H2S-Mediated Mechanism

Subjects: Plant Sciences

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Hydrogen sulfide (H2S) is predominantly considered as a gaseous transmitter or signaling molecule in plants.

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1. Introduction

Hydrogen sulfide 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. H2S 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 H2S-Cys-cycle during abiotic stressed conditions followed by post-translational modifications of cysteine residues. Besides their role during abiotic stresses, crosstalk of H2S with other biomolecules such as NO and phytohormones (abscisic acid, salicylic acid, melatonin, ethylene, etc.) have also been explored in plant signaling.

2. 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 [1]. The persulfidation mechanism of various proteins have been well documented in case of mammals [2][3]. Hydrogen sulfide mediated persulfidation of proteins in case of plants for the proper functioning of biological processes has been discussed as follows.

2.1. 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. [4][5][6]. 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 [7][8]. 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 [3].

2.1.1. 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 [9][10]. 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 [1]. 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 [1] 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 1**) [11][12]. This reversion reaction of protein persulfidation evades the chances of irreversible oxidative damage that normally occurs at the thiol group of the protein [13][12][10].

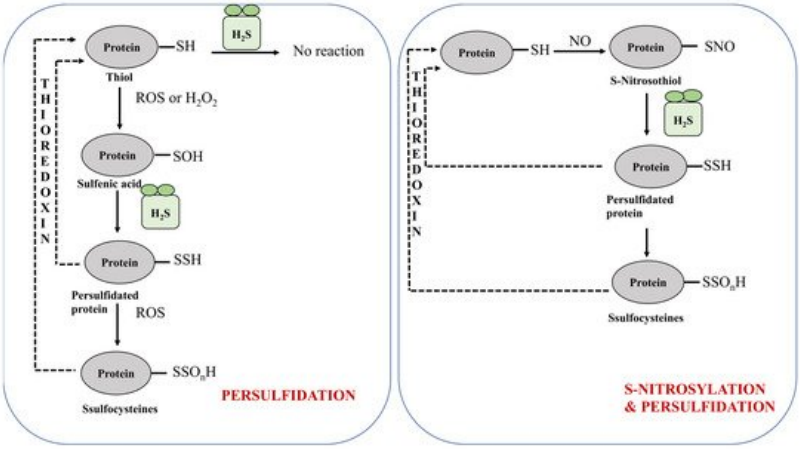

Figure 1. A brief model explaining persulfidation and S-nitrosylation in plants. The thiol group of protein undergo oxidation in presence of ROS and form sulfenic acid, which then undergo persulfidation in the presence of hydrogen sulfide to form persulfidated protein. If the persulfidated protein is 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.

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 [14]. The products formed as a result of this reaction are known as S-nitrosothiols [15][16]. 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 [17]. The main electrophilic agents include S-4bromobenzyl methanothiosulfonate (BBMTS), methanethiosufonate (MMTS), and methylsulfonylbenzothiazole (MSBT) (**Figure 1**).

2.1.2. 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. [8]. 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 [18].

Similarly, as per the reports of Li et al. [19], 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 [20]. However, whenever there is an overaccumulation ofH_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 [19]. 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 [19]. 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 [21].

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 [8][22]. 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) [22]. 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 [23]. 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 [23]. 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 1**).

3. 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.

3.1. 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 [24]. 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 [25]. A study affirmed by Zhu et al. [26], 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 [27]. 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 [25]. 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 [27].

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 [28]. 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 [29]. Sodium nitroprusside show similar action to NaHS in mitigating metal toxicity, depicting the closed relation among NO and H_2S, respectively [30]. 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 [31]. Strikingly, NaHS modulated CDPK-transcripts in zucchini exposed to Ni-stress [28]. 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 [32]. All these components trigger H_2S-pathway or H_2S-producing enzymes or endogenous H_2S [32]. 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 [32]. Likewise, ZIP-transcript *TGA* also increase the production of H_2S during metal toxicity [33].

3.2. 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 [34]. Another study reported by Kaya et al. [35], 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 [36]. 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 [37]. 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 [37]. 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.

3.3. 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 [38]. 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 [39]. 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 [40]. 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 [41]. 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 [41]. In addition to this, plants with H_2S treatment also reduced oxidative stress markers such as MDA and H_2O_2 [41]. 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 [32]. 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.

3.4. 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. [42], 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 [43]. 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 [44]. 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 [37].

3.5. 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 [44]. 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 [44]. Moreover, H_2S application also improved the seed germination rate of tomato during nitrate stress through improvement in the levels of antioxidants [45]. Furthermore, Kaya and Ashraf [46] 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.

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