Something smells bad to plant pathogens: Production of hydrogen sulfide in plants and its role in plant defence responses

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GRAPHICAL ABSTRACT

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Background: Sulfur and diverse sulfur-containing compounds constitute important components of plant defences against a wide array of microbial pathogens. Among them, hydrogen sulfide (H$_2$S) occupies a prominent position as a gaseous signalling molecule that plays multiple roles in regulation of plant growth, development and plant responses to stress conditions. Although the production of H$_2$S in plant cells has been discovered several decades ago, the underlying pathways of H$_2$S biosynthesis, metabolism and signalling were only recently uncovered.

Aim of the review: Here we review the current knowledge on the biosynthesis of H$_2$S in plant cells, with special attention to L-cysteine desulfhydrase (DES) as the key enzyme controlling H$_2$S levels biosynthesis in the cytosol of plant cells during plant growth, development and diverse abiotic and biotic stress conditions.

Key Scientific Concepts of Review: Recent advances have revealed molecular mechanisms of DES properties, functions and regulation involved in modulations of H$_2$S production during plant responses to abiotic and biotic stress stimuli. Studies on des mutants of the model plant Arabidopsis thaliana uncovered molecular mechanisms of H$_2$S action as a signalling and defence molecule in plant-pathogen interactions. Signalling pathways of H$_2$S include S-persulfidation of protein cysteines, a redox-based post-translational modification leading to activation of downstream components of H$_2$S signalling. Accumulated evidence shows DES and H2S implementation into salicylic acid signalling and activation of pathogenesis-related proteins and autophagy within plant immunity. Obtained knowledge on molecular mechanisms of H$_2$S action in plant defence responses opens new prospects in the search for crop varieties with increased resistance to bacterial and fungal pathogens.

Introduction

Hydrogen sulfide (H$_2$S) has been recognized as a multifaceted gasotransmitter involved in a diverse array of biological processes across all kingdoms including plants [1–3]. Since the first observation of H$_2$S emissions from leaves of several plant species by Wilson et al. 1978 [4], H$_2$S has emerged as a vital signalling
molecule involved in the regulation of multiple mechanisms in plant growth and development and responses to external stimuli. H2S was identified as an important component of signalling pathways regulating stomatal closure [5–7], root organogenesis [8] and photosynthesis [9]. Hydrogen sulfide also plays important roles in fruit biology and freshness and regulation of postharvest senescence of horticultural products. Application of H2S in the form of aqueous solutions of sodium hydrosulfide (NaHS) or sodium sulfide (Na2S) can decelerate fruit ripening and senescence in numerous fruits, partially by inhibiting the growth of fungal spores [10–11].

Under stress conditions, H2S can shape plants ability of adaptation to diverse environmental stimuli by alleviating stress-induced injuries and activation of defence mechanisms [12–14]. Hydrogen sulfide can mediate enhancement of plant tolerance to salinity, drought, heavy metal and high-temperature stress, based on priming effect of H2S on plant redox signalling, antioxidant capacity and specific components of cellular defence, [15–17]. Exogenous application of H2S induces plant cross-adaptation to multiple abiotic stresses [18].

Signalling functions of H2S in animal cells are known to be mediated namely by protein persulfidation of protein cysteines, interactions with metal centres in the protein active sites and reactions with S-nitrosothiols and electrophilic compounds [3]. Importantly, the biological functions of H2S in plants involve interactions and cross-talk with signalling pathways of other plant gasotransmitters and reactive nitrogen and oxygen species [19–25]. Complex interactions of signalling pathways of H2S and nitric oxide (NO) were revealed to regulate stomatal movement in plant leaves [19] where 8-mercapto-cGMP was shown as the active component of H2S-mediated guard cell signalling [26]. Furthermore, biological functions of H2S in plant growth, development and responses to abiotic stresses are determined by a dual role of H2S in interactions with phytohormones. Endogenous H2S levels are regulated by phytohormones, whereas H2S can influence the production, transport, and signalling pathways of diverse plant hormones in plant physiological responses [27].

The role of H2S in plant signalling and responses to abiotic stresses has attracted considerable attention and has been extensively reviewed elsewhere [14,18,21,28]. Within this special issue, Corpas and Palma [29] provide an excellent overview of the current state of knowledge on H2S signalling in plants and potential application to increased plant performance under conditions of diverse environmental stresses.

It has become evident that similarly to reactive nitrogen and oxygen species, H2S can perform a dual role in plant pathogenesis, i.e. signalling functions and direct inhibitory or toxic actions towards penetrating pathogens [30]. Crop fertilization with sulfur has been known for long to stimulate plant resistance, which is actually known to be mediated by H2S. Pathogen resistance can be also potentiated by H2S by induced expression of salicylic acid-dependent pathogen-related genes [30]. A substantial part of available published reports comprises rather descriptive studies performed by plant or fruit treatments with exogenous sulfide that has not provided deeper mechanistic insights into H2S biological roles. L-cysteine desulphydrase (DES1) has been shown to act as the key enzyme in the control of H2S production and signalling in physiological conditions during plant growth and development as well as during plant-pathogen interactions. Moreover, H2S has been recently reported to play a role in the regulation of plant autophagy, a key mechanism of plant innate immunity [31,32]. However, compared to H2S role in plant responses to abiotic stresses, the sources, targets and mechanisms of H2S action in diverse processes during plant-pathogen interactions are only partially uncovered. Major advances in this field have been achieved using des1 mutant of Arabidopsis thaliana and need to be replicated and extended also in plant crop species and their relevant pathogens.

Up to our best knowledge, the actual state of the art in the field of H2S functions in plant biotic interactions has not been previously reviewed. In this review, we focus namely to H2S role in plant-pathogen interactions and we summarize in more detail the specific involvement of H2S in plant responses to pathogen infection, with special attention dedicated to H2S-producing enzyme L-cysteine desulphydrase and its connections to plant sulfur metabolism.

**L-cysteine and sulfide: A central role in plant sulfur metabolism and defence responses**

In a central position within the plant primary metabolism and plant responses to stress conditions, amino acid L-cysteine (L-Cys) serves as a precursor of essential biomolecules and defence sulfur-containing metabolites [33,34]. L-Cys incorporated into peptide and protein molecules plays an outstanding role in redox-based signalling in various plant cell compartments. In a prominent place, the cysteine-containing tripeptide glutathione (GSH, γ-glutamyl-cysteinyl glycine) plays a crucial role in the maintenance of redox homeostasis and cellular protection to oxidative stress [35]. Protein cysteine thiols are targets of diverse post-translational modifications, which can strongly affect protein structure, activity, functions, and localization [36]. Oxidative modifications of thiol groups (−SH) in protein cysteines include a reversible formation of disulfides (−S−S−), sulfenic (−SOH) or sulfinic (−SO2H) groups, whereas modifications caused by the action of signalling molecules NO and H2S are represented by S-nitrosation and S-persulfidation, respectively [37–39]. Other examples of plant metabolites derived from L-Cys include amino acid methionine, enzyme cofactors like biotin and Fe-S clusters and S-adenosyl methionine, which provides methyl groups for methylation reactions in the biosynthesis of polyamines, phytosiderophores and phytohormone ethylene.

In the last decades, the involvement of diverse types of sulfur-containing compounds in plant defences and resistance to microbrial pathogens has been widely uncovered. Besides the well-established role of GSH and GSH-dependent enzymes [40,41], L-Cys and its metabolites sulfide and carbonyl sulfide have been recognized within plant resistance mechanisms [42–44]. Sulfur-rich proteins, such as thionins, contribute to the disintegration of pathogen cell walls and induce the formation of ion channels in pathogen membranes [45,46]. Plant tissue challenge with microbial pathogens induces phytoalexins de novo [47–49]. Isocyanates as degradation products of glucosinolates represent another important group of antimicrobial compounds [50]. Elemental sulfur (S0) is known to accumulate in vascular tissue upon fungal infections and to inhibit pathogen germination, respiration, and metabolism, possibly through interaction with protein thiol groups [51–53].

Collectively, a chemically diverse group of sulfur-containing metabolites, including elemental sulfur, glutathione, glucosinolates, phytoalexins and gaseous H2S are involved in pathogen resistance. Their occurrence is species-specific and in a large extent influenced by the sulphur nutritional status of the plant.

**Synthesis and catabolism of L-cysteine as H2S precursor in the plant cytosol**

L-Cysteine, as a potential donor molecule of reduced sulfur, is produced in the last step of sulfate assimilation in plants by incorporation of sulfide into O-acetylserine catalysed by O-acetylserine (thiol)lyase (OAS-TL), which is found in various isoforms in the cytosol, mitochondria and chloroplasts [33,54–56]. The cytosol
plant cells is thus the main cellular compartment of L-Cys biosynthesis. As a result of very high activity of cysteoyclic OAS-TL isoforn OAS-A1, usual cysteoyclic L-Cys concentrations range around 300 μM, whereas in other compartments L-Cys is found at levels lower than 10 μM [57]. Due to its high reactivity, increasing concentrations of L-Cys potentially cause toxic effects to plant cells. L-Cys is an effective reductor of iron (III) to iron (II) ions, which participate in Fenton-type reactions with reactive oxygen species (ROS) causing oxidative damage of cellular components [58,59]. For this reason, the maintenance of L-Cys homeostasis by the coordinate action of key enzymes of its biosynthesis and catabolism, i.e. OAS-A1 and L-cysteine desulphhydrase (DES), is of utmost importance for the proper functioning of plant metabolism under physiological and stress conditions.

The most abundant cysteoyclic OAS-TL isoform OAS-A1 is involved in plant responses to abiotic stress, namely to heavy metal exposure, through metal-chelating activity of phytochelatins, synthesized from L-Cys with GSH as an intermediate [35]. A major contribution to understanding the role of the key enzymes in L-Cys biosynthesis and catabolism was provided by studies of Arabidopsis oas-a1 and des1 mutants, respectively [33,60,61]. The knockout oas-a1.1 and oas-a1.2 mutants were characterized by decreased intracellular L-Cys and glutathione levels. Compromised antioxidant capacity results in perturbation of H2O2 homeostasis, as documented by spontaneous cell death lesions occurring in leaves of oas mutants [60]. Mutation of the DES1 gene results in elevated total Cys content caused by reduced total Cys desulfuration activity in leaves. Arabidopsis des1 mutants show premature leaf senescence, whereas enhanced antioxidant defences and tolerance to oxidative stress [61].

A 25% decrease of L-Cys concentration was observed in the oas-a1 mutant, in contrast to a 25% increase of L-Cys levels in a des1 mutant which did not cause any toxic effects to the mutant plants. OAS-A1 down-regulation resulted in higher ROS levels, likely asociated with defective homeostasis of H2S, whereas the DES1 deficit was accompanied with decreased ROS. In consequence, decreased GSH levels in OAS-A1 deficient plants were associated with an increased ratio of oxidized glutathione. Subsequently, oas-a1 mutants showed compromised tolerance to cadmium exposure, in comparison to wild-type plants. The overall oxidative stress induced by OAS-A1 deficiency was evident even under control growth conditions when plants showed the spontaneous formation of leaf cell death lesions [33,60,61].

The chloroplastic OAS-TL isoform in Arabidopsis thaliana has been described as an S-sulfocysteine synthase (SSCS) enzyme which has a crucial role in the proper photosynthetic performance of the chloroplast under long-day growth conditions. SSCS is located in the thylakoid lumen and it was suggested to function as a sensor to detect accumulated thiosulfate caused by ineffective removal of ROS under conditions of excess light [62–64].

Together with plant OAS-TL, mitochondrial β-cyanoalanine synthases (CAS-C1) belong to the large superfamily of pyridoxal 5’-phosphate-dependent enzymes together with OAS-TL. As a mechanism of cyanide detoxification, CAS catalyses the biosynthesis of the nonprotein amino acid β-cyano-Ala from L-Cys and cyanide, producing sulfide as a product [65,66]; however, the contribution of CAS to H2S production is highly variable among plant species. Nine CAS genomic sequences were reported in A. thaliana [67]. Using T-DNA insertion mutants, cytosolic Basa1.1, plastidic Basa2.1, and mitochondrial Basa2.2 were found to play important roles in L-Cys biosynthesis, with a major contribution of cytosolic Basa1.1 in leaves and root, and mitochondrial Basa2.2 in the root.

Addition of O-acetylserine inhibits emissions of gaseous H2S from plant tissues and increased L-Cys levels [68,69]. On the other hand, compounds known to inhibit GSH biosynthesis induce H2S emission, suggesting that under conditions when biosynthetic pathways consuming L-Cys are inhibited, sulfides are emitted in the form of gaseous H2S [70]. A correlation between enzymatically-produced H2S and the total amount of sulfur was observed in Brassica napus [71]. With increased sulfur content, DES1 activity was observed to decrease whereas OAS-TL activity decreased. However, later reports found that under sulfur deficiency, plants showed up-regulation of both OAS-TL and DES1 [72].

The schematic overview of known biochemical pathways of H2S biosynthesis and conversion in plant cells highlight the central role of DES1 in cytosolic H2S productions (Fig. 1). Other enzymes such as CAS-C1, D-cysteine desulphydrase (β-DCD), L-cysteine desulphurase (DSF) and ferredoxin are capable to contribute to H2S production in other plant cell compartments, but to which extent this might occur in different plant species under specific growth or stress conditions is largely unknown. Current knowledge thus demonstrates that L-Cys occupies a central position in plant sulphur metabolism as a reduced sulfur donor in the biosynthesis of defence compounds. Besides the involvement of L-Cys and its metabolites in redox signalling within plant cell compartments, cysteoyclic and mitochondrial L-Cys play crucial roles in plant immunity and cyanide detoxification, respectively. The functions of L-Cys in plant responses to pathogen challenge are in a large extent mediated by L-Cys-derived H2S, as discussed in the next sections of this review.

**L-cysteine desulphydrase: A key enzyme of H2S production in plants**

L-Cysteine degradation in plant cytosol is known to proceed in a reaction catalysed by L-cysteine desulphydrase (DES), leading to H2S, pyruvate and ammonia. Cysteoyclic levels of L-Cys and also of H2S are therefore controlled by activities of OAS-TL and DES [72,73]. Detailed characterization of des1 mutants of *A. thaliana*, together with pharmacological approaches using DES1 inhibitors as well as H2S donors and scavengers, have recently provided valuable insights into the role of DES1 and H2S in signalling pathways of plant responses to biotic and abiotic stress stimuli [44,74–76].

L-cysteine desulphydrase (DES1, EC 4.4.1.1.1.) is the main enzyme of L-Cys catabolism in plant cytosol, which catalyses L-Cys decomposition to pyruvate, ammonia and H2S in a stoichiometric ratio 1:1:1. DES1 regulates cysteoyclic L-Cys levels together with major cysteoyclic OAS-TL, highly active OAS-A1 involved in L-Cys biosynthesis [33,54]. Plant DES1 belongs to the family of O-acetylserin (thiol)lases (OATL), which in *A. thaliana* comprises 8 described members involved in sulfur metabolism (Table 1).

This enzyme was described for the first time based on sequence homology to other members of OAS-TL family during Arabidopsis genome sequencing. Originally, the enzyme was termed as CS-LIKE according to its cysteine synthase-like activity. Alvarez et al. [61] achieved production and purification of recombinant Arabidopsis DES1 in *E. coli* and performed detailed in vitro characterization of enzyme molecular properties. Similarly to other members of OAS-TL family, DES1 requires pyridoxal phosphate (PLP) as a cofactor and contains all conserved amino acid residues involved in PLP binding. On the other hand, DES1 differs from other members of OAS-TL family in the sequence of β8A–β9A loop, which is otherwise highly conserved in OAS-TLs due to its role in protein interaction with serine O-acetyltransferases [55,77].

Purification of recombinant DES1 by affinity chromatography results in increased cysteine desulphydrase activity of the protein but decreased O-acetylserine lyase activity, in agreement with enzyme primary role in cysteine degradation. This is also supported by enzyme higher activity towards L-Cys, measured by the value of Michaelis constant Km, which is 14-times lower for L-Cys in DES reaction compared to Km value for O-acetylserine in OAS-TL reaction. However, the limit reaction rate is quite low, determined as 0.04 μmol.min⁻¹.mg⁻¹ for purified Arabidopsis
DES1. Purified AtDES1 also possesses D-cysteine desulfhydrase activity, but one order of magnitude lower compared to L-Cys desulfhydrase activity [61].

A homologous DES1 gene was isolated from rapeseed (Brassica napus) and sequenced, showing 85% homology to Arabidopsis gene and coding a 323 amino acid polypeptide of 34.5 kDa molecular weight [78]. BnDES1 was found highly expressed in rapeseed flowers, whereas the expression levels in vegetative tissues were much lower. Similarly to AtDES1, BnDES12 also shows a minor O-acetylserine (thiol)lyase activity. A recent report revealed OAS-TL family in Solanum lycopersicum, where measurable DES activity was found in some isoforms, namely SIOAS4 and SIOAS6 [79]. Reported pH optimum values for plant DES range from 8.0 in tobacco [80], 9.0 in Brassica [78] to 10.0 in A. thaliana [81]; however, the physiological relevance of these differences in DES pH optima is not known. Based on the published reports, DES1 enzyme catalysing L-Cys desulphydratation has been established as the main sources of the endogenous production of H2S in the plant cytosol, at least in A. thaliana. Moreover, it has been shown that DES1 is regulated by plant hormones on the transcriptional level, enabling DES1 and H2S level regulation in response to stress stimuli.

L-cysteine desulphydrase activity has not been detected in animals, where H2S production has been demonstrated to be catalysed by cystathionine-γ-lyase and cystathionine-β-synthase [82] or 3-mercaptopropionate sulfur transferase [83]. However, L-cysteine desulphydrase was discovered to operate in some bacteria, e.g. Escherichia coli [84,85].

It should be noted that D-cysteine desulphydrase (EC 4.4.1.15), which catalyses the conversion of D-cysteine to the same products as DES1, including H2S, represent a completely different enzyme both in protein structure and biochemical properties [86]. D-cysteine desulphydrase activity has been observed in multiple plant species including Arabidopsis, where two genes At3g26115 and At1g48420 coding proteins with D-cysteine desulphydrase activity were identified [87,88]. Interestingly, the biological function of D-cysteine as well as of D-enantiomers of other amino acid is still not known. One of the suggested functions of D-cysteine desulphydrase might be degradation of malformin, a phytotoxic peptide produced by Aspergillus niger containing D-cysteine [89].

Production of H2S can be catalysed also by cytosolic L-cysteine desulhydrase (EC 2.8.1.7) when using L-cysteine methyl ester as a substrate [90]. The main role of L-cysteine desulhydrases is to catalyse L-Cys desulhydration to give L-alanine and elemental sulfur. Proteins showing L-cysteine desulhydrase have been identified in the cytosol, chloroplast and mitochondria, where it provides elemental sulfur for the biosynthesis of biotin, thiamine and Fe-S clusters [91,92].

**DES1 regulates H2S production and signalling during plant growth and abiotic stresses**

As already mentioned, DES1 is the specific source for the production of cytotoxic H2S, involved in signalling pathways of vital plant processes like autophagy and stomatal regulation. During the development of Arabidopsis plants, the highest DES1 gene expression was found in leaves of 14-days old seedlings and 35-old plants just after the termination of flowering, whereas the lowest expression levels were observed in rosettes of 20-days old plants before the appearance of visible buds [93]. At the tissue level, DES1 transcripts were abundant in mesophyll and epidermal cells, including guard cells, in cells surrounding hydathode pores, in trichomes and flowers.

It is known that mutations in AtDES1 induce leaf senescence, accompanied by higher expression of genes involved in plant ageing and increase levels of related transcription factors. Absence of
DES1 activity leads to substantially decreased overall desulfurase activity in leaves, associated with increased L-Cys levels [61]. Furthermore, DES1 deficiency in Arabidopsis results in an accumulation of various isoforms of autophagy-related proteins 8 (ATG8), as a sign of activated autophagy processes [31]. The DES1 reaction product, H$_2$S, is likely involved in autophagy regulation, as suggested by the inhibitory effect of exogenous H$_2$S to the accumulation of ATG8 proteins in des1 mutants.

Metabolic rates of L-Cys and H$_2$S production are closely related to the plant nitrogen uptake. Plants under high nitrogen nutrition conditions contain higher activities of OAS-TL and DES1, increased level of sulfur-containing compounds and decreased sulfate levels compared to plants grown in nitrogen-deficient conditions. These findings suggest that sufficient nitrogen uptake enables a higher rate of sulfur incorporation into proteins. The observed higher DES1 activity can serve as a protective factor to avoid excessive L-Cys accumulation [72].

Interestingly, decreased levels of nitric oxide (NO) were observed in des1 mutant, suggesting DES1 is involved by an unknown mechanism in production this gaseous signalling compound in stomata [94]. Currently, it has been recognized that H$_2$S regulates multiple developmental processes and stress responses in interaction with signalling pathways plant hormones [27] including signalling gasotransmitters NO [95] and carbon monoxide [96] or reactive oxygen species like hydrogen peroxide [28].

The role of H$_2$S in stomata closure has been extensively studied [5–7,94]. DES1 is involved in the signalling pathway of abscisic acid (ABA) in the leaf stomata, where it participates in the regulation of guard cell movements in stomata closure and opening. ABA is known to induce DES1 expression in wild-type plants, whereas in the ABA-nonresponsive des1 mutant stomata closure can be induced by exogenous H$_2$S [97].

Treatment with H$_2$S, provided as an aqueous solution of NaHS, promotes lateral roots in tomato seedlings with increased auxin levels, suggesting H$_2$S produced by DES1 is involved in the auxin signalling pathway regulating lateral roots formation [98]. A role for DES1 has been proposed in phytohormone-induced programmed cell death in the aleurone layer of wheat [99]. This was evidenced by observations that gibberellins cause decreased H$_2$S to the accumulation of various isoforms of autophagy-related proteins 8 (ATG8), as a sign of activated autophagy processes [31]. The DES1 reaction product, H$_2$S, is likely involved in autophagy regulation, as suggested by the inhibitory effect of exogenous H$_2$S to the accumulation of ATG8 proteins in des1 mutants.

Current accumulated knowledge support a functional implementation of DES1 into signalling pathways of salicylic acid (SA), the key plant hormones in responses to microbial pathogens as well as in plant growth and development [104,105], WRKY transcription factors are key regulators of specific plant developmental processes, including seed dormancy, seed germination, and senescence and also plant responses to biotic and abiotic stresses [106]. Expression levels of multiple WRKY members were previously found to be modulated by pathogen infection or SA treatment [107] and WRKY transcription factors are known to down-regulate the expression of DES in A. thaliana [108]. Transcript levels of WRKY54, which serves as a transcription factor regulating gene expression of SA proteins were increased in des1 mutants and decreased in oas-a1 mutants [60]. Simultaneously, des1 mutants showed a lower degree of L-glutathione oxidation compared to oas-a1 mutants. Higher levels of L-Cys in des1 mutants, resulting in lower intracellular redox potential, thus can contribute to increased plant resistance to invading pathogens. This has been further confirmed in a subsequent study of Alvarez et al. [42], which characterized Arabidopsis des1 mutants as more resistant to both biotrophic and necrotrophic pathogens, whereas oas-a1 mutants showed compromised pathogen resistance. In parallel, higher levels of SA, putatively involved in long-distance plant signalling, were observed in des1 mutants. Transcriptomic analysis showed induction of four PR proteins including defensins PDF1-2a and PDF1-2b. Collectively, high Cys-associated decreased intracellular redox potential might play an important role within plant defence to pathogens; however, this mechanism requires further experimental investigations in Arabidopsis and other plant species.

Arabidopsis cad2-1 mutants show approx. 70% decreased levels of L-glutathione but unchanged levels of L-Cys, as compared to wild-type plants, unlike oas-a1 mutants where both L-Cys and glutathione levels are reduced. As the repression of WRKY54 was not found in cad2-1 mutants, suggesting the inhibition of PR expression in oas-a1 mutants was caused by decreased L-Cys levels. Interestingly, members of the WRKY family were recently identified to pose binding capacity to the promoter of DES1 gene and regulate its expression [108]. Furthermore, oas-a1 mutant plants lack the capacity of the hypersensitive reaction, which can be restored by addition of L-Cys but not glutathione, in agreement with a specific requirement for L-Cys in incompatible interactions in plant pathogenesis [33,42]. The involvement of L-Cys in plant immunity was tested on oas-a1 plants exposed to a virulent bacterial pathogen
Pseudomonas syringae pv. tomato DC3000, which produces effectors suppressing plant immunity induced by pathogen molecular patterns. Arabidopsis oas-1 mutants plants showed increased susceptibility to this pathogen [109].

Development of Arabidopsis mutants in two genes of cysteine desulphydrases enabled to study the function of DES1 and H2S in plant tolerance biotic and abiotic stress stimuli [75]. Transcript levels of DES1 and D-cysteine desulphhydrase (EC 4.4.1.15) were increased by bacterial pathogen as well as diverse abiotic stress conditions including cold, dehydration, salt and hydrogen peroxide treatment; however, with different timing. The highest increase in DES1 expression and activity was detected from 1 to 3 h after pathogen infection, whereas increased DES1 mRNA continued several hours after the abiotic stress treatment. Compared to wild-type plants, DES1 overexpressors showed lower counts of bacterial cells in infected tissues, in contrast to increased counts of pathogen cells in DES1 knock-outs. Plant defence responses were induced and suppressed by NaHS or H2S scavenger hypotaurine, respectively. Moreover, expression of PR protein genes was induced in DES overexpressors by NaHS treatment, while it was found increased in DES1 knock-down mutants and by H2S scavenger. Collectively, these data support the functional role of DES1 enzyme in H2S production involved in the regulation of plant resistance mechanisms, putatively mediated by activation of salicylic acid-dependent signalling and defence genes [75].

Interestingly, plants under sulfur deficient conditions are able to uptake gaseous H2S or carbonyl sulfide (OCS) from the environment, whereas H2S and OCS are released on pathogen infection, whereas increased DES1 mRNA continued several hours after the abiotic stress treatment. Compared to wild-type plants, DES1 overexpressors showed lower counts of bacterial cells in infected tissues, in contrast to increased counts of pathogen cells in DES1 knock-outs. Plant defence responses were induced and suppressed by NaHS or H2S scavenger hypotaurine, respectively. Moreover, expression of PR protein genes was induced in DES overexpressors by NaHS treatment, while it was found increased in DES1 knock-down mutants and by H2S scavenger. Collectively, these data support the functional role of DES1 enzyme in H2S production involved in the regulation of plant resistance mechanisms, putatively mediated by activation of salicylic acid-dependent signalling and defence genes [75].

In summary, the use of transgenic plants with modulated DES1 activity and endogenous H2S level, in combination with exogenous treatments with H2S donors or scavengers, confirm proposed protective role of H2S in biotic stress resistance.

Mechanisms of H2S action in plant-pathogen interactions

As evident, major advances in the detailed understanding of H2S sources and functions in plant metabolism and stress responses have been obtained in the model plant A. thaliana, with available mutants of key enzymes of sulphur metabolism. In contrast, the understanding of the role and mechanisms of H2S action in defence responses of crop plants is quite limited (Table 2).

Early studies found killing grape mildews by H2S fumigation in a close jar [112] and completely inhibited germination of Botrytis cinerea spores sowed in a saturated solution of H2S [113]. This was confirmed by a more extensive study on a set of fungal phytopathogens (including B. cinerea, Cladosporium herbarum, Fusiscladium dendriticum, Monilia cinerea and M. fructigena, Penicillium verdidatum, Physalospora miyabeana) which showed H2S acting as a general poison toxic at a low concentration to all used fungi [114]. Importantly, this study noted the conversion of sulphur to volatile H2S, which mediates the toxic effects previously attributed to the sulphur treatments.

The enzyme DES1 was identified as the H2S source in rapeseed plants infected by a fungal pathogen Pyrenopeziza brassicae, which resulted in a 50% increase of DES1 activity [72]. Fungal infection of the grapevine by grape powdery mildew (Uncinula necator) induced an increased release of H2S, namely during the early phase of the infection; however, it strongly decreased 10 days after infection [43]. An application of elemental sulfur to powdery mildew-infected grape leaves showed the highest efficiency when applied in the early phase of pathogenesis prior to the formation of fungal appressoria in penetrated leaf cells [115]. It was estimated that uptake of 10 μM/h of H2S by the pathogen would provide a fungicidal effect. Role of H2S in plant defence against fungal pathogen was evidenced by significant increase of H2S emissions from crops challenged by fungal infection [72,111]. Collectively, in a similar manner to model plants, crops have been demonstrated to exert capabilities to respond to fungal infection by modulations of L-Cys metabolism, H2S emissions and increased levels of GSH and phytoalexins [116].

Recently, it was found that H2S could extend postharvest storage of fresh-cut pears and inhibit the growth of fungal pathogens Aspergillus niger and Penicillium expansum [117]. The inhibitory effects of H2S to these fungal pathogens both on inoculated fruits and in vitro culture was confirmed for several fruits including apple, lemon, kiwi and tomato [118]. Fumigation with H2S released from NaHS solution inhibited the growth of fungal pathogens Rhizopus migricans, Mucor rouxianus and Geotrichum candidum on slices of sweet potato (Ipomoea batatas); however, the molecular mechanism of the antifungal H2S action has not been elucidated [119]. Similarly, strawberry (Fragaria ananassa) fruits treated with NaHS solution, or with a combination of NaHS and a NO donor, resulted in increased activities of potentially antifungal enzymes chitinase and beta-1,3-glucanase; however, if this effect can contribute to fruit resistance to fungal contamination and decay has not been tested [120].

Recently, the antimicrobial effect of H2S was corroborated also for a microbial pathogen using Arabidopsis plants infected with P. syringae pv. tomato DC3000 [75]. Plants overexpressing LCD and DCD1 had lower bacterial counts compared to WT plants, unlike LCD and DCD1 knockdown plants exhibiting higher bacterial infection. Furthermore, both LCD and DCD1 overexpressors and plants treated with a NaHS solution showed higher levels of transcription of pathogenesis-related genes. In vitro, P. syringae pv. phaseolicola were found to be resistant to low levels of H2S, whereas high doses of NaHS, NaS and a mitochondria-targeted H2S donor AP39 inhibited cell growth, which was mediated by excision of a genomic island from the bacterial genome [121]. It has been suggested that H2S emitted from the plants in response to bacterial challenges can modify the genomic structure of invading bacteria and thus affect their virulence, which might be exploited to increase crops resistance.

It would be not surprising to found that pathogens have evolved mechanisms for efficient H2S removal and detoxification. H2S is known to block cell respiration as a strong inhibitor of cytochrome c oxidase [122], so its elimination is vital to enable the growth of the microbial pathogen in a low oxygen environment, as in bacterial biofilms, plant xylem vessels or root tissues. In plant pathogens Xylella fastidiosa and Agrobacterium tumefaciens, the biofilm growth-associated repressor (BigR) regulates transcription of the bigR operon, which is important for H2S detoxification through the action of a sulfur dioxygenase in conjunction with a sulfite exporter [123]. It was shown that the respiratory oxidase cytochrome bd in the model microorganism Escherichia coli is resistant to H2S inhibition [124]; however, it seems that this mechanism of respiration resistance to H2S inhibition is present only in enterobacteria.

Hydrogen sulfide in biological systems occurs as diprotonated gaseous H2S as well as HS− anion, which co-exist in a chemical equilibrium [1,37,94]. Gaseous H2S can diffuse freely across the cell membranes and migrate outside of the plant tissues, which will
result in HS⁻ protonation and formation of H₂S to re-establish the equilibrium. Some previous studies concluded that amounts of H₂S produced by plants were not sufficient to exert its toxic effect to plant pathogens [25]. H₂S fumigation experiments showed that even relatively high 20 μM concentrations of H₂S (i.e. two orders of magnitude higher than levels known to decrease plant growth) reduced the growth of fungal pathogen Rhizoctonia solani only by 17%; moreover, prolonged fumigation resulted in increased growth of bacterial colonies [125]. In contrast, other results demonstrated that plant were capable to reduce fungal growth through localized high H₂S production at the site of infection and on the leaf surface. In Arabidopsis thaliana, H₂S concentrations in leaf mesophyll were reported within the range of 4–10 μM [126]. Still, it has not been decisively demonstrated if pathogen destruction is the primary role of H₂S emission or whether it is just its side effect.

Another unresolved issue concerns the capability of plant-produced H₂S to enter pathogen cells. H₂S can be transported from chloroplasts to the cytosol by directed transport enabled by specific transporter proteins. It is supposed that cystolic HS⁻, representing at pH 7.4 approx. 75% of hydrogen sulfide, can be transported to the apoplastic space, although the HS⁻ transporters in plant membranes have not been characterized yet [44]. Apoplastic pH is known to increase during plant-pathogen interactions, which can ensure that the equilibrium is shifted toward HS⁻ anion, thus avoiding diffusion of H₂S back into the plant cells [127]. Nevertheless, the molecular mechanism of how H₂S enters pathogen cells remains unresolved.

Locally increased H₂S concentrations in the site of pathogen attack were suggested to inhibit spore germination or to decrease the growth rate of fungal hyphae. H₂S can be oxidized in presence of electron acceptor or by the catalytic action of superoxide dismutases to elemental sulfur, which is known to be toxic in significantly lower levels compared to H₂S itself [41,88]. Besides its direct toxic effect to plant pathogens, H₂S is involved in the activation of signalling pathways regulating plant responses to pathogen recognition and penetration. Among these mechanisms, protein persulfidation (previously termed also as S-sulfhydration) as a post-translational protein modification can strongly affect protein biological activity [29,39,97]. On reaction with H₂S, persulfidated proteins have cysteine thiol groups modified to –SSH group. In Arabidopsis, OAS-A1 and DES1 is necessary for plant sulfur metabolism and plant responses to stress conditions. Modulations of enzyme activities of OAS-A1 and DES1 are crucial for plant sulfur metabolism and plant responses to stress conditions. Modulations of enzyme activities of OAS-A1 and DES1 were associated with different biological processes [29,39,97].

**Table 2**

| Plant species | Pathogen | Treatment | Observed effects | Source |
|---------------|----------|-----------|------------------|--------|
| Vitis vinifera (grapes) | Uncinula necator | Elemental sulfur | Fungicidal effects of sulfur-derivied H₂S | [112] |
| n.a. | Botrytis cinerea | Saturation of dissolved H₂S to HS⁻ | Inhibition of spore germination | [113] |
| n.a. | Botrytis cinerea, Cladosporium herbarum, Fusarium ducitrium, Monilia cinerea, Monilia fructigena, Penicillium verdicatum, Phytophthora miyabeana | Fumigation with H₂S | Fungicidal effect | [114] |
| Brassica napus | Pyrenopeziza brassicae | n.a. | Increased DES1 activity | [72] |
| Vitis vinifera | Uncinula necator | n.a. | Increased H₂S release in the early phase of infection | [43] |
| Vitis vinifera (leaves) | Uncinula necator | Elemental sulfur applied in the early phase of pathogenesis | Uptake of 10 μM/h of H₂S by the pathogen provides fungicidal effect | [115] |
| Brassica napus | Sclerotinia sclerotiorum | Fumigation with H₂S released from NaHS solution | Increased H₂S release | [111] |
| Actinidia delicosa | Citrus sinensis, Citrus reticulata, Malus domestica, Pyrus bretschneideri, Solanum lycopersicum | Increased H₂S release | Reduced postharvest decay of fruits induced by fungal pathogen | [118] |
| Ipomoea batatas | Rhizopus nigricans, Macor rouxianus, Geotrichum candidum | Fumigation with H₂S released from NaHS solution | Inhibition of fungal growth | [119] |
| Pyrus pyrifolia | Aspergillus niger, Penicillium expansum | H₂S fumigation | Inhibition of fungal growth | [117] |
| Fragaria ananassa (strawberry) | n.a. | Fruit immersion in NaHS solution alone or in combination with a NO donor | Accumulation of antifungal enzymes chitinase and beta-glucanase | [120] |
| Arabidopsis thaliana | Pseudomonas syringae pv. tomato DC3000 | DES1 and DCD overexpression, H₂S donor NaHS, DCD, knock-down, H₂S scavenger hypotaurine | Decreased bacteria count in infected tissues | [75] |
| n.a. | Pseudomonas syringae pv. phaseolicola (Pph) 1302A | H₂S donors (NaHS, Na₂S, AP39 – mitochondria-targeted H₂S donors) | Inhibition of cell growth, increased virulence | [121] |

n.a., not applicable.

Conclusions and future perspectives

Precise regulation of L-Cys homeostasis in the cytosol of plant cells by OAS-A1 and DES1 is necessary for plant sulfur metabolism and plant responses to stress conditions. Modulations of enzyme activities of OAS-A1 and DES during plant development and in reaction to environmental conditions regulate the levels of L-cysteine and H₂S [128]. It should be noted that many published reports describing the effects of H₂S in biological systems including plants were obtained using solutions of NaHS or Na₂S as “H₂S donors”. In solution, these inorganic sulfides are H₂S equivalents, but their dissolution results in a fast formation of high H₂S levels, unlike in case of synthetic H₂S releasing compounds that can...
mimic low and steady H$_2$S levels occurring in living tissues. Moreover, in an aerobic environment, NaHS and Na$_2$S solutions are known to contain numerous sulfur species, including polysulfides, S$_0$ as well as thyl radicals as products of sulfide autoxidation [129]. Introduction and validation of reliable methods for the quantitative analysis of H$_2$S and its metabolites, already widely used within animal H$_2$S research, is required to solve controversies on biological effects of H$_2$S in plants under physiological and stress conditions.

In plant systems, rigorous studies focused to the identification of the active agent and analysis of reaction mechanisms have been lacking, including the proposed insertion of sulfur atom(s) into sulfhydryl groups [130,131]. The first report on the Arabidopsis persulfidome established the prominent role of this post-translational modification in plant H$_2$S signalling [39,97,132]. The recently developed dimedone switch method provided deeper insights into the mechanistic details of protein persulfidation, which occurs on sulfenylated cysteine residues and thus protects proteins from over-oxidation under stress conditions [133]. This mechanism is evolutionary conserved from the bacteria to humans and represent a putative interconnection of signalling pathways of H$_2$S, ROS and NO through diverse cysteine post-translational modifications; however, if this persulfidation mechanism operates also in plants has not been tested yet. Significant gaps exist in the knowledge of the regulation of endogenous H$_2$S levels, the sources and their modulation for H$_2$S signalling as well as the molecular targets of H$_2$S both in plant and pathogen cells. So to fully understand the regulatory and signalling roles of DES1 as well as the molecular targets of H$_2$S in plant cell compartments varying in pH values and levels of ROS, thiol and other reaction partners [28,134].

The major part of our actual knowledge on the role of L-Cys and H$_2$S in plant resistance to phytopathogens has been obtained on model plant species A. thaliana using specific mutants with down- or up-regulated enzymes of L-Cys metabolic pathways. Thus further experiments on agriculturally relevant crops, ideally in field conditions, can contribute to transfer the knowledge on molecular mechanisms of the involvement of sulfur-containing compounds in plant biotic interaction into their practical application towards increased crop resistance. In this regard, new technologies available for direct plant genome editing [135] can be considered a promising tools to further understand plant pathosystems by modulations of genes coding plant proteins and enzymes involved in H$_2$S metabolism, signalling and defence mechanisms activated upon pathogen challenge.

Compliance with ethics requirements

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

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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