress tolerance in barley [70]. Strikingly, $H_2S$ involve the use of NADPH-oxidases derived $H_2O_2$ to trigger tomato root architecture as well as lateral root formation [71].

### *4.2. Antioxidant Defense System of $H_2S$*

The increment in the levels of ROS due to numerous stresses are directly co-linked to oxidative damage of various biomolecules such as cell membrane integrity, nucleic acids, and base pairing of DNA, protein structures, etc. Plants tend to cope with ROS and its adverse effects of oxidative damage specifically through two different pathways. Firstly, it comprises of scavenging mechanism in which ROS generated within plants is scavenged by a series of antioxidant related compounds such as ascorbate and glutathione and various antioxidative enzymes related to ascorbate-glutathione cycle [1]. A study conducted in *Brassica rapa* subjected to Cd stress showed a stimulated content of $H_2O_2$, $O_2^{\bullet -}$ and lipid peroxidation due to excessive malondialdehyde content [72]. Followed by that, the ROS accrual was considerably reduced by exogenously supplied $H_2S$. This reduction in ROS is mainly attributed to the higher activities of antioxidant enzymes CAT and SOD, respectively. Likewise, the SOD and APOX activity was also stimulated in barley after application of $H_2S$ donor NaHS to alleviate Al-toxicity [73]. In addition, the protein expression of *APOX* and *APX1* gene was upregulated by $H_2S$ along with the modulated expression levels of C/Zn SOD. All these findings speculated that $H_2S$-mediated antioxidant enzyme activities regulate the expression of protein transcripts followed by reducing the ROS accrual due to Al-toxicity [73].

In addition, the enzymatic activities of CAT and glycolate oxidase was also observed to be reduced under glyphosate-mediated oxidative stresses in *Arabidopsis* [33]. With the aid of NaHS gradients and biotin method, it has been reported that CAT reduction is most likely due to persulfidation and post-translational modifications in the conversion of thiol groups into persulfide groups of proteins, respectively [38]. Furthermore, ascorbate-glutathione cycle has been observed to directly contribute towards waning off of ROS and oxidative stress generated, through a series of reactions underlying antioxidative enzymes. Majorly, APOX, MDHAR, DHAR, and GR act concomitantly in a coordinated manner for $H_2O_2$ quenching and maintaining cellular redox homeostasis [4]. Treatment using NaHS enhanced APOX and GR activities along with induced APOX and glutathione levels in *Zea mays* subjected to temperature stress [53]. Similar to this, NaHS also triggered the APOX, GR, and DHAR activities with mitigating the declined ratios of ascorbate/DHA and GSH/GSSG in *Z. mays* under salt stress [74]. Although, the positive action of $H_2S$ on ascorbate-glutathione pools was also annulated by the addition of hypotaurine, $H_2S$ scavenger, thereby depicting the role of $H_2S$ in regulating redox homeostasis in plants under Cd stress via ascorbate-glutathione [12]. All the above discussed studies provided with the fact that exogenously applied $H_2S$ induces resistance against various biotic as well as abiotic stresses through the regulation of ascorbate and glutathione metabolism. Additionally, exogenous $H_2S$ also incline the levels of endogenous $H_2S$ in plants under stressed conditions. Further, a study formulated that $H_2S$ mitigated salt stress and the rendered growth of root elongation in alfalfa plants. Meanwhile, this positive impact was disturbed by an inhibitor or $H_2S$ scavenger. Moreover, they also determined that $H_2S$-modulated the protein transcripts and gene expressions of genes encoding SOD, CAT, glutathione, ascorbate, etc., which nullified the effects caused by lipid peroxidation in plants [40]. Altogether, $H_2S$ regulates ROS homeostasis and maintain membranal integrity in plants by regulating the plant metabolic activities associated with antioxidant enzymes and enzymes associated with ascorbate-glutathione pools. Henceforth, plant tolerance towards various abiotic stresses is achieved through $H_2S$-regulated antioxidant defense system of plants (Figure 3).

### *4.3. Role of $H_2S$ in Metal Uptake and Transport*

Heavy metals restrict crop quality and productivity due to their toxicity, as it has adverse effect on various physiological processes of plants. Under heavy metal stress, there is alteration in the absorption and transport of metal ions in plants. It has been reported that $H_2S$ acts as a regulator of plant resistance against heavy metal stress. In rice seedlings, the toxicity imposed by mercury (Hg) is reduced by $H_2S$, by inhibiting Hg transport to shoots and its sequestering in roots [75]. This is due to the increased levels of metallothioneins and non-protein thiol induced by $H_2S$, which can further chelate with ions of heavy metal. Phytochelatins (PCs) and metallothionein's (MTs) are important compounds which act as heavy metal chelators and regulate the solubility and toxicity of heavy metals [76]. Zinc (Zn), one of the essential elements required for growth and development of plant impose toxic effects on plants when in excess. Transport of $Zn^{2+}$ in the cytoplasm is through a specific zinc transporter [77]. Sodium hydrosulfide (NaHS), a $H_2S$ donor that not only inhibits the expression of natural-resistance associated macrophage protein 1 (*NRAMP*), iron-regulated transporter (*IRT*), zinc-regulated transporter (*ZRT*), heavy metal ATPase 4(*HMA4*), and metal tolerance proteins (*MTP*) genes (homeostasis related genes), but also decreases the accumulation and uptake of zinc in the roots and shoots of *Solanum nigrum.*

Cadmium, a water soluble non-redox toxic heavy metal, absorbed through the plant roots and accumulated in edible plants parts, impose harmful effects on human health [78]. In the cytoplasm of *Populus euphratica,* accumulation of cadmium ion can be significantly reduced by the exogenous application of $H_2S$, through its increased vacuolar Cd sequestration, further decreasing the cadmium influx across the plasma membranes [27]. Acceleration of cadmium influx in *P. euphratica* cells by $H_2O_2$ was also reported in this study. In cell, the influx of Cd across the plasma membrane was also reduced by CAT, suggesting that plasma calcium channels could get activated by $H_2O_2$, which allows the Cd influx. Additionally, activity of anti-oxidant enzymes was enhanced by $H_2S$, resulting in inhibition of $H_2O_2$accumulation in *P. euphratica* cells [27]. Collectively, these results indicate that $H_2O_2$ mediated Cd influx through calcium channels is regulated by $H_2S$.

Furthermore, $Al^{3+}$ toxicity is one of the most common environmental factors that limits crop productivity by root growth inhibition, especially in acidic soil. Under $Al^{3+}$ toxicity, an increase in root length and decrease in $Al^{3+}$ content in root tip of rice can be achieved by exogenous application of $H_2S$ [27]. $H_2S$ scavenger (HT) reversed these effects, suggesting that $H_2S$ is involved in the alleviation of $Al^{3+}$ toxicity in rice. Pectin, a polysaccharide component of cell wall was involved in the tolerance of $Al^{3+}$ in plants and $Al^{3+}$ is mainly accumulated in the hemicellulose component of the cell wall in plants [54]. Hemicellulose and pectin level was significantly decreased by $H_2S$ in order to reduce the $Al^{3+}$ content in the cell wall of rice root [79]. Furthermore, the expression of genes which encode certain proteins necessary for the detoxification of $Al^{3+}$ in plants is regulated by $H_2S$. On the other hand, the protein level can be enhanced by $H_2S$ to prevent $Al^{3+}$ entry in cytoplasm. $Al^{3+}$ deposition is reduced by UDP glucose, which is transported to the cell wall from cytoplasm via an ATP binding cassette transporter STAR1-STAR2 complex [80]. *OsSTAR1* and *OsSTAR2* expression was significantly enhanced by $H_2S$, suggesting that $Al^{3+}$ resistance is increased by $H_2S$. Additionally, in rice $Al^{3+}$ toxicity can be increased by citric acid secretion [81]. A citrate efflux transport involved in Al-induced citrate secretion is encoded by *OsFRDL4* [82]. Under $Al^{3+}$ stress in rice exogenous application of $H_2S$ improved *OsFRDL* expression and significantly increased citrate content in root exudates. These results suggest that in rice, citrate secretion could be regulated by $H_2S$ to enhance resistance to $Al^{3+}$ toxicity. Similarly, expression of *OSNRAT1*, which encodes aluminum transporter 1 (*NRAMP*) is decreased by $H_2S$ by decreasing the $Al^{3+}$ amount, which enter the root cells [54]. These results indicate that activity of some proteins is regulated by $H_2S$ by blocking the $Al^{3+}$ entry in the root cells, resulting in enhanced $Al^{3+}$ tolerance in rice. On the other hand, sequestration of $Al^{3+}$ from cytoplasm to vacuole depends on half- sized ATP binding cassette transporter. *OsALS1* stimulated by $H_2S$ results in $Al^{3+}$ sequestration in the vacuole, suggesting that $Al^{3+}$ can be alleviated by $H_2S$ through the reduction of the $Al^{3+}$ content in symplast and apoplast of rice root. Collectively, it would be safe to accord the involvement of $H_2S$ in plant metal tolerance essentially by influencing the absorption of metal ions and their transport [61] (Figure 4).

### 4.4. Role of $H_2S$ in $Na^+/K^+$ Homeostasis

Salt stress has affected the growth, development, and survival of plants. Numerous negative effects such as oxidative stress and ionic stress (accumulation of $Na^+$) are induced by excess salinity [83]. Recently, some studies reported that the salt tolerance of plants can be enhanced by $H_2S$that has been reported to maintain homeostasis of $Na^+/K^+$. In case of rice plant growing under salt stress, the $K^+$ content in the cell decreased while the $Na^+$ content was found to increase. In other words, the $Na^+/K^+$ ratio increased in the roots and leaves of rice. Whereas, exogenous application of$H_2S$ resulted in an increase in $K^+$ and decrease in $Na^+$ levels, thus maintaining the homeostasis of $Na^+$ and $K^+$ ions in rice. However, the addition of HT ($H_2S$ scavenger) inhibited the ameliorative effect of $H_2S$ [57]. In further studies, reduction in NaCl-induced transient $K^+$ efflux by $H_2S$ was also observed. It has been documented that under salt stress, $H_2S$ significantly suppresses the expression of *SKOR* (gene involved in encoding of outward $K^+$ rectifying channel), thus ultimately suggesting that $H_2S$-restored $K^+$ efflux might be dependent on $K^+$ rectifying channel. In addition, the level of plasma membrane bounded NADPH oxidase mediated $H_2O_2$was reported to enhance under the influence of $H_2S$ [84]. However, the activity of $H_2S$-induced $H_2O_2$ accumulation was reported to suppress by a non-specific suppressor of plasma membrane bound NADPH oxidase in the roots of *Arabidopsis* This indicates that $H_2S$ increases the salt tolerance in *A. thaliana* by maintaining $H_2O_2$ mediated $Na^+/K^+$ homeostasis [85].

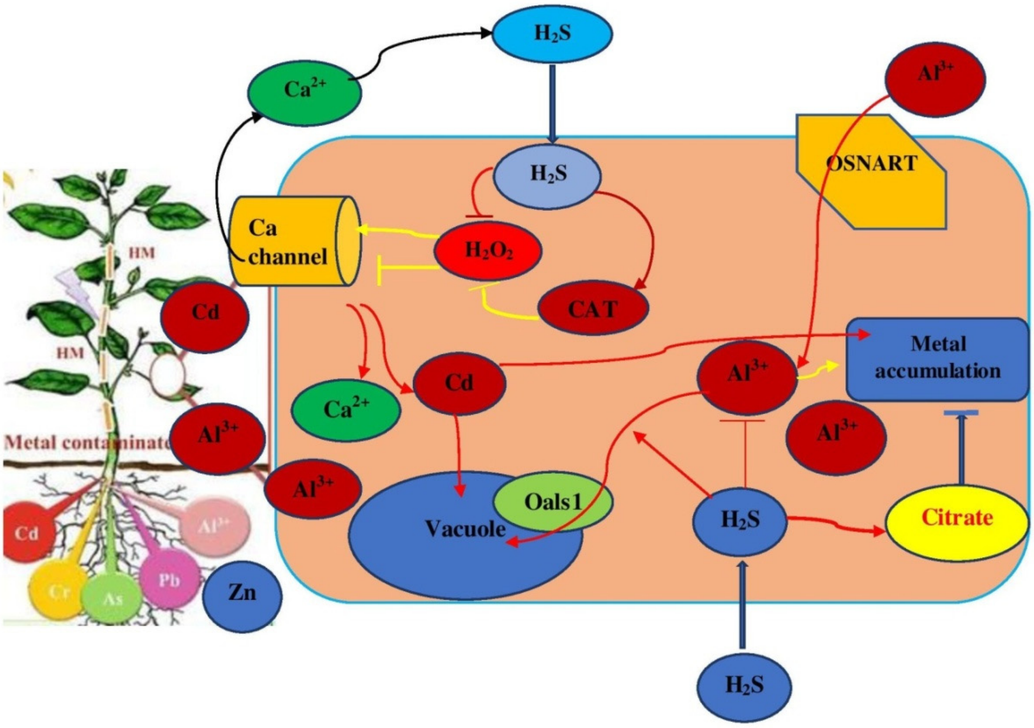


**Figure 4.** Schematic representation of $H_2S$-mediated responsive pathway in plants towards heavy metal stresses. Various heavy metals such as Cd, Pb, Zn, Al, As, Cu, Cr, etc., enter the plants and after entering the plant cell Ca-channels induce $H_2S$ responses in plants that further induce the antioxidative enzyme activities. The free radicals produced are also scavenged through different metabolites such as citrate activated by $H_2S$ and tend to chelate metal ion or stabilize them to prevent their accumulation. In addition, $H_2S$ promotes metal ion sequestration in vacuoles.

Homeostasis of ions in the cytoplasm is maintained by the $Na^+/H^+$ antiporter, Salt-Overly-Sensitive 1 (SOS1) present on plasma membrane by reducing the concentration of $Na^+$ in cytoplasm. The kinetic energy required for this transportation is provided by $H^+$-ATPase derived $H^+$ gradient [86]. The efficacy of $H_2S$ on maintenance of $Na^+/K^+$ homeostasis has been reported to impede by Vanadate (an PM $H^+$-ATPase inhibitor) and Amiloride (an SOSI inhibitor), thus suggesting that the ion homeostasis is regulated by $H_2S$ through plasma membrane $Na^+/H^+$ antiporter system [87]. Furthermore, in case of *Arabidopsis* growing under salt stress, $H_2S$ has been reported to have a positive impact on gene expression and phosphorylation level of $H^+$-ATPase and moreover this effect was suppressed by N,N-dimethylthiourea, which works by inhibiting the endogenous production of $H_2O_2$ [88]. These results suggest that $H_2S$ regulates the homeostasis of ions through $H_2O_2^-$ mediated signaling pathway to synchronize the expression of plasma membrane $Na^+/H^+$ antiporter and activity of $H^+$-ATPase in *Arabidopsis* roots [87].

## 5. $H_2S$-Mediated Mechanism of Action in Plants

It has been reported that hydrogen sulfide is generated for performing various important physiological functions, usually by post-translational oxidation of cysteine moiety to per sulfide form [89]. The persulfidation mechanism of various proteins have been well documented in case of mammals [90,91]. Hydrogen sulfide mediated persulfidation of proteins in case of plants for the proper functioning of biological processes has been discussed as follows.

### *5.1. Role of $H_2S$ in Post-Translational Modification of Cysteine Residues and Protein Sulfidation*

Since, $H_2S$ is a type of gasotransmitter in plants as well as in animal cells, it is known to be equally important as other signaling molecules such as carbon monoxide (CO), nitric oxide (NO), and hydrogen peroxide ($H_2O_2$), etc. [92–94]. Moreover, $H_2S$ has been reported to have a significant role in plant growth and also in plant protection against various types of stresses such as drought, heat, heavy metal toxicity, etc. Despite of all this, the main function of $H_2S$ is its potential of acting as a signaling molecule [95,96]. Its role as a signaling molecule can be explained through a post-translational modification of protein, which is frequently known as 'persulfidation', which is characterized by upgradation of thiol group of cysteine residues (-SH group) of protein into persulfide (-SSH) group. Previously, this modification was termed as 'S-sulfhydration', but in actual practice, there is no hydration reaction that occurs to complete the process, so the process was renamed as persulfidation. Moreover, it has also been documented that modified cysteine has greater reactivity when compared to the unmodified thiol form [91].

#### 5.1.1. Protein Persulfidation

As discussed earlier, $H_2S$ perform its function by promoting the persulfidation of active cysteine moiety of protein into persulfide form via covalent conversion of thiol group into persulfide group [97,98]. However, the studies suggests that there is no direct reactivity between the thiol group of protein and $H_2S$ group. The reason behind this non-reactivity is due to the oxidation of both hydrogen and sulfur atoms in the reaction, the electrons thus produced end up as protons that are not able to form hydrogen gas [89]. Despite this, when the thiol group of protein reacts with hydrogen peroxide, the oxidized product formed is Sulfenic acid (R-SOH), which further reacts with $H_2S$ to form persulfidated (R-SSH) product. Furthermore, the resultant component thus formed reacts with reactive oxygen species (ROS) and generate the product, perthiosulfenic acid (R-SSOH), which has low stability. Further, it has been documented by Filipovic [89] that if the excess number of oxidants are present, R-SSOH may get further converted to two products via oxidation namely, perthiosulfinic (R-$SSO_2H$) and perthiosulfonic acid (R-$SSO_3H$). It has been reported that within the cell the level of persulfidation is regulated by thioredoxin, i.e., thioredoxin is involved in catalyzing the reverse reaction of persulfidation (Figure 5) [58,66]. This reversion reaction of protein persulfidation evades the chances of irreversible oxidative damage that normally occurs at the thiol group of the protein [5,66,98].

In addition to this, other signaling molecules such as NO are also capable of manipulating proteins by a process called S-nitrosylation (R-SNO). This reaction involves the covalent attachment of thiol group of cysteine moiety in protein to the NO [99]. The products formed as a result of this reaction are known as S-nitrosothiols [100,101]. These S-nitrosothiols are capable of reacting with $H_2S$, thus ultimately resulting in protein persulfidation (R-SSG).

Furthermore, it has been well documented that the modified or persulfidated proteins have high reactivity in comparison to normal unmodified form. The valid reason for this reactivity is the enhanced nucleophilicity of -SSH group that can undergo easy chemical reaction with the electrophiles [102]. The main electrophilic agents include S-4bromobenzyl methanothiosulfonate (BBMTS), methanethiosufonate (MMTS), and methylsulfonylbenzothiazole (MSBT) (Figure 5).

#### 5.1.2. Protein Persulfidation in Plants

The first report of persulfidation in plants was reported in *Arabidopsis* with about 106 protein that are modified at cysteine residues by Aroca et al. [96]. Furthermore, 2015 persulfidated proteins were reported from wild type and des1 mutant *Arabidopsis* plants with the help of an assay in which an electrophile MSBT was used as blocking agent. All the reported proteins were found mainly involved in amino acid metabolism, protein biosynthesis, glycolysis, and in response to various stress conditions [103].

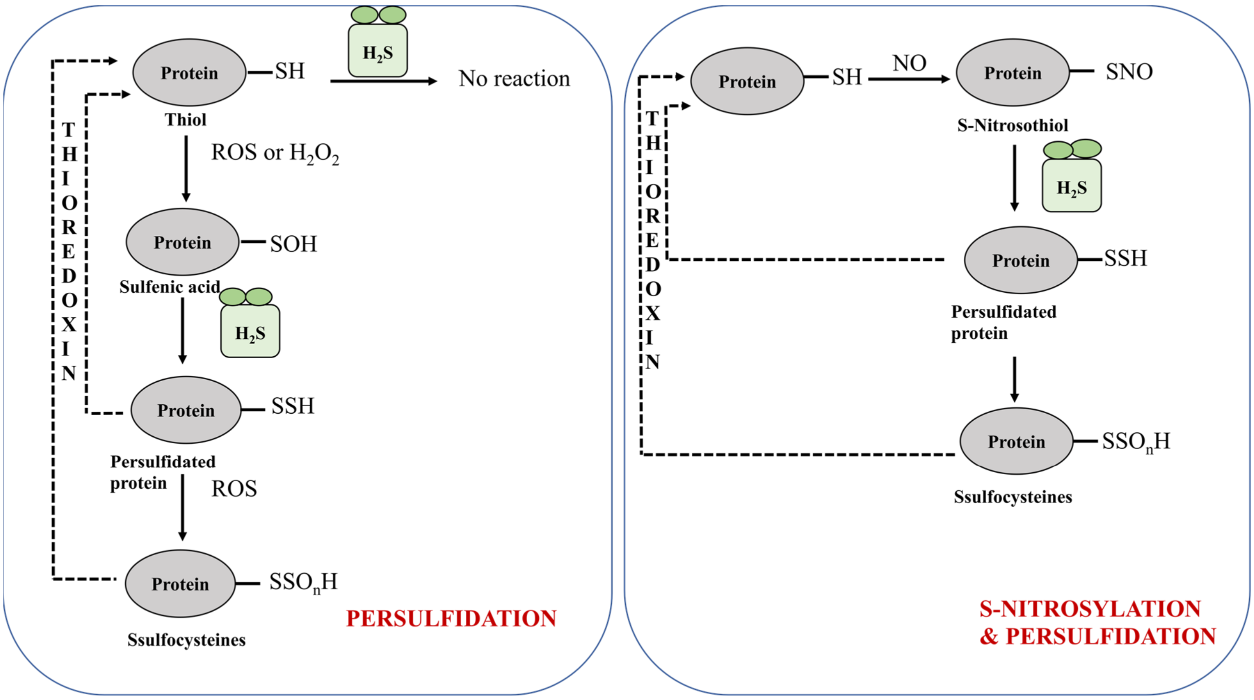


**Figure 5.** A brief model explaining persulfidation and S-nitrosylation in plants. The thiol group of protein undergo oxidation in presence of ROS and form sulfenic acid, which then undergo persulfidation in the presence of hydrogen sulfide to form persulfidated protein. If the persulfidated protein is exposed to ROS, S-sulfocysteines are formed. Both S-sulfocysteines and persulfidated proteins can revert back to thiol form by thioredoxin. In S-nitrosylation, nitric oxide (NO) combines with thiol group of protein to form S-nitrosothiol, which can further react with hydrogen sulfide to form persulfidated protein, which can then revert back to thiol form in presence of thioredoxin.

Similarly, as per the reports of Li et al. [104], $H_2S$ has a role in the regulation of actin and thus ultimately has effect on root hair growth. Basically, the genome of *Arabidopsis* consists of 8 ACTIN genes, which are further categorized into two major groups on the basis of their functioning in reproductive and vegetative organs [105]. However, whenever there is an overaccumulation of$H_2S$, persulfidation at cys 287 residue of one of the vegetative gene, i.e., ACTIN2 occurs. This persulfidation leads to depolymerization of actin cytoskeleton and thus ultimately resulting in root hair growth inhibition [104]. These findings were further proved by introduction of *actin* 2-1 mutant with Cys-287 mutated ACTIN2; the outcome of the study is the partial suppression of root hair inhibition, which is $H_2S$ dependent [104]. Moreover, according to the literature, $H_2S$ suppresses the activity of aminocyclopropane carboxylate oxidase (ACC oxidase) enzyme (rate limiting enzyme in ethylene biosynthesis), thus ultimately inhibiting the elongation of root hairs [44].

Moreover, $H_2S$ has also reported to have role in the persulfidation of various enzymes that are involved in signaling of abscisic acid (ABA), which make $H_2S$, a contributor in stomatal closure [96,106]. This whole cascade of interactions has been studied in *Arabidopsis*, a model plant, and it has been reported that when the level of ABA increases in the cell, it binds to the receptor PYR/PYL/RCAR (PYRABACTIN RESISTANCE/PYR-LIKE/REGULATORY COMPONENT OF ABA RECEPTOR) and suppresses the activity of PP2C (clade A protein phosphatases) [106]. Further, the *SnRK2.6* (SNF1-RELATED PROTEIN KINASE2.6) or *OST1* (OPEN STOMATA 1) is stimulated to initiate multiple downstream signaling pathways. This is the stage from where $H_2S$ regulates the ABA signaling by persulfidation of Cys-131 and Cys-137 residues of *SnRK2.6*, present in guard cell [107]. The persulfidation of cysteine residues enhances the kinase potential of *SnRK2.6* and also promotes its interaction with *ABF2* (ABA RESPONSE ELEMENT-BINDING FACTOR 2), thus resulting in the phosphorylated ABF2, which further stimulates the downstream genes that control the closure of stomata [107]. In addition to this, $H_2S$ is also found involved in persulfidation of Cys-44 and Cys-205 of DES1 in the presence of ABA, resulting in the enhanced level of $H_2S$ in the guard cell. This increment further promotes overproduction of ROS through persulfidation at Cys-825 and Cys-890 residues of RBOHD (NADPH oxidase RESPIRATORY BURST OXIDASE HOMOLOG D). The excessive production of ROS resulted in suppressing the activity of ABA signaling. Conclusively, all these potencies of $H_2S$ have a role in the regulation of ABA signaling in plant tissue (Figure 5).

## 6. $H_2S$-Signaling during Abiotic Stresses

Under stressed conditions, various signaling molecules such as ABA, $Ca^{2+}$, various phytohormones, $H_2O_2$, etc., come into action. Likewise, $H_2S$ levels are also triggered in plants in response to various stressors. This $H_2S$- is triggered in response to many stresses and forms a signaling cascade. Following sections describe the role of $H_2S$-signaling pathway under diverse stress conditions.

### *6.1. $H_2S$-Signaling during Heavy Metal Stresses*

It has been observed that there is an accumulation of $H_2S$ in plants subjected to heavy metal stresses due to their extreme toxic nature. $H_2S$ enhances the number of mitochondria, endoplasmic reticulum, and golgi bodies in plants [108]. Moreover, it also stimulates metal ion fixation, co-related to cell wall functioning, transporter regulation, and closed association of chelators with specific signals. The cell wall acts as a barrier to external metals, and $H_2S$ in turn induces pectin and pectin methylesterases for strengthening the cell wall [109]. A study affirmed by Zhu et al. [54], revealed that Al-stressed rice plants showed stability in the cell wall towards metals by $H_2S$-mediated reduction of negative charges in cell wall along with plummeting pectin methylesterases, pectins and hemicelluloses within roots and shoots, respectively. Plants also possess specific mechanism to mitigate metal toxicity via transporting metals into vacuoles through $H^+$-ATPases and citrate transporters localized onto vacuolar membrane. This is further amplified by $H_2S$ with upregulation of $H^+$-ATPases expression in tonoplast followed by reducing cytoplasmic metal accumulation [110]. Alongside, induced expression of *MATE13, MATE47,* and *FRPL4* genes in soybean and rice by $H_2S$ not only alleviates Cd and Al toxicity but also enhanced citrate exudation [109]. Another study reported that rice exposed to Al-stress showed upregulation in *NRT1* and *ALS1* genes after $H_2S$ treatment along with controlling Al level in cytoplasm by transporting it to vacuoles [110].

Nevertheless, one of the most efficacious mechanism possessed by plants to counteract metal toxicity is to momentarily pause the metals through PCs and MTs, having a close connection to sulfur metabolism ($H_2S$-cysteine-core). Cysteine is crucial for GSH-biosynthesis through different enzymes, therefore, $H_2S$-induces the expression profile of genes encoding MTs and PCs through transcriptional regulation [111]. A certain co-related factor of $H_2S$ that works during heavy metal stresses is NO, which is considered a principal partner of $H_2S$ [112]. Sodium nitroprusside show similar action to NaHS in mitigating metal toxicity, depicting the closed relation among NO and $H_2S$, respectively [113]. In addition, $H_2S$ also works along with $Ca^{2+}$ ions for metal stress amelioration. This is most likely due to blocking of Ca-channels by metal ions followed by their detoxification through $Ca^{2+}$-pathway [39]. Strikingly, NaHS modulated CDPK-transcripts in zucchini exposed to Ni-stress [111]. Apart from this, $H_2S$-mediated metal stress alleviation is also accompanied by phytohormones such as salicylic acid, jasmonic acid, gaseous molecules, and different mineral elements [114]. All these components trigger $H_2S$-pathway or $H_2S$-producing enzymes or endogenous $H_2S$ [114]. The regulatory action of transcripts in promoter sites of vital genes encoding $H_2S$-biosynthesis have been observed. Certain transcripts such as *WRKY18*, *WRKY13*, *WRKY60*, etc., are enhanced, which further induces $H_2S$-levels under metal stressed conditions [114]. Likewise, ZIP-transcript *TGA* also increase the production of $H_2S$ during metal toxicity [115].

### 6.2. $H_2S$-Signaling during Salinity Stress

Salinity has caused many adversities towards agricultural crops by reducing plant growth and productivities. Climatic disturbances have altered the agricultural practices, specifically at coastal sites. $H_2S$ has been known to play a pivotal role in ongoing cellular responses in plants against salinity, therefore considered a powerful agricultural intervention. It has been observed that exogenously applied $H_2S$ enhanced salinity resistance through regulating $Na^+/K^+$-homeostasis along with endogenous $H_2S$ levels with boosted antioxidant activities in cucumber [116]. Another study reported by Kaya et al. [117], showed that melatonin mediated salinity tolerance in pepper through triggering $H_2S$ and antioxidant levels. In addition, NaHS induced salinity in cabbage via enhancing antioxidants and enzymes involved in ascorbate/glutathione cycle [118]. Further, it has also been observed that NaHS stimulated salt tolerance and osmotic stress in strawberry through antioxidants and ascorbate/glutathione redox states, thereby minimizing oxidative/nitrosative stress [119]. Interestingly, it has been revealed that $H_2S$ play key role in regulating antioxidants and various transcription factors namely, dehydration responsive element binding factor, ascorbate/glutathione biosynthesis along with salt overly sensitive genes [119]. $H_2S$ on combination with NO also mitigate salt toxicity as $H_2S$ acts downstream of NO in the signaling pathway. Henceforth, accrual of $H_2S$ has a direct impact on the stress-mediated signaling pathway under salt conditions with the motive to alleviate the toxicity.

### 6.3. $H_2S$-Signaling during Drought/Osmotic Stress

As climatic conditions are altering on global scale and precipitation is therefore altering due to such weather conditions. Few areas experience high rain, while others perceive lower or very minimal rainfall depending on where there is disaster in the form of either drought or flooding. Overall agriculture faces a huge impact and treatments to such conditions are required. Strategies such as NO-based molecules, $H_2S$ compounds, etc., act as impactful adjuncts. Drought stress has seriously impacted horticultural crops and impediment towards achieving productivity targets [120]. Additionally, limited rainfall and higher evaporation due to enhanced temperature also induces the impact of drought. Therefore, plants possess adaptive measures to survive during such unfavorable situations through regulating stomatal activities by reducing the transpiration rate so as to retain the water within for regulating physiological activities. $H_2S$ also acts up/down stream in NO-signaling pathways, based on activities such as stomatal movement, closure, etc., during stressed conditions [15]. The role of $H_2S$ in stomatal activities has been observed and studies are further conducted to understand its exact mechanism. To elucidate, $H_2S$ causes stomatal opening and closing under varied conditions in response to adverse conditions. Another study reported that short $H_2S$-exposure in plants led to induce stomatal closure whereas long exposure led to stimulate stomatal activities and $H_2S$ was also mediated by 8-mercapto-cGMP, respectively [121]. cGMP also acts as a downstream mediator of NO in plants and therefore both of them work in corroboration with one another. $H_2S$ treatment in plants regulates the relative water content of plants subjected to drought, however, the $H_2S$ acts as a donor during such conditions followed by inducing the metabolic profiles of plants in the form of polyamines, glycine betaine, osmolytes, proline and $H_2S$-biosynthesis [122]. Additionally, genes encoding soluble sugars, aquaporins, polyamines, choline monooxygenases, and betaine aldehyde dehydrogenases, etc., are also upregulated after $H_2S$ application in drought stressed plants [122]. In addition to this, plants with $H_2S$ treatment also reduced oxidative stress markers such as MDA and $H_2O_2$ [122]. NaHS treatment in Bermuda grass also stimulated tolerance against salt, osmotic, and chilling stress and this is mainly due to increased activities of antioxidants and osmolytes [114]. Further, proteomic approaches were used in $H_2S$-mediated drought resistance. They reported the imperative role of proteins namely, S-nitrosated proteins, photosynthetic proteins, etc., induced by $H_2S$. Henceforth, the plant-water relations, plant movements, stomatal opening/closing, etc., act as suitable target sites for $H_2S$ for modulating different physiological activities in plants. $H_2S$-formulations act as the most suitable molecules for stress resistance in plants.

### 6.4. $H_2S$-Signaling during Temperature Stress

Global warming has been observed to be the most adverse effect of climate change, basically due to the enhanced average temperature of the Earth. However, there are various regions where temperature extremity is observed both in the form of warming as well as freezing, therefore affecting the normal agricultural patterns. Certainly, there are $H_2S$ based compounds that participate in counteracting temperature extremities. Plants being sessile have to tolerate the varying temperatures of environment. $H_2S$ has been observed to cope up in mediating tolerance towards high/low temperature conditions. To illustrate, Tang, et al. [123], reported that exogenous $H_2S$ and hypotaurine, $H_2S$-scavenger mediated cooling stress tolerance in blueberry plants. This improvement is mainly due to enhanced tolerance after NaHS treatment owing to regulated activities of leaf gaseous exchange parameters, declined photoinhibition of PSI/PSII and higher proline levels. Concomitantly, the oxidative stress markers such as $H_2O_2$, MDA, etc., were also declined after $H_2S$ treatment. Meanwhile, hypotaurine enhanced the negative effects of cooling stress. Another study reported exogenously applied NaHS boosted chilling tolerance in cucumber and the most probable reason behind this was crosstalk among $H_2S$ and auxins during stressed conditions along with higher flavin monooxygenases (FMO) and FMO-like proteins. This in turn inclined auxins that further reduced chilling stress-generated electrolyte leakage and ROS-generation with higher expression of photosynthetic enzymes. They concluded that auxins act downstream in $H_2S$-mediated chilling stress tolerance in plants [124]. Further, $H_2S$-mediated chilling stress tolerance also revealed stimulation in cucurbitacin C, a secondary metabolite that enhanced tolerance as well as bitter taste in cucumber [52]. Contrastingly, $H_2S$ also determined an ameliorating agent in high temperature stress that could prove lethal towards agricultural crops. A study carried out in strawberry raised under induced temperatures showed declined oxidative damage and higher heat show defense upon $H_2S$ application. $H_2S$-mediated heat tolerance was found by antioxidants, aquaporins and heat shock proteins along with upregulation of genes encoding these components along with ascorbate/glutathione pools [119].

### 6.5. $H_2S$-Signaling during Nutritional Stress

Agricultural crops are susceptible to nutritional stresses, nutrient deprivation or excessive of nutrients, therefore, plants have to tolerate such conditions when there are either excessive nutrients or there is shortage. Climatic disturbances often alter $CO_2$ but may also affect the nutritional availability by interfering micro-biotic associations among plants. Exogenously applied NaHS lowers oxidative stresses in plants raised under nitrate stress conditions [52]. The ROS was observed to decline with aggravated activities of antioxidants via mitogen-activated protein kinases and NO-signaling. Sidewise, the expression levels of *CsNMAPK* transcripts were also found to upregulate in cucumber after $H_2S$ treatment [52]. Moreover, $H_2S$ application also improved the seed germination rate of tomato during nitrate stress through improvement in the levels of antioxidants [125]. Furthermore, Kaya and Ashraf [126] depicted that Fe-deprivation caused chlorosis, which was ameliorated by NaHS. Subsequently, oxidative stress markers were also reduced with promoted plant growth and metabolism. Hence, the inter-relationship among plants, microbiotic environment, and nutrient availability mediated by $H_2S$ is an interesting art of work that should be further explored.

## 7. Challenges of Utilizing $H_2S$ in Crop Protection

It is quite challenging to understand that $H_2S$ and sulfane sulfur are two of the most reactive species that coexist, and it has been speculated that sulfane sulfur is the signaling molecule that performs biological actions rather than $H_2S$ [127]. Moreover, sulfane sulfur comprises of many reactive molecules such as polysulfides, polythionates, persulfides, elemental sulfur, etc., along with the products of cysteine metabolism such asthiocysteine, thiotaurine, etc. [128]. These compounds alter cysteine residues through S-sulfhydration to generate protein persulfides that are incorporated during the translation of proteins [129]. Henceforth, these molecules have attained much attention due to their signaling, antioxidant and regulatory actions. These compounds also act as a storage unit for $H_2S$ that further releases a gasotransmitter during biological signals. Therefore, this co-relation is of biological significance and needs to be explored. There are many studies going on to unravel this research.

$H_2S$ has been found to play a predominant role in plants as a signaling molecule but sometimes it may become disruptive for cells. This is mainly because of their generation in excess endogenously within plants or they may arrive from exterior and become accumulated within the cells. Their higher concentrations may either affect plays in positive or negative manner [130]. However, organisms possess many ways to remove $H_2S$ generated for proper functioning of cells. Therefore, due to these reasons it is quite challenging for their usage in plants for stress amelioration. Moreover, it also hinders the enzymatic activities in plants of pertinent enzymes such as cytochrome oxidase [131]. Consequently, plants have O-acetylserine thiol lyase enzyme to work against such kind of $H_2S$. In some cases, mitochondria also metabolizes $H_2S$ to further use it as electron source for electron transport chain through ubiquinone for utilizing $H_2S$ for ATP generation [132]. In addition to this, $H_2S$ is exceeded to such levels that it also impairs complex IV functioning, making mitochondria non-functional due to the lack of electron flow. Accordingly, mitochondria along with controlling $H_2S$-generation also blocks its working. Meanwhile, $H_2S$ in mitochondria is also maintaining $H_2S$ at optimum levels so that its concentration does not impinge ROS. Therefore, balancing such actions is very substantial so that these reactive molecules could work in accordance with one another. Yet no such literature explains the mitochondrial and $H_2S$ metabolism, therefore, studies are still going on to effectively understand these mechanisms.

## 8. Conclusions and Future Perspectives

The role of $H_2S$ in plants has been widely known during various abiotic stresses where their accumulation is gradually increased. Various $H_2S$ donors play predominant role in plants and studies pertaining to the same are still required to be strengthened in terms of their suitability and compatibility with no adverse effects towards plants. $H_2S$ regulates the metal uptake and transport of various mineral nutrients to mitigate different abiotic stresses such as heavy metals by inducing chelation through metallothioneins and phytochelatins. $H_2S$ accumulation also alters protein persulfidome, which is crucial in stress signaling, making this molecule a most imperative part to study. The redox changes in protein cysteine residues during initial stress responses are also co-linked to stress-mediated redox perturbation. The mechanistic role and regulatory framework of $H_2S$-regulated redox regulation and signaling is also a primitive part of this study. Apart from proteomics, the identification of substantial $H_2S$ sensors and donors is quite prevalent. Besides, the sidewise detailed investigations associated with persulfidation is the topic of interest in near future.

Since decades, the complexed role of $H_2S$ during oxidative stress has been studied. $H_2S$-associates with ROS-induced oxidative stress at various levels to form a network for regulating ROS-processing system at a translational, transcriptional, as well as post-translational level. In addition, the induced activities of antioxidant enzymes were found to be persulfidated, therefore, comprehensive molecular understanding about underlying redox mechanisms that curbs their activities are required to be explored further. In order to unravel such instances, the elucidation of an inter-relationship among persulfidation, sulfenylation, and nitrosation should be untangled with regard to their functional role in activities of redox enzymes and those which are subjected to several modifications. The persulfidation process in mitigating oxidative stress through changes in the cell sulfenylation process through ROS should also be explored further. There are various evidential studies that we have discussed in regard to ROS and $H_2S$ in autophagy, but the fine-tuned molecular understanding pertaining to same would provide us valuable knowledge and novel functions of $H_2S$ and their signaling mechanisms for regulating oxidative stresses. However, the transcripts of genes encoding antioxidant enzymes also adds to the protective role of $H_2S$ against various stresses and these factors are needed to be studied in detail. $H_2S$ also mediate stress responses in plants during stomatal movement and $H_2S$-targeted signaling molecules are also required to be identified. Uncovering the complexity of $H_2S$ interactions will enable us to gain knowledge about the intricate redox reactions in plants against stresses. The progress in gaining all the information and uncovering the related aspects of $H_2S$ with ROS and stresses in plants have been observed to be accelerated by working on model plants such as *Arabidopsis*. Therefore, improving our knowledge about such interactions is not just essential for rudimentary research but also for its implementation in crop improvement and breeding programmes in context to provide resistance against fluctuating environment.

**Author Contributions:** Conceptualization, K.K. and P.O.; validation, R.B., P.O. and K.K.; writing—original draft preparation, K.K., N.S., S.K., M.A.; writing—review and editing, K.K., N.S., S.K., M.A.; visualization, R.B., P.O., K.K.; supervision, R.B.; project administration, P.O., R.B. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Huang, D.; Huo, J.; Liao, W. Hydrogen Sulfide: Roles in Plant Abiotic Stress Response and Crosstalk with Other Signals. *Plant Sci.* **2020**, *302*, 110733. [CrossRef] [PubMed]
2. Choudhury, F.K.; Rivero, R.M.; Blumwald, E.; Mittler, R. Reactive oxygen species, abiotic stress and stress combination. *Plant J.* **2017**, *90*, 856–867. [CrossRef] [PubMed]
3. Kleine, T.; Leister, D. Retrograde signaling: Organelles go networking. *Biochim. Biophys. Acta Bioenerg.* **2016**, *1857*, 1313–1325. [CrossRef] [PubMed]
4. Gill, S.S.; Tuteja, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* **2010**, *48*, 909–930. [CrossRef] [PubMed]
5. Filipovic, M.R.; Zivanovic, J.; Alvarez, B.; Banerjee, R. Chemical biology of $H_2S$ signaling through persulfidation. *Chem. Rev.* **2018**, *118*, 1253–1337. [CrossRef]
6. Parker, E.T.; Cleaves, H.J.; Dworkin, J.P.; Glavin, D.P.; Callahan, M.; Aubrey, A.; Lazcano, A.; Bada, J.L. Primordial synthesis of amines and amino acids in a 1958 Miller H2S-rich spark discharge experiment. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 5526–5531. [CrossRef] [PubMed]
7. Lefer, D. Redox pioneer: Professor hideo kimura. *Antioxid. Redox Signal.* **2019**, *30*, 1699–1708. [CrossRef] [PubMed]
8. Li, Z.G.; Min, X.; Zhou, Z.H. Hydrogen sulfide: A signal molecule in plant cross-adaptation. *Front. Plant Sci.* **2016**, *7*, 1621. [CrossRef]
9. Wang, R.U.I. Two's company, three's a crowd: Can H2S be the third endogenous gaseous transmitter? *FASEB J.* **2002**, *16*, 1792–1798. [CrossRef] [PubMed]
10. Luo, S.; Calderon-Urrea, A.; Jihua, Y.U.; Liao, W.; Xie, J.; Lv, J.; Feng, Z.; Tang, Z. The role of hydrogen sulfide in plant alleviates heavy metal stress. *Plant Soil* **2020**, *449*, 1–10. [CrossRef]
11. Ali, S.; Farooq, M.A.; Hussain, S.; Yasmeen, T.; Abbasi, G.H.; Zhang, G. Alleviation of chromium toxicity by hydrogen sulfide in barley. *Environ. Toxicol. Chem.* **2013**, *32*, 2234–2239. [CrossRef]
12. Mostofa, M.G.; Rahman, A.; Ansary, M.M.U.; Watanabe, A.; Fujita, M.; Tran, L.S.P. Hydrogen sulfide modulates cadmium-induced physiological and biochemical responses toalleviate cadmium toxicity in rice. *Sci. Rep.* **2015**, *5*, 14078. [CrossRef]
13. Jin, Z.; Shen, J.; Qiao, Z.; Yang, G.; Wang, R.; Pei, Y. Hydrogen sulfide improves drought resistance in *Arabidopsis thaliana*. *Biochem. Biophys. Res. Commun.* **2011**, *414*, 481–486. [CrossRef]
14. Li, Z.G.; Yang, S.Z.; Long, W.B.; Yang, G.X.; Shen, Z.Z. Hydrogen sulphide may be a novel downstream signal molecule in nitric oxide-induced heat tolerance of maize (*Zea mays* L.) seedlings. *Plant Cell Environ.* **2013**, *36*, 1564–1572. [CrossRef] [PubMed]
15. Corpas, F.J. Hydrogen sulfide: A new warrior against abiotic stress. *Trends Plant Sci.* **2019**, *11*, 983–988. [CrossRef]
16. Mathai, J.C.; Missner, A.; Kügler, P.; Saparov, S.M.; Zeidel, M.L.; Lee, J.K.; Pohl, P. No facilitator required for membrane transport of hydrogen sulfide. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 16633–16638. [CrossRef]
17. Zhao, Y.; Biggs, T.D.; Xian, M. Hydrogen sulfide ($H_2S$) releasing agents: Chemistry and biological applications. *Chem. Commun.* **2014**, *50*, 11788–11805. [CrossRef]

18. Mondal, U.; Sen, S.; Singh, G. Advances in hydrogen sulphideutilisation: Phase transfer catalysed selective reduction of nitronaphthalene. *RSC Adv.* **2015**, *5*, 102942–102952. [CrossRef]
19. Pla-Tolos, J.; Moliner-Martinez, Y.; Verdu-Andres, J.; Casanova-Chafer, J.; Molins-Legua, C.; Campins-Falco, P. New optical paper sensor for in situ measurement of hydrogen sulphide in waters and atmospheres. *Talanta* **2016**, *156*, 79–86. [CrossRef] [PubMed]
20. Tan, B.; Jin, S.; Sun, J.; Gu, Z.; Sun, X.; Zhu, Y.; Huo, K.; Cao, Z.; Yang, P.; Xin, X.; et al. New method for quantification of gasotransmitter hydrogen sulfide in biological matrices by LC-MS/MS. *Sci. Rep.* **2017**, *7*, 46278. [CrossRef] [PubMed]
21. Chen, T.; Tian, M.; Han, Y. Hydrogen sulfide: A multi-tasking signal molecule in the regulation of oxidative stress responses. *J. Exp. Bot.* **2020**, *71*, 2862–2869. [CrossRef] [PubMed]
22. Yamasaki, H.; Cohen, M.F. Biological consilience of hydrogen sulfide and nitric oxide in plants: Gases of primordial earth linking plant, microbial and animal physiologies. *Nitric Oxide* **2016**, *55-56*, 91–100. [CrossRef] [PubMed]
23. Banerjee, A.; Tripathi, D.A.; Roychoudhury, A. Hydrogen sulphide trapeze: Environmental stress amelioration and phytohormone crosstalk. *Plant Physiol. Bioch.* **2018**, *132*, 46–53. [CrossRef]
24. Cuevasanta, E.; Denicola, A.; Alvarez, B.; Moller, M.N. Solubility and permeation of hydrogen sulfide in lipid membranes. *PLoS ONE* **2012**, *7*, e34562. [CrossRef]
25. Riahi, S.; Rowley, C.N. Why can hydrogen sulfide permeate cell membranes? *J. Am. Chem. Soc.* **2014**, *136*, 15111–15113. [CrossRef]
26. Li, Z.G. Hydrogen sulfide: A multifunctional gaseous molecule in plants. *Russ. J. Plant Physiol.* **2013**, *60*, 733–740. [CrossRef]
27. Sun, J.; Wang, R.; Zhang, X.; Yu, Y.; Zhao, R.; Li, Z.; Chen, S. Hydrogen sulfidealleviatescadmium toxicity through regulations of cadmium transport across the plasma and vacuolarmembranes in *Populuseuphratica* cells. *Plant Physiol. Biochem.* **2013**, *65*, 67–74. [CrossRef]
28. Jin, Z.; Xue, S.; Luo, Y.; Tian, B.; Fang, H.; Li, H.; Pei, Y. Hydrogen sulfide interacting with abscisic acid in stomatal regulation responses to drought stress in Arabidopsis. *Plant Physiol. Biochem.* **2013**, *62*, 41–46. [CrossRef] [PubMed]
29. Wilson, L.G.; Bressan, R.A.; Filner, P. Light-dependent emission of hydrogen sulfide from plants. *Plant Physiol.* **1978**, *61*, 184–189. [CrossRef]
30. Zhang, J.; Zhou, M.; Zhou, H.; Zhao, D.; Gotor, C.; Romero, L.C.; Shen, J.; Ge, Z.; Zhang, Z.; Shen, W.; et al. Hydrogen sulfide, a signaling molecule in plant stress responses. *J. Integr. Plant Biol.* **2021**, *63*, 146–160.
31. Sekiya, J.; Wilson, L.G.; Filner, P. Resistance to injury by sulfur dioxide: Correlation with its reduction to, and emission of, hydrogen sulfide in Cucurbitaceae. *Plant Physiol.* **1982**, *70*, 437–441. [CrossRef]
32. Calderwood, A.; Kopriva, S. Hydrogen sulfide in plants: From dissipation of excess sulfur to signaling molecule. *Nitric Oxide* **2014**, *41*, 72–78. [CrossRef]
33. Corpas, F.J.; Gonzalez-Gordo, S.; Canas, A.; Palma, J.M. Nitric oxide and hydrogen sulfide in plants: Which comes first? *J. Exp. Bot.* **2019**, *70*, 4391–4404. [CrossRef] [PubMed]
34. Kabil, O.; Banerjee, R. Redox biochemistry of hydrogen sulfide. *J. Biol. Chem.* **2010**, *285*, 21903–21907. [CrossRef] [PubMed]
35. Hohner, R.; Aboukila, A.; Kunz, H.H.; Venema, K. Proton gradients and proton-dependent transport processes in the chloroplast. *Front. Plant Sci.* **2016**, *7*, 218. [CrossRef] [PubMed]
36. Gotor, C.; Garcia, I.; Aroca, A.; Laureano-Marin, A.M.; Arenas-Alfonseca, L.; Jurado-Flores, A.; Moreno, I.; Romero, L.C. Signaling by hydrogen sulfide and cyanide through post-translational modification. *J. Exp. Bot.* **2019**, *70*, 4251–4265. [CrossRef] [PubMed]
37. Álvarez, C.; García, I.; Moreno, I.; Pérez-Pérez, M.E.; Crespo, J.L.; Romero, L.C.; Gotor, C. Cysteine-generated sulfide in the cytosol negatively regulates autophagy and modulates the transcriptional profile in *Arabidopsis*. *Plant Cell* **2012**, *24*, 4621–4634. [CrossRef]
38. Corpas, F.J.; Barroso, J.B.; Gonzalez-Gordo, S.; Munoz-Vargas, M.A.; Palma, J.M. Hydrogen sulfide: A novel component in *Arabidopsis* peroxisomes which triggers catalase inhibition. *J. Integr. Plant Biol.* **2019**, *61*, 871–883. [CrossRef]
39. Fang, H.; Liu, Z.; Long, Y.; Liang, Y.; Jin, Z.; Zhang, L.; Liu, D.; Li, H.; Zhai, J.; Pei, Y. The $Ca^{2+}$/calmodulin2-binding transcription factor TGA3 elevates LCD expression and $H_2S$ production to bolster $Cr^{6+}$ tolerance in *Arabidopsis*. *Plant J.* **2017**, *91*, 1038–1050. [CrossRef]
40. Lai, D.W.; Mao, Y.; Zhou, H.; Li, F.; Wu, M.Z.; Zhang, J.; He, Z.Y.; Cui, W.T.; Xie, Y.J. Endogenous hydrogen sulfide enhances salt tolerance by coupling the reestablishment of redox homeostasis and preventing salt-induced $K^+$ loss in seedlings of *Medicago sativa*. *Plant Sci.* **2014**, *225*, 117–129. [CrossRef] [PubMed]
41. Guo, H.; Zhou, H.; Zhang, J.; Guan, W.; Xu, S.; Shen, W.; Xu, G.; Xie, Y.; Foyer, C.H. L-Cysteine desulfhydrase-related $H_2S$ production is involved in OsSE5-promoted ammonium tolerance in roots of *Oryza sativa*. *Plant Cell Environ.* **2017**, *40*, 1777–1790. [CrossRef]
42. Kabala, K.; Zboinska, M.; Glowiak, D.; Reda, M.; Jakubowska, D.; Janicka, M. Interaction between the signaling molecules hydrogen sulfide and hydrogen peroxide and their role in vacuolar $H^+$-ATPase regulation in cadmium-stressed cucumber roots. *Physiol. Plant.* **2019**, *166*, 688–704. [CrossRef] [PubMed]
43. Scuffi, D.; Alvarez, C.; Laspina, N.; Gotor, C.; Lamattina, L.; Garcia-Mata, C. Hydrogen sulfide generated by L-cysteine desulfhydrase acts upstream of nitric oxide to modulate abscisic acid-dependent stomatal closure. *Plant Physiol.* **2014**, *166*, 2065–2076. [CrossRef]
44. Jia, H.; Chen, S.; Liu, D.; Liesche, J.; Shi, C.; Wang, J.; Ren, M.; Wang, X.; Yang, J.; Shi, W.; et al. Ethylene-induced hydrogen sulfide negatively regulates ethylene biosynthesis by persulfidation of ACO in tomato under osmotic stress. *Front. Plant Sci.* **2018**, *9*, 1517. [CrossRef] [PubMed]

45. Pantaleno, R.; Scuffi, D.; Garcia-Mata, C. Hydrogen sulphide as a guard cell network regulator. *New Phytol.* **2020**, *230*, 451–456. [CrossRef]
46. Du, X.; Jin, Z.; Liu, Z.; Liu, D.; Zhang, L.; Ma, X.; Yang, G.; Liu, S.; Guo, Y.; Pei, Y. $H_2S$ Persulfidated and Increased Kinase Activity of MPK4 to Response Cold Stress in *Arabidopsis*. *Front. Mol. Biosci.* **2021**, *8*, 81. [CrossRef] [PubMed]
47. Liu, Q.; Zhou, Y.; Li, H.; Liu, R.; Wang, W.; Wu, W.; Yang, N.; Wang, S. Osmotic stress-triggered stomatal closure requires Phospholipase Dδ and hydrogen sulfide in *Arabidopsis thaliana*. *Biochem. Biophys. Res. Commun.* **2021**, *534*, 914–920. [CrossRef] [PubMed]
48. Liu, Y.L.; Shen, Z.J.; Simon, M.; Li, H.; Ma, D.N.; Zhu, X.Y.; Zheng, H.L. Comparative proteomic analysis reveals the regulatory effects of H2S on salt tolerance of mangrove plant *Kandeliaobovata*. *Int. J. Mol. Sci.* **2020**, *21*, 118. [CrossRef]
49. Li, H.; Shi, J.; Wang, Z.; Zhang, W.; Yang, H. $H_2S$ pretreatment mitigates the alkaline salt stress on *Malus hupehensis* roots by regulating $Na^+/K^+$ homeostasis and oxidative stress. *Plant Physiol. Biochem.* **2020**, *156*, 233–241. [CrossRef] [PubMed]
50. Khan, M.N.; Siddiqui, M.H.; AlSolami, M.A.; Alamri, S.; Hu, Y.; Ali, H.M.; Al-Amri, A.A.; Alsubaie, Q.D.; Al-Munqedhi, B.M.; Al-Ghamdi, A. Crosstalk of hydrogen sulfide and nitric oxide requires calcium to mitigate impaired photosynthesis under cadmium stress by activating defense mechanisms in *Vigna radiata*. *Plant Physiol. Biochem.* **2020**, *156*, 78–290. [CrossRef]
51. Jiang, J.; Ren, X.; Li, L.; Hou, R.; Sun, W.; Jiao, C.; Yang, N.; Dong, Y. $H_2S$ Regulation of Metabolism in Cucumber in Response to Salt-Stress through Transcriptome and Proteome Analysis. *Front. Plant Sci.* **2020**, *11*, 1283. [CrossRef]
52. Liu, Z.; Li, Y.; Cao, C.; Liang, S.; Ma, Y.; Liu, X.; Pei, Y. The role of $H_2S$ in low temperature-induced cucurbitacin C increases in cucumber. *Plant Mol. Biol.* **2019**, *99*, 535–544. [CrossRef] [PubMed]
53. Zhou, Z.H.; Wang, Y.; Ye, X.Y.; Li, Z.G. Signaling molecule hydrogen sulfide improves seed germination and seedling growth of maize (*Zea mays* L.) under high temperature by inducing antioxidant system and osmolyte biosynthesis. *Front. Plant Sci.* **2018**, *9*, 1288. [CrossRef] [PubMed]
54. Zhu, C.Q.; Zhang, J.H.; Sun, L.M.; Zhu, L.F.; Abliz, B.; Hu, W.J.; Zhong, C.; Bai, Z.G.; Sajid, H.; Cao, X.C.; et al. Hydrogen sulfide alleviates aluminum toxicity via decreasingapoplast and symplast Al contents in rice. *Front. Plant Sci.* **2018**, *9*, 294. [CrossRef]
55. Kharbech, O.; Houmani, H.; Chaoui, A.; Corpas, F.J. Alleviation of Cr (VI)-induced oxidative stress in maize (*Zea mays* L.) seedlings by NO and $H_2S$ donors through differential organ-dependent regulation of ROS and NADPH-recycling metabolisms. *J. Plant Physiol.* **2017**, *219*, 71–80. [CrossRef] [PubMed]
56. Ma, D.; Ding, H.; Wang, C.; Qin, H.; Han, Q.; Hou, J.; Lu, H.; Xie, Y.; Guo, T. Alleviation of drought stress by hydrogen sulfide is partially related to the abscisic acid signaling pathway in wheat. *PLoS ONE* **2016**, *11*, 0163082. [CrossRef]
57. Mostofa, M.G.; Saegusa, D.; Fujita, M.; Tran, L.S.P. Hydrogen sulfide regulates salt tolerance in rice by maintaining $Na^+/K^+$ balance, mineral homeostasis and oxidative metabolism under excessive salt stress. *Front. Plant Sci.* **2015**, *6*, 1055. [CrossRef]
58. Ren, X.; Zou, L.; Zhang, X.; Branco, V.; Wang, J.; Carvalho, C.; Holmgren, A.; Lu, J. Redox signaling mediated by thioredoxin and glutathione systems in the central nervous system. *Antioxidants Redox Signal.* **2017**, *27*, 989–1010. [CrossRef]
59. Paulsen, C.E.; Carroll, K.S. Cysteine-mediated redox signaling: Chemistry, biology, and tools for discovery. *Chem. Rev.* **2013**, *113*, 4633–4679. [CrossRef]
60. Calabrese, G.; Peker, E.; Amponsah, P.S.; Hoehne, M.N.; Riemer, T.; Mai, M.; Bienert, G.P.; Deponte, M.; Morgan, B.; Riemer, J. Hyperoxidation of mitochondrial peroxiredoxin limits $H_2O_2$-induced cell death in yeast. *EMBO J.* **2019**, *38*, e101552. [CrossRef]
61. Huang, J.; Willems, P.; Wei, B.; Tian, C.; Ferreira, R.B.; Bodra, N.; Gache, S.A.M.; Wahni, K.; Liu, K.; Vertommen, D.; et al. Mining for protein S-sulfenylation in *Arabidopsis* uncovers redox-sensitive sites. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 21256–21261. [CrossRef]
62. Rizwan, M.; Mostofa, M.G.; Ahmad, M.Z.; Zhou, Y.; Adeel, M.; Mehmood, S.; Ahmad, M.A.; Javed, R.; Imtiaz, M.; Aziz, O.; et al. Hydrogen sulfide enhances rice tolerance to nickel through the prevention of chloroplast damage and the improvement of nitrogen metabolism under excessive nickel. *Plant Physiol. Biochem.* **2019**, *138*, 100–111. [CrossRef] [PubMed]
63. Pérez-Pérez, M.E.; Lemaire, S.D.; Crespo, J.L. Reactive oxygen species and autophagy in plants and algae. *Plant Physiol.* **2012**, *160*, 156–164. [CrossRef]
64. Shibata, M.; Oikawa, K.; Yoshimoto, K.; Kondo, M.; Mano, S.; Yamada, K.; Hayashi, M.; Sakamoto, W.; Ohsumi, Y.; Nishimura, M. Highly oxidized peroxisomes are selectively degraded via autophagy in *Arabidopsis*. *Plant Cell* **2013**, *25*, 4967–4983. [CrossRef] [PubMed]
65. Alvarez, C.; Garcia, I.; Romero, L.C.; Gotor, C. Mitochondrial sulfide detoxification requires a functional isoform O-acetylserine(thiol)lyase C in *Arabidopsis thaliana*. *Mol. Plant* **2012**, *5*, 1217–1226. [CrossRef] [PubMed]
66. Wedmann, R.; Onderka, C.; Wei, S.; Szijártó, I.A.; Miljkovic, J.L.; Mitrovic, A.; Lange, M.; Savitsky, S.; Yadav, P.K.; Torregrossa, R.; et al. Improved tag-switch method reveals that thioredoxin acts as depersulfidase and controls the intracellular levels of protein persulfidation. *Chem. Sci.* **2016**, *7*, 3414–3426. [CrossRef]
67. Zhang, J.; Zhou, M.; Ge, Z.; Shen, J.; Zhou, C.; Gotor, C.; Romero, L.C.; Duan, X.; Liu, X.; Wu, D.; et al. Abscisic acid-triggered guard cell l-cysteine desulfhydrase function and in situ hydrogen sulfide production contributes to heme oxygenase-modulated stomatal closure. *Plant Cell Environ.* **2020**, *43*, 624–636. [CrossRef] [PubMed]
68. Du, X.; Jin, Z.; Zhang, L.; Liu, X.; Yang, G.; Pei, Y. $H_2S$ is involved in ABA-mediated stomatal movement through MPK4 to alleviate drought stress in *Arabidopsis thaliana*. *Plant Soil* **2019**, *435*, 295–307. [CrossRef]
69. Zhang, T.Y.; Li, F.C.; Fan, C.M.; Li, X.; Zhang, F.F.; He, J.M. Role and interrelationship of MEK1-MPK6 cascade, hydrogen peroxide and nitric oxide in darkness-induced stomatal closure. *Plant Sci.* **2017**, *262*, 190–199. [CrossRef]

70. Li, Z.; Zhu, Y.; He, X.; Yong, B.; Peng, Y.; Zhang, X.; Ma, X.; Yan, Y.; Huang, L.; Nie, G. The hydrogen sulfide, a downstream signaling molecule of hydrogen peroxide and nitric oxide, involves spermidine-regulated transcription factors and antioxidant defense in white clover in response to dehydration. *Environ. Exp. Bot.* **2019**, *161*, 255–264. [CrossRef]
71. Mei, Y.; Chen, H.; Shen, W.; Shen, W.; Huang, L. Hydrogen peroxide is involved in hydrogen sulfide-induced lateral root formation in tomato seedlings. *BMC Plant Biol.* **2017**, *17*, 162. [CrossRef]
72. Zhang, L.; Pei, Y.; Wang, H.; Jin, Z.; Liu, Z.; Qiao, Z.; Fang, H.; Zhang, Y. Hydrogen sulfide alleviates cadmium-induced cell death through restraining ROS accumulation in roots of *Brassica rapa* L. ssp. pekinensis. *Oxidative Med. Cell. Longev.* **2015**, *2015*, 804603. [CrossRef] [PubMed]
73. Chen, J.; Wang, W.H.; Wu, F.H.; You, C.Y.; Liu, T.W.; Dong, X.J.; He, J.X.; Zheng, H.L. Hydrogen sulfide alleviates aluminum toxicity in barley seedlings. *Plant Soil* **2013**, *362*, 301–318. [CrossRef]
74. Shan, C.; Liu, H.; Zhao, L.; Wang, X. Effects of exogenous hydrogen sulfide on the redox states of ascorbate and glutathione in maize leaves under salt stress. *Biol. Plant* **2014**, *58*, 169–173. [CrossRef]
75. Chen, Z.; Chen, M.; Jiang, M. Hydrogen sulfide alleviates mercury toxicity by sequestering itin roots or regulating reactive oxygen species productions in rice seedlings. *Plant Physiol. Biochem.* **2017**, *111*, 179–192. [CrossRef] [PubMed]
76. He, H.; Li, Y.; He, L.F. The central role of hydrogen sulfide in plant responses to toxic metal stress. *Ecotoxicol. Environ. Saf.* **2018**, *157*, 403–408. [CrossRef]
77. Sinclair, S.A.; Krämer, U. The zinc homeostasis network of land plants. *Biochim. Biophys. Acta Mol. Cell Res.* **2012**, *1823*, 1553–1567. [CrossRef]
78. Sun, H.; Wang, D.; Zhou, Z.; Ding, Z.; Chen, X.; Xu, Y.; Huang, L.; Tang, D. Association of cadmium in urine and blood with age in a general population with low environmental exposure. *Chemosphere* **2016**, *156*, 392–397. [CrossRef]
79. Yang, J.L.; Zhu, X.F.; Peng, Y.X.; Zheng, C.; Li, G.X.; Liu, Y.; Shi, Y.Z.; Zheng, S.J. Cell wall hemicellulose contributes significantly to aluminum adsorption and root growth in *Arabidopsis*. *Plant Physiol.* **2011**, *155*, 1885–1892. [CrossRef]
80. Huang, C.F.; Yamaji, N.; Mitani, N.; Yano, M.; Nagamura, Y.; Ma, J.F. A bacterial-type ABC transporter is involved in aluminum tolerance in rice. *Plant Cell* **2009**, *21*, 655–667. [CrossRef]
81. Ishikawa, S.; Wagatsuma, T.; Sasaki, R.; Ofei-Manu, P. Comparison of the amount of citric and malic acids in Al media of seven plant species and two cultivars each in five plantspecies. *Soil Sci. Plant Nutr.* **2000**, *46*, 751–758. [CrossRef]
82. Yokosho, K.; Yamaji, N.; Fujii-Kashino, M.; Ma, J.F. Retrotransposon-mediated aluminum tolerance through enhanced expression of the citrate transporter OsFRDL4. *Plant Physiol.* **2016**, *172*, 2327–2336. [CrossRef]
83. Gupta, B.; Huang, B. Mechanism of salinity tolerance in plants: Physiological, biochemical, and molecular characterization. *Int. J. Genom.* **2014**, *2014*, 701596. [CrossRef]
84. Levine, A.; Tenhaken, R.; Dixon, R.; Lamb, C. $H_2O_2$ from the oxidative burst orchestrates the plant hypersensitive disease resistance response. *Cell* **1994**, *79*, 583–593. [CrossRef]
85. Li, J.; Jia, H.; Wang, J.; Cao, Q.; Wen, Z. Hydrogen sulfide is involved in maintaining ion homeostasis via regulating plasma membrane $Na^+/H^+$ antiporter system in the hydrogen peroxide-dependent manner in salt-stress *Arabidopsis thaliana* root. *Protoplasma* **2014**, *25*, 899–912. [CrossRef]
86. Flowers, T.J.; Troke, P.F.; Yeo, A.R. The mechanism of salt tolerance in halophytes. *Annu. Rev. Plant Physiol.* **1977**, *28*, 89–121. [CrossRef]
87. Jian, W.; Zhang, D.W.; Zhu, F.; Wang, S.X.; Pu, X.J.; Deng, X.G.; Luo, S.S.; Lin, H.H. Alternative oxidase pathway is involved in the exogenous SNP-elevated tolerance of *Medicago truncatula* to salt stress. *J. Plant Physiol.* **2016**, *193*, 79–87. [CrossRef]
88. Amooaghaie, R.; Enteshari, S. Role of two-sided crosstalk between NO and $H_2S$ onimprovement of mineral homeostasis and antioxidative defense in *Sesamum indicum* under lead stress. *Ecotoxicol. Environ. Saf.* **2017**, *139*, 210–218. [CrossRef] [PubMed]
89. Filipovic, M.R. Persulfidation (S-sulfhydration) and $H_2S$. *Handb. Exp. Pharmacol.* **2015**, *230*, 29–59. [PubMed]
90. Krishnan, N.; Fu, C.; Pappin, D.J.; Tonks, N.K. $H_2S$—Induced sulfhydration of the phosphatase PTP1B and its role in the endoplasmic reticulum stress response. *Sci. Sign.* **2011**, *4*, ra86. [CrossRef] [PubMed]
91. Paul, B.D.; Snyder, S.H. $H_2S$: A novel gasotransmitter that signals by sulfhydration. *Trends Biochem. Sci.* **2015**, *40*, 687–700. [CrossRef]
92. Vandiver, M.; Snyder, S. Hydrogen sulfide: A gasotransmitter of clinical relevance. *J. Mol. Med.* **2012**, *90*, 255–263. [CrossRef] [PubMed]
93. Garcia-Mata, C.; Lamattina, L. Hydrogen sulphide, a novel gasotransmitter involved in guard cell signalling. *New Phytol.* **2010**, *188*, 977–984. [CrossRef] [PubMed]
94. Kimura, H. The physiological role of hydrogen sulfide and beyond. *Nitric Oxide* **2014**, *41*, 4–10. [CrossRef]
95. Mustafa, A.K.; Gadalla, M.M.; Sen, N.; Kim, S.; Mu, W.; Gazi, S.K.; Barrow, R.K.; Yang, G.; Wang, R.; Synder, S.H. $H_2S$ signals through protein S-sulfhydration. *Sci. Signal.* **2009**, *2*, ra72. [CrossRef] [PubMed]
96. Aroca, A.; Serna, A.; Gotor, C.; Romero, L.C. S-sulfhydration: A cysteine posttranslational modification in plant systems. *Plant Physiol.* **2015**, *168*, 334–342. [CrossRef]
97. Aroca, A.; Gotor, C.; Romero, L.C. Hydrogen sulfidesignaling in plants: Emerging roles of protein persulfidation. *Front. Plant Sci.* **2018**, *9*, 1369. [CrossRef] [PubMed]

98. Shen, J.; Zhang, J.; Zhou, M.; Zhou, H.; Cui, B.; Gotor, C.; Romero, L.C.; Fu, L.; Yang, J.; Foyer, C.H.; et al. Persulfidation-based modification of cysteine desulfhydrase and the NADPH Oxidase RBOHD controls guard cell abscisic acid signaling. *Plant Cell* **2020**, *32*, 1000–1017. [CrossRef]
99. Feng, J.; Chen, L.; Zuo, J. Protein S-nitrosylation in plants: Current progresses and challenges. *J. Integr. Plant Biol.* **2019**, *61*, 1206–1223. [CrossRef] [PubMed]
100. Stamler, J.S.; Simon, D.I.; Jaraki, O.; Osborne, J.A.; Francis, S.; Mullins, M.; Singel, D.; Loscalzo, J. S-nitrosylation of tissue-type plasminogen activator confers vasodilatory and antiplatelet properties on the enzyme. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 8087–8091. [CrossRef]
101. Hess, D.T.; Matsumoto, A.; Kim, S.O.; Marshall, H.E.; Stamler, J.S. Protein S-nitrosylation: Purview and parameters. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 150–166. [CrossRef] [PubMed]
102. Zhao, D.D.; Zhang, J.; Zhou, M.J.; Zhou, H.; Gotor, C.; Romero, L.C.; Shen, J.; Yuan, X.X.; Xie, Y.J. Current approaches for detection of hydrogen sulfide and persulfidation in biological systems. *Plant Physiol. Biochem.* **2020**, *155*, 367–373. [CrossRef]
103. Aroca, A.; Benito, J.M.; Gotor, C.; Romero, L.C. Persulfidation proteome reveals the regulation of protein function by hydrogen sulfide in diverse biological processes in *Arabidopsis*. *J. Exp. Bot.* **2017**, *68*, 4915–4927. [CrossRef] [PubMed]
104. Li, J.; Chen, S.; Wang, X.; Shi, C.; Liu, H.; Yang, J.; Shi, W.; Guo, J.; Jia, H. Hydrogen sulfide disturbs actin polymerization via S-sulfhydration resulting in stunted root hair growth. *Plant Physiol.* **2018**, *178*, 936–949. [CrossRef]
105. Mcdowell, J.M.; Huang, S.; Mckinney, E.C.; An, Y.; Meagher, R.B. Structure and evolution of the actin gene family in *Arabidopsis thaliana*. *Genetics* **1996**, *142*, 587–602. [CrossRef]
106. Gong, Z.; Xiong, L.; Shi, H.; Yang, S.; Herrera-Estrella, L.R.; Xu, G.; Chao, D.Y.; Li, J.; Wang, P.Y.; Qin, F.; et al. Plant abiotic stress response and nutrient use efficiency. *Sci. China Life Sci.* **2020**, *63*, 635–674. [CrossRef] [PubMed]
107. Chen, L.; Wu, R.; Feng, J.; Feng, T.; Wang, C.; Hu, J.; Zhan, N.; Li, Y.; Ma, X.; Ren, B.; et al. Transnitrosylation mediated by the non-canonical catalase ROG1 regulates nitric oxide signaling in plants. *Dev. Cell* **2020**, *53*, 444–457. [CrossRef]
108. Ali, B.; Gill, R.A.; Yang, S.; Gill, M.B.; Ali, S.; Rafiq, M.T.; Zhou, W. Hydrogen sulfide alleviates cadmium-induced morpho-physiological and ultrastructural changes in *Brassica napus*. *Ecotoxicol. Environ. Saf.* **2014**, *110*, 197–207. [CrossRef] [PubMed]
109. Yu, Y.; Zhou, X.; Zhu, Z.; Zhou, K. Sodium hydrosulfide mitigates cadmium toxicity by promoting cadmium retention and inhibiting its translocation from roots to shoots in *Brassica napus*. *J. Agric. Food Chem.* **2018**, *67*, 433–440. [CrossRef] [PubMed]
110. Wang, H.; Ji, F.; Zhang, Y.; Hou, J.; Liu, W.; Huang, J.; Liang, W. Interactions between hydrogen sulphide and nitric oxide regulate two soybean citrate transporters during the alleviation of aluminium toxicity. *Plant Cell Environ.* **2019**, *42*, 2340–2356. [CrossRef]
111. Valivand, M.; Amooaghaie, R.; Ahadi, A. Interplay between hydrogen sulfide and calcium/calmodulin enhances systemic acquired acclimation and antioxidative defense against nickel toxicity in zucchini. *Environ. Exp. Bot.* **2019**, *158*, 40–50. [CrossRef]
112. Shi, H.; Ye, T.; Chan, Z. Nitric oxide-activated hydrogen sulfide is essential for cadmium stress response in bermudagrass (*Cynodondactylon* (L). Pers.). *Plant Physiol. Biochem.* **2014**, *74*, 99–107. [CrossRef]
113. Shivaraj, S.M.; Vats, S.; Bhat, J.A.; Dhakte, P.; Goyal, V.; Khatri, P.; Kumawat, S.; Singh, A.; Prasad, M.; Sonah, H.; et al. Nitric oxide and hydrogen sulfide crosstalk during heavy metal stress in plants. *Physiol. Plant.* **2020**, *168*, 437–455. [CrossRef]
114. Kaya, C.; Ashraf, M.; Alyemeni, M.N.; Ahmad, P. Responses of nitric oxide and hydrogen sulfide in regulating oxidative defence system in wheat plants grown under cadmium stress. *Physiol. Plant.* **2020**, *168*, 345–360. [CrossRef]
115. Liu, Z.; Fang, H.; Pei, Y.; Jin, Z.; Zhang, L.; Liu, D. WRKY transcription factors down-regulate the expression of $H_2$ S-generating genes, LCD and DES in *Arabidopsis thaliana*. *Sci. Bull.* **2015**, *60*, 995–1001. [CrossRef]
116. Jiang, J.L.; Tian, Y.; Li, L.; Yu, M.; Hou, R.P.; Ren, X.M. H2S alleviates salinity stress in cucumber by maintaining the $Na^+/K^+$ balance and regulating $H_2S$ metabolism and oxidative stress response. *Front. Plant Sci.* **2019**, *10*, 678. [CrossRef] [PubMed]
117. Kaya, C.; Higgs, D.; Ashraf, M.; Alyemeni, M.N.; Ahmad, P. Integrative roles of nitric oxide and hydrogen sulfide in melatonin-induced tolerance of pepper (*Capsicum annuum* L.) plants to iron deficiency and salt stress alone or in combination. *Physiol. Plant.* **2020**, *168*, 256–277. [CrossRef] [PubMed]
118. Zulfiqar, F.; Hancock, J.T. Hydrogen sulfide in horticulture: Emerging roles in the era of climate change. *Plant Physiol. Biochem.* **2020**, *155*, 667–675. [CrossRef]
119. Christou, A.; Manganaris, G.A.; Papadopoulos, I.; Fotopoulos, V. Hydrogen sulfide induces systemic tolerance to salinity and non-ionic osmotic stress in strawberry plants through modification of reactive species biosynthesis and transcriptional regulation of multiple defence pathways. *J. Exp. Bot.* **2013**, *64*, 1953–1966. [CrossRef]
120. Kopta, T.; Sekara, A.; Pokluda, R.; Ferby, V.; Caruso, G. Screening of chilli pepper genotypes as a source of capsaicinoids and antioxidants under conditions of simulated drought stress. *Plants* **2020**, *9*, 364. [CrossRef]
121. Honda, K.; Yamada, N.; Yoshida, R.; Ihara, H.; Sawa, T.; Akaike, T.; Iwai, S. 8-Mercapto-cyclic GMP mediates hydrogen sulfide-induced stomatal closure in Arabidopsis. *Plant Cell Physiol.* **2015**, *56*, 1481–1489. [CrossRef]
122. Chen, J.; Shang, Y.T.; Wang, W.H.; Chen, X.Y.; He, E.M.; Zheng, H.L.; Shangguan, Z. Hydrogen sulfide-mediated polyamines and sugar changes are involved in hydrogen sulfide-induced drought tolerance in Spinacia oleracea seedlings. *Front. Plant Sci.* **2016**, *7*, 1173. [CrossRef] [PubMed]
123. Tang, X.; An, B.; Cao, D.; Xu, R.; Wang, S.; Zhang, Z.; Liu, X.; Sun, X. Improving photosynthetic capacity, alleviating photosynthetic inhibition and oxidative stress under low temperature stress with exogenous hydrogen sulfide in blueberry seedlings. *Front. Plant Sci.* **2020**, *11*, 108. [CrossRef] [PubMed]

124. Zhang, W.; Cao, J.; Fan, X.; Jiang, W. Applications of nitric oxide and melatonin in improving postharvest fruit quality and the separate and crosstalk biochemical mechanisms. *Trends Food Sci. Technol.* **2020**, *99*, 531–541. [CrossRef]
125. Li, S.; Yan, J.P.; Yang, E.; Bai, X.G.; Long, J.; Li, K.Z.; Xu, H.N. Effects of exogenous H2S on the germination of tomato seeds under nitrate stress. *J. Hortic. Sci. Biotechnol.* **2015**, *90*, 39–46. [CrossRef]
126. Kaya, C.; Ashraf, M. The mechanism of hydrogen sulfide mitigation of iron deficiency-induced chlorosis in strawberry (Fragaria × ananassa) plants. *Protoplasma* **2019**, *256*, 371–382. [CrossRef] [PubMed]
127. Toohey, J.I. Sulphane sulphur in biological systems: A possible regulatory role. *Biochem. J.* **1989**, *264*, 625. [CrossRef]
128. Iciek, M.; Bilska-Wilkosz, A.; Górny, M. Sulfanesulfur–new findings on an old topic. *Acta Biochim. Pol.* **2019**, *66*, 533–544.
129. Kabil, O.; Motl, N.; Banerjee, R. $H_2S$ and its role in redox signaling. *BBA Proteins Proteom.* **2014**, *1844*, 1355–1366. [CrossRef] [PubMed]
130. Bouillaud, F.; Ransy, C.; Andriamihaja, M.; Blachier, F. PL11 Sulfide and mitochondrial bioenergetics. *Nitric Oxide* **2013**, *31*, S15–S16. [CrossRef]
131. Dorman, D.C.; Moulin, F.J.M.; McManus, B.E.; Mahle, K.C.; James, R.A.; Struve, M.F. Cytochrome oxidase inhibition induced by acute hydrogen sulfide inhalation: Correlation with tissue sulfide concentrations in the rat brain, liver, lung, and nasal epithelium. *Toxicol. Sci.* **2002**, *65*, 18–25. [CrossRef] [PubMed]
132. Qabazard, B.; Li, L.; Gruber, J.; Peh, M.T.; Ng, L.F.; Kumar, S.D.; Rose, P.; Tan, C.H.; Dymock, B.W.; Wei, F.; et al. Hydrogen sulfide is an endogenous regulator of aging in *Caenorhabditis elegans*. *Antioxid. Redox Signal.* **2014**, *20*, 2621–2630. [CrossRef] [PubMed]