H$_2$S signaling in plants and applications in agriculture

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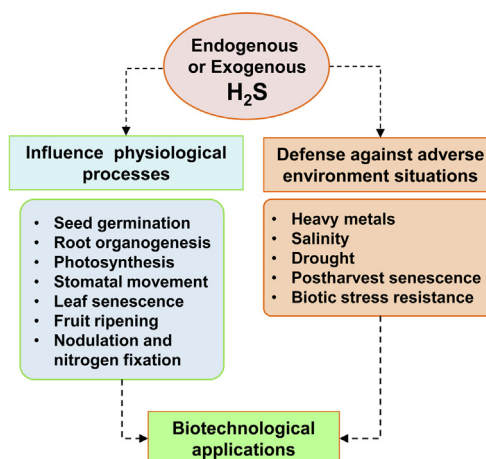
HIGHLIGHTS

- Hydrogen sulfide (H$_2$S) plays a signaling role in higher plants.
- It mediates persulfidation, a post-translational modification.
- It regulates physiological functions ranging from seed germination to fruit ripening.
- The beneficial effects of exogenous H$_2$S are mainly caused by the stimulation of antioxidant systems.

GRAPHICAL ABSTRACT

Summary of the main physiological or adverse environmental situations in higher plants where the hydrogen sulfide (H$_2$S) participates.

ABSTRACT

The signaling properties of the gasotransmitter molecule hydrogen sulfide (H$_2$S), which is endogenously generated in plant cells, are mainly observed during persulfidation, a protein post-translational modification (PTM) that affects redox-sensitive cysteine residues. There is growing experimental evidence that H$_2$S in higher plants may function as a mechanism of response to environmental stress conditions. In addition, exogenous applications of H$_2$S to plants appear to provide additional protection against stresses, such as salinity, drought, extreme temperatures and heavy metals, mainly through the induction of antioxidant systems, in order to palliate oxidative cellular damage. H$_2$S also appears to be involved in regulating physiological functions, such as seed germination, stomatal movement and fruit ripening, as well as molecules that maintain post-harvest quality and rhizobium–legume symbiosis. These properties of H$_2$S open up new challenges in plant research to better understand its functions as well as new opportunities for biotechnological treatments in agriculture in a changing environment.
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Introduction

The description of the gasotransmitter hydrogen sulfide (H$_2$S), with its toxic impact on the metabolism of animal and plant cells,

changed drastically when this molecule was shown to be endogenously generated in cells. However, its signaling capacity has particularly fascinated researchers in many fields of investigation [1–5]. A number of studies in the field of plants began to show that H_2S is directly or indirectly involved in a wide range of physiological processes including seed germination [6], root organogenesis [7,8], photosynthesis [9], stomatal movement [10–13], fruit ripening [14,15], as well as senescence in leaves, flowers and fruits [16,17]. H_2S has also been shown to be involved in the mechanism of response to adverse biotic and abiotic environmental conditions [18,19]. Research has shown a significant correlation between the functions of H_2S and nitric oxide (NO), another simple molecule, whose metabolisms appear regulate each other [4]. Fig. 1 summarizes the principal functions of H_2S in higher plants. The main aim of this review is to provide a broad overview of the major role played by H_2S in higher plants, with particular attention paid to the beneficial effects of its biotechnological application in crop plants, especially under adverse stressful conditions.

Plant biochemistry of H_2S: An overview

The study of H_2S as a signaling molecule has focused on its capacity to interact with thiol (-SH) groups present in protein cysteine residues through the post-translational modification (PTM) persulfidation [4,20]. It is important to point out the major regulatory role played by protein thiol groups involved in multiple interactions which can activate or inhibit the function of the target proteins [21,22]. H_2S competes with other molecules, such as nitric oxide (NO), glutathione (GSH), cyanide and fatty acids, which generate the PTMs *S*-nitrosation [4,23], *S*-glutathionylation [24,25], *S*-cyanylation [26] and *S*-acylation [27–29], respectively. Fig. 2 shows a simple model of these PTMs involving protein thiol groups. However, fewer studies have explored the potential protein targets of persulfidation, previously known as *S*-sulfhydration, and how this PTM affects up-regulates and down-regulates these proteins.

Information garnered from initial plant proteomic analyses focusing on the model plant *Arabidopsis thaliana* [30,31] and that obtained from animal cells [32,33], as well as complementary studies, have facilitated the evaluation of the *in vitro* effect of H_2S on a
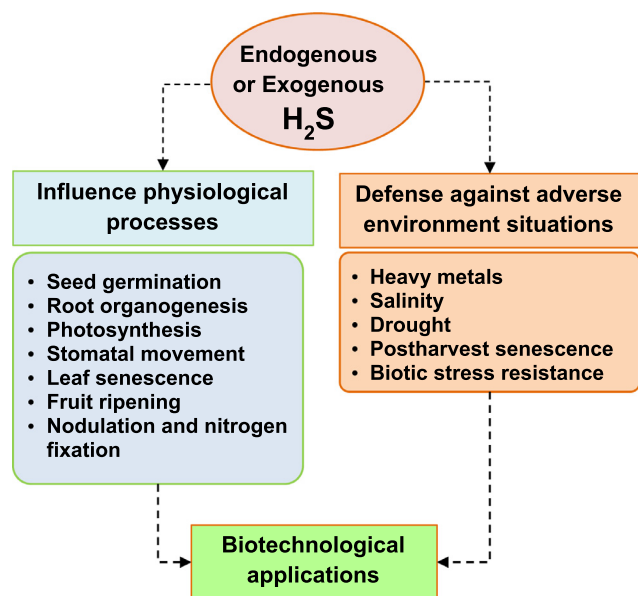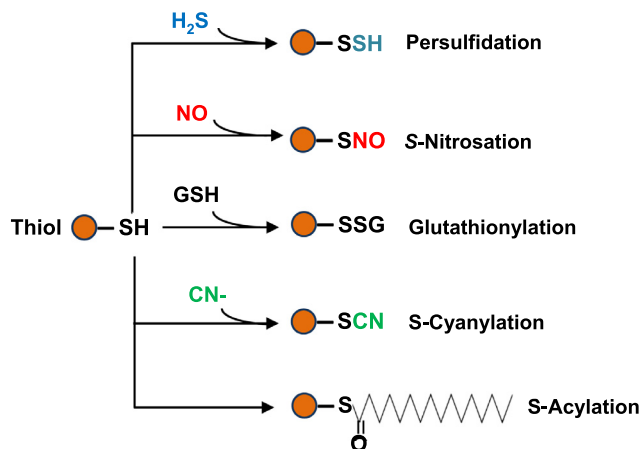
Fig. 2. Protein thiol (-SH) modifications mediated by either the incorporation of H_2S (persulfidation), NO (S-nitrosation), glutathione (GSH) (S-glutathionylation), cyanide (S-cyanylation) or fatty acid (S-acylation).

specific plant protein using different H_2S donors [15,34,35]. Table 1 shows a list of plant proteins, which have been observed to undergo persulfidation, and how their protein function is modulated [36,37]. In some cases, a specific purified protein can behave differently under *in vitro* conditions depending on whether the H_2S donor is applied to the whole plant, added to the nutrient solution or growth media or sprayed on the aerial part of the plant. This is due to the complex action of H_2S characterized by its functional interaction/competition in whole cells with other molecules including nitric oxide (NO) [4], melatonin [38] and phytohormones such as ethylene, auxin and abscisic acid [39,40].

Although the precise mechanisms involved remain unknown, H_2S has been shown to regulate gene expression [41,42]. Exogenous applications of H_2S to grapevine (*Vitis vinifera* L.) plants trigger gene expression involved in the synthesis of secondary metabolites as well as various defensive compounds which boosts plant development and abiotic resistance [43]. In addition, microarray analysis of differentially expressed genes of tomato plants supplemented with NaHS has shown that 5349 genes were up-regulated, while 5536 were down-regulated [44].

However, any precise biochemistry of endogenous H_2S in plant cells, as well as how and where H_2S is produced and its metabolic interactions with other molecules, is still in its infancy. In higher plant systems, several enzymes involved in cysteine metabolism present in subcellular compartments (the cytosol, chloroplasts, mitochondria and peroxisomes) are available for the production of H_2S [35,45,46]. These enzymes include L-cysteine desulfhydrase (L-DES), L-cysteine desulfhydrase 1 (DES1), previously known as Cys synthase-like (CS-LIKE), and cysteine synthase (CS) in the cytosol; D-cysteine desulfhydrase (D-DES) and cyano alanine synthase (CAS) in mitochondria; and sulfite reductase (SiR) in the chloroplast [3,46–48]. However, given its highly lipophilic nature, the H_2S molecule can spread with ease throughout the lipid bilayer of cell membranes [49]. New promising data also show how activities, such as cysteine desulfhydrases, in some of these enzymes are up-regulated under red light and down-regulated by blue and white light [50].

Potential biotechnological applications of exogenously applied H_2S

Although further basic research on H_2S is required, sufficient experimental data show that the exogenous application of H_2S to different plant species at different stages of development can

Fig. 1. Summary of the main physiological or adverse environmental situations in higher plants where the endogenous or exogenous H_2S seems to participate which could also have biotechnological applications.

Table 1
Examples of plant protein targets which function is affected by H$_2$S and consequently they undergo persulfidation.

Enzyme	Function	Effect	Ref.
RuBISCO	Photosynthesis	Activity up-regulated	[9]
O-acetylserine(thiol)lyase (OAS-TL)	Sulfur metabolism	Activity up-regulated	[9]
L-cysteine desulphydrase (LCD)	Sulfur metabolism	Activity up-regulated	[9]
Ascorbate peroxidase (APX)	Antioxidant	Activity up-regulated	[30]
Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	Energy production in the glycolysis	Activity up-regulated	[30]
Glutamine synthetase (GS)	Metabolism of nitrogen	Activity down-regulated	[30]
Actin	Involved in organelle movement, in cell division and expansion	Inhibite actin polymerization	[36]
1-aminocyclopropane-1-carboxylic acid oxidase (ACO)	Ethylene biosynthesis	Activity down-regulated	[37]
NADP-isocitrate dehydrogenase (NADP-ICDH)	Provides NADPH as a reducing agent	Activity down-regulated	[15]
NADP-malic enzyme (NADP-ME)	Provides NADPH as a reducing agent	Activity down-regulated	[34]
Catalase	Antioxidant	Activity down-regulated	[35]
SNF1-RELATED PROTEIN KINASE2.6 (SnRK2.6)	Promote ABA signaling.	Promote ABA-induced stomatal closure	[12]
Respiratory burst oxidase homolog protein D (RBOHD)	Generation of superoxide radical	Activity up-regulated	[13]

palliate damage caused by abiotic stress and enhance physiological features such as seed germination, root development and post-harvest preservation of vegetables [4,51,52]. However, an empirical evaluation of how H$_2$S is to be applied and appropriate dosages is also required. Up to now, exogenous applications have been carried out using chemicals capable of delivering H$_2$S. In animal research on biomedical applications, different families of chemicals, with the capacity to slowly release H$_2$S into cells, have been developed. This has led to the development of water-soluble molecules such as (p-methoxyphenyl)morpholino-phosphinodithioic acid (GYY4137) and a family of cysteine-activated H$_2$S donors (5a, 8l, and 8o) [53]. Few plant studies have used these chemicals [54] which are comparatively more expensive to produce than standard chemicals such as sodium hydrosulfide (NaHS) and inorganic sodium polysulfides (Na$_2$S$_n$) such as Na$_2$S$_2$, Na$_2$S$_3$, and Na$_2$S$_4$. Thus, in aqueous solutions, delivery of H$_2$S by these polysulfides depends on medium pH and the corresponding pKa [55]. In plant research, the cheaper NaHS is exogenously added to hydroponic solutions and *in vitro* growth media or is sprayed directly on plants. NaHS, which is a short-lived donor and does not mimic the slow continuous process of H$_2$S generation *in vivo,* is used in a wide range of concentrations. The chemical dialkyldithiophosphate, which is capable of slowly releasing H$_2$S [56], has recently been demonstrated to increase corn plant weight by up to 39% after 4.5 weeks of treatment. Other compounds, which are capable of releasing NO combined with H$_2$S, are being used in anti-inflammatory pharmaceutical treatments [57].

H$_2$S and abiotic stress

Many adverse external conditions are well known to negatively affect plant growth, development and productivity [58]. To palliate these effects, plants have developed various strategies which differ according to the type of stress and plant species involved. In many cases, these stresses are associated with unregulated overproduction of reactivate oxygen and nitrogen species (ROS/RNS) which can trigger nitro-oxidative stress [59] characterized by an increase in key parameters such as lipid peroxidation, protein tyrosine nitration and oxidative damage to proteins and nucleic acids. Table 2 shows different examples of the beneficial effects of the exogenous application of H$_2$S through the use of different donors on a wide range of agronomically important plants affected by stresses such as heavy metals (cadmium, aluminum, chromium, copper, iron, zinc), metalloids (arsenic), salinity, drought, as well as high and low temperatures [60–84]. Apart from certain specific

responses, in most cases, the application of exogenous H$_2$S appears to cause an increase in the different components of antioxidant systems, such as catalase, superoxide dismutase (SOD) isozymes, as well as enzymatic and non-enzymatic components of the ascorbate-glutathione cycle, which enables H$_2$O$_2$ levels and lipid peroxidation content to be reduced.

H$_2$S in fruit ripening and post-harvest damage to fresh produce

Information available on endogenous H$_2$S metabolism in fruits and vegetables is highly limited. Recently, endogenous H$_2$S content in non-climacteric sweet pepper (*Capsicum annumm* L) fruits was reported to increase during the transition from green immature to red ripe [15]. However, the number of studies focusing on the economic impact of biotechnological applications of H$_2$S on fruit ripening and post-harvest storage, which prevent the loss of fresh produce caused by fungi, bacteria, viruses and low temperatures used to store fruits and vegetables, has increased over the last ten years. Given that all these factors are usually associated with oxidative stress, many studies have shown that the exogenous application of H$_2$S could have a beneficial effect on the shelf life of a diverse range of fruits, vegetables and flowers [14,16,38,85,86]. Table 3 provides representative examples of the exogenous application of H$_2$S to fruits and vegetables [87–94] which enables their quality to be maintained. Another common effect observed following exogenous treatment with H$_2$S is an increase in antioxidant systems which prevent ROS overproduction and consequently oxidative damage.

Implication of H$_2$S in rhizobium–legume symbiosis

In agriculture and natural ecosystems, a major source of nitrogen-fixation is throughout the nodule formation during the plant-rhizobia interaction [95]. As happened with the NO that was seen to be involved in the interaction rhizobium–legume symbiosis [96–98], H$_2$S seems to be also involved in different ways in this process. A recent report indicates that exogenous H$_2$S promotes plant growth, nodulation and nitrogenase activity in the functional symbiosis between rhizobium (*Sinorhizobium fredii*) and soybean (*Glycine max*) plants [99]. Furthermore, the synergy between H$_2$S and rhizobia allowed the increase of soybean nitrogen contents by the regulation of related enzymes at different levels (activity, protein, and gene expression) as well as senescence-associated genes which were also regulated [100]. Moreover, new data obtained during the *Mesorhizobium–Lotus*

Table 2

Main effects of the exogenous application of H2S to plants exposed to diverse environmental stresses. ABA, abscisic acid. APX, ascorbate peroxidase. AsA, ascorbate. CAT, catalase. GR, glutathione reductase. GSH, reduced glutathione. GSNOR, S-nitrosoglutathione reductase. HT, high temperature. MDA, malondialdehyde. POD, peroxidase. NaHS, sodium hydrosulfide. PIP, plasma membrane intrinsic proteins. PM, plama membrane. SOD, superoxide dismutase.

Environmental stress	H2S donor(µM)	Plant species	Effects	Ref.
Aluminum	NaHS(2)	Rice (Oryza sativa L.)	Increases root elongation and decrease Al contents in rice root tips. Increase antioxidant enzyme activities. Decrease MDA and H_2O_2 content in roots	[60]
	NaHS(50)	Soybean (Glycine max L.)	Reduce Al accumulation. H2S function downstream of NO and induce citrate secretion through the upregulation of PM H^+-ATPase-coupled citrate transporter cotransport systems	[61]
Cadmium (Cd)	NaHS(100)	Alfafa (Medicago sativa L.)	Reduces the accumulation of MDA and H_2O_2. Increase the content of GSH and the activity of antioxidant enzymes (SOD, CAT and POD)	[62]
	NaHS(500)	Bermudagrass (Cynodon dactylon L)	Alleviates Cd damages by modulating enzymatic and non-enzymatic antioxidants.	[63]
	NaHS(200)	Barley (Hordeum vulgare L.)	Reduces the accumulation of H_2O_2 and superoxide ions in roots	[64]
	NaHS(200)	Wheat (Triticum aestivum)	Increases the activities of antioxidant enzymes. Inhibits Cd uptake and reduce proline content	[65]
	Endogenous H2S	Arabidopsis (Arabidopsis thaliana)	Overexpression of D-Cysteine desulfhydrase (DCD) decreases Cd and ROS content	[66]
Chromium(Cr)	NaHS(500)	Maize (Zea mays L.)	Alleviate chromium toxicity and enhances antioxidant activities (CAT, SOD, APX)	[67]
	NaHS(200)	Caulifower (Brassica oleracea L.)	Decreases Cr content, H_2O_2 and MDA concentrations. Increases activity of antioxidant enzymes	[68]
Copper (Cu)	NaHS(1,400)	Wheat (Triticum aestivum L.)	Lowers levels of MDA and H_2O_2 in germinating seeds. Increases SOD and CAT activities, and decreases lipoxygenase	[6]
Iron deficiency	NaHS(200)	Strawberry (Fragaria × ananassa)	Reduces electrolyte leakage, and content of H_2O_2 and MDA. Upregulate activities of antioxidant enzymes. Improved Fe uptake	[69]
Zinc (Zn)	NaHS (200)	Pepper (Capsicum annuum L.)	Increases plant growth, fruit yield, water status and proline content. Enhances the activity of antioxidant enzymes	[70]
Arsenic (As)	NaHS(100)	Pea (Pisum sativum L.	Increases of AsA and GSH contents and activities of the AsA–GSH cycle enzymes	[71]
Salinity	NaHS(50)	Rice (Oryza sativa L.)	Decreases the uptake of Na^+ and the Na^+/K^+ ratio	[72]
	NaHS(50)	Wheat (Triticum aestivum L.)	Suppresses ROS accumulation by increasing antioxidant defense	[73]
	NaHS(20)	Cucumber (Cucumis sativus L.)	Keeps Na^+ and K^+ homeostasis by the gene expression of plasma membrane Na+/ H + antiporter (SOS1). Decrease lipid peroxidation content and ROS generation. Increases activity of antioxidant system	[74]
	NaHS(200)	Mangrove plant (Kandelia obovata)	Enhances the quantum efficiency of photosystem II (PSII) and the membrane lipid stability	[75]
Drought	NaHS(500)	Wheat (Triticum aestivum L.)	Increases antioxidant enzyme activities, reduces MDA and H_2O_2 contents in both leaves and roots. Increases of the transcription levels of genes encoding ABA receptors.	[40]
	NaHS(400)	Wheat (Triticum aestivum L.)	Induction of genes that code for antioxidant enzymes	[76]
	NOSH[1] compounds (100)	Alfalfa (Medicago sativa L.)	Lowers MDA. Induce Cu/ZnSOD, FeSOD genes	[77]
Osmotic stress	NaHS(150)	Arabidopsis (Arabidopsis thaliana)	Increase phospholipase Dα1 and the antioxidant enzyme system. Reduce ROS and MDA content and reduce electrolyte leakage	[78]
Low temperature	NaHS(50)	Cucumber (Cucumis sativus L.)	Increases GSH and cucurbitacin C content	[79]
	NaHS(500)	Lowbush blueberry (Vaccinium angustifolium)	Alleviate the degradation of chlorophyll and carotenoids and reduce the photoinhibition of PSII and PSI.	[80]
High temperature	NaHS(100)	Strawberry (Fragaria × ananassa cv. 'Camarosa')	Induction of gene expression ocoding for antioxidant enzymes (cAPX, CAT, MnSOD, GR), heat shock proteins (HSP70, HSP80, HSP90) and aquaporins (PIP)	[81]
	NaHS(500)	Maize (Zea mays L.)	Improves seed germination and increases antioxidant enzymes. Accumulation of proline	[82]
	NaHS (50) or MGYY4137 (10)	Poplar (Populus trichocarpa)	Increases GSNOR activity and reduce HT-induced damage to the photosynthetic system	[83]
	NaHS (100) or GYY4137 (10)	Arabidopsis thaliana	Enhances seed germination rate under HT.Increases gene expression of ABI5 (ABA-INSENSITIVE 5).	[84]

[1] Resulted in the Utility Patent Pub. No.: WO/2015/123273.

symbiosis indicate that this interaction is regulated by the cross-talk among H2S with other signaling molecules including NO and ROS [101].

Conclusions and future perspectives

H2S, which is part of the plant sulfur metabolism, is a new signal molecule whose regulatory function acts through redox interactions, especially the protein post-translational modification persulfidation. The application of exogenous H2S, involving a signaling mechanism, causes an increase in different components of the antioxidant system at both the gene and protein level. Nevertheless, the precise biochemical and molecular mechanisms involved in these processes need to be further investigated in future research. However, the exogenous application of H2S undoubtedly has a beneficial effect on different plant species, especially those of considerable agronomic interest under adverse environmental conditions. Therefore, the use of H2S alone or combined with other molecules, such as nitric oxide, melatonin, thiourea, silicon, chitosan and calcium, which appear to beneficially affect crop plants, needs to be explored in light of climate change [102–108]. Thus, additional research is necessary in order to decipher the unknowns of H2S and its interaction with the metabolism of ROS and RNS under physiological and stressful conditions [109], as well as to establish biotechnological strategies to combat these stresses,

Table 3
Representative examples of the main beneficial effects of the exogenous application of H_2S in fruits and vegetables.

Fruit/vegetable	H_2S donor	Effects	Ref.
Strawberry (*Fragaria* × *ananassa* Duch.)	0.8 mM NaHS	Prolongs postharvest shelf life and reduces fruit rot disease	[87]
Broccoli (*Brassica oleracea*)	2.4 mM NaHS	Alleviates senescent symptoms	[88]
Grape (*Vitis vinifera* L. × *V. labrusca* L. cv. Kyoho)	1 mM NaHS	Alleviates postharvest senescence of grape and maintain high fruit quality	[89]
Banana (*Musa acuminata*, AAA group)	1 mM NaHS	Alleviates fruit softening. Antagonizes ethylene effects	[14]
Tomato (*Solanum lycopersicum* L.) 'Micro Tom'	0.9 mM NaHS	Postpones ripening and senescence of postharvest tomato fruits by antagonizing the effects of ethylene	[90]
Hawthorn (*Crataegus oxyacantha*) fruit	1.5 mM NaHS	Confers tolerance to chilling. Triggers H_2S accumulation, increase antioxidant enzyme activities of and promote phenolics accumulation	[91]
Avocado (*Persea americana* Mill, cv. 'Hass')	200 μMNaHS	Protects against frost and day high light	[92]
Kiwifruit (*Actinidia chinensis*)	20 μM H_2S	Delays ripening and senescence. Inhibits ethylene production. Increases antioxidant activities. Regulates the cell wall degrading enzyme gene	[42]
Daylily (*Hemerocallis fulva*)	4 mMNaHS	Delays senescence of postharvest daylily flowers. Increases antioxidant capacity to maintain the redox balance	[93]
Tomato (*Solanum lycopersicum* L.).	1 M NaHS	Inhibits ethylene-induced petiole abscission	[94]

which are responsible for major losses in plant yield and crop productivity.

Compliance with Ethics requirements

This article does not contain any studies with human or animal subjects.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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