Signaling by hydrogen sulfide and cyanide through posttranslational modification

Cecilia Gotor, Irene García, Ángeles Aroca, Ana M. Laureano-Marín, Lucía Arenas-Alfonseca, Ana Jurado-Flores, Inmaculada Moreno, Luis C. Romero

Instituto de Bioquímica Vegetal y Fotosíntesis. Consejo Superior de Investigaciones Científicas and Universidad de Sevilla. Avenida Américo Vespucio, 49. 41092 Seville. Spain

E-mails: gotor@ibvf.csic.es (Cecilia Gotor), irene.garcia@ibvf.csic.es (Irene García), mangelesaroca@yahoo.es (Ángeles Aroca), analaureano85@gmail.com (Ana Laureano-Marín), lucia.arenas@ibvf.csic.es (Lucía Arenas-Alfonseca), ana.jurado@ibvf.csic.es (Ana Jurado-Flores), inmaculada@ibvf.csic.es (Inmaculada Moreno), lromero@ibvf.csic.es (Luis C. Romero)

Corresponding author: Cecilia Gotor. Phone: 34-954489516

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Highlights

Novel aspects of the plant sulfur research are focused on the roles of sulfide and cyanide in signaling. Their mechanisms of action are related to chemical features, taking place through posttranslational modifications.

Abstract

A new concept has arisen regarding two cysteine metabolism-related molecules, hydrogen sulfide and hydrogen cyanide, which are considered toxic but have now been established as signaling molecules. Hydrogen sulfide is produced in chloroplasts through the sulfite reductase activity and in the cytosol and mitochondria by the action of sulfide-generating enzymes and regulates/affects essential plant processes such as plant adaptation, development, photosynthesis, autophagy and stomatal movement, where interplay with other signaling molecules occurs. The mechanism of action of sulfide, which modifies protein cysteine thiols to form persulfides, is related to its chemical features. This posttranslational modification, called persulfidation, could play a protective function for thiols against oxidative damage. Hydrogen cyanide is produced during the biosynthesis of ethylene and camalexin in noncyanogenic plants and is detoxified by the action of sulfur-related enzymes. Cyanide functions include the breaking of seed dormancy, modifying the plant responses to biotic stress, and inhibition of root hair elongation. The mode of action of cyanide is under investigation, although it has recently been demonstrated to perform posttranslational modification of protein cysteine thiols to form thiocyanate, a process called *S*-cyanylation. Therefore, the signaling roles of sulfide and most probably of cyanide are performed through the modification of specific cysteine residues, thus altering protein functions.

Keywords: β-cyanoalanine synthase, cyanide, L-cysteine desulfhydrase, persulfidation, redox regulation, *S*-cyanylation, sulfide, thiol group

Abbreviations: CAS, β-cyanoalanine synthase; H_2O_2, hydrogen peroxide; L-CDES, L-cysteine desulfhydrase; $NO^{\bullet}$, nitric oxide; OASTL, *O*-acetylserine(thiol)lyase; RNS, reactive nitrogen species; ROS, reactive oxygen species

Introduction

Cysteine is the reduced sulfur-containing metabolite that is first synthesized by plants from the most abundant inorganic oxidized sulfur molecule in soil, sulfate (Garcia *et al.*, 2015; Gotor *et al.*, 2017; Takahashi *et al.*, 2011). In all living systems, cysteine is fundamental as a proteinogenic amino acid because it defines the structure and function of proteins through the conversion of cysteine thiol groups into disulfide bridges (Tridevi *et al.*, 2009). Specifically, the cysteine-based redox modifications are the basis of different posttranslational modifications that affect and regulate the functions of many proteins (Buchanan and Balmer, 2005; Chung *et al.*, 2013). Protein thiols are also crucial to many enzymatic reactions that require the involvement of cysteines in active sites (Richau *et al.*, 2012), or the binding of metals in specific proteins involved in electron transfer reactions (Balk and Schaedler, 2014). Another very important feature of cysteine is its role as a precursor molecule from which the majority of sulfur-containing metabolites are synthesized. A representative of this type of metabolite is glutathione, which plays major roles in biosynthetic pathways, detoxification, transport, redox signaling and reactive oxygen species (ROS) metabolism (Noctor *et al.*, 2012).

Due to the significance of sulfur-containing compounds in plant metabolism, an intense investigation has been progressively conducted since the late 1980s to the present. Important breakthroughs, such as the elucidation of the entire genome sequence of the model plant *Arabidopsis thaliana*, the development of research tools to perform functional genomics, and the blossoming of omics technologies, have allowed relevant advances in knowledge of the sulfate assimilation pathway and, in general, sulfur metabolism in plants (Garcia *et al.*, 2015; Gotor *et al.*, 2017; Koprivova and Kopriva, 2014; Ravilious and Jez, 2012; Rennenberg and Herschbach, 2014; Takahashi *et al.*, 2011).

Currently, the most novel aspect of research on the role of sulfur in plants is focused on plant signaling. A fundamental change in the concept of sulfur compounds and related molecules performing signaling roles and thus regulating/affecting essential processes in the plant has occurred (Gotor *et al.*, 2015; Romero *et al.*, 2014). In this review, we focus on two molecules related to the metabolism of cysteine, sulfide and cyanide, which have been very recently shown to be involved in signaling of different plant processes. A comparison with other signaling molecules such as nitric oxide, hydrogen peroxide and ethylene shows many similarities between them and hydrogen sulfide and cyanide. Like

the other established signaling molecules, sulfide and cyanide are low-molecular-weight molecules with high to moderate chemical reactivity that are able to modify specific targets. They are also gases that can cross membranes to reach different cell compartments and perform their roles inside. Another common feature between all of them is the duality that they show; that is, above a certain concentration threshold, they are toxic molecules, but below that threshold, they are important signaling molecules.

Sulfide: from toxic to signaling molecule

Hydrogen sulfide has long been considered a poisonous substance hazardous to the life and the environment. Although it was known to be present in mammalian tissues, it was not until the late 20th century that the endogenous production and signaling role of hydrogen sulfide as a neuromodulator was first established (Abe and Kimura, 1996). Intense research followed; this molecule is now accepted as a relevant signaling molecule in physiology, and it is included in the family of gasotransmitters in addition to nitric oxide ($NO^{\bullet}$) and carbon monoxide (CO) (Gadalla and Snyder, 2010; Lowicka and Beltowski, 2007; Wang, 2002; Wang, 2014). Hydrogen sulfide is produced and metabolized by the cells in an accurate way, and the physiological functions for which it has been implicated are continuously increasing. Thus, it plays important biological roles in numerous systems of the body such as the cardiovascular, nervous, endocrine, gastrointestinal, immune, and respiratory systems. Moreover, H_2S has clinical relevance because the alteration of H_2S metabolism is often associated with different pathologies such as diabetes and cancer (Olas, 2015; Paul and Snyder, 2015; Wang, 2012).

Similar to animal systems, the change in the concept of hydrogen sulfide as a toxic molecule to a regulator has also occurred in plant systems. An exponential increase in the number of plant studies in recent decades has led H_2S to be considered to have the same relevance as the signaling molecules $NO^{\bullet}$ and hydrogen peroxide (H_2O_2) (Calderwood and Kopriva, 2014; Garcia-Mata and Lamattina, 2013; Guo *et al.*, 2015; Jin and Pei, 2015; Lisjak *et al.*, 2013). Hydrogen sulfide has been shown to produce physiological effects on a wide range of processes vital for plant performance. Thus, it has been studied in the plant responses to many different plant stresses, mainly abiotic stresses, ranging from metal stresses to drought, salinity, hypoxia, heat, and many others (Table 1). H_2S allows for plant adaptation against these adverse environmental conditions, and its beneficial

effects affect important aspects of development such as seed germination, root elongation, and plant survival. In many cases, hydrogen sulfide alleviates oxidative damage through the increase of antioxidative defenses. The activity of several enzymes involved in ROS detoxification or the level of the antioxidants glutathione and ascorbic acid were increased by H_2S treatments in stressed cucumber seedlings (Yu *et al.*, 2013), maize (Shan *et al.*, 2014), wheat (Khan *et al.*, 2017; Shan *et al.*, 2018), alfalfa (Wang *et al.*, 2012), and rice (Mostofa *et al.*, 2015) or during tomato fruit ripening (Yao *et al.*, 2018) at concentrations ranging from 25 μM to 600 μM. (Table 1). The role of H_2S in plant resistance to pathogens has not been extensively studied, although it has been reported that the release of H_2S correlated with an increased resistance to fungal infection. Therefore, the previously described concept of sulfur-induced resistance (SIR), which proposed that the sulfur fertilization of crops reduces sensitivity to pathogens, was suggested to be mediated by hydrogen sulfide (Bloem *et al.*, 2004). In addition, acquired pathogen resistance has been suggested to be related to an increase in endogenous sulfide content (Alvarez *et al.*, 2012a; Gotor *et al.*, 2015; Shi *et al.*, 2015).

Hydrogen sulfide also exerts physiological effects on processes that are critical for adequate plant performance including different aspects of the plant developmental program such as seed germination (Baudouin *et al.*, 2016; Dooley *et al.*, 2013), root development (Fang *et al.*, 2014; Jia *et al.*, 2015; Li *et al.*, 2014b), leaf senescence (Alvarez *et al.*, 2012b), and postharvest senescence and fruit ripening (Huo *et al.*, 2018; Ziogas *et al.*, 2018). Other essential plant process like photosynthesis is enhanced by H_2S through promotion of chloroplast biogenesis, photosynthetic enzyme expression and thiol redox modification (Chen *et al.*, 2011). Hydrogen sulfide also delays programmed cell death by the modulation of glutathione homeostasis and heme oxyenase-1 expression. (Xie *et al.*, 2014). Moreover, the progression of autophagy is negatively regulated by H_2S in a way unrelated of sulfur nutrition and by mechanism of action independent of redox conditions (Alvarez *et al.*, 2012b; Gotor *et al.*, 2015; Laureano-Marín *et al.*, 2016; Laureano-Marin *et al.*, 2016). Of particular interest is that hydrogen sulfide regulates the stomatal movement, which has important implications for countering the osmotic and drought stress conditions. Numerous studies have demonstrated that H_2S is a component of the abscisic acid signaling network in guard cells and specifically targets ion channels. The existence of complex crosstalk with the other signaling molecules $NO^{\bullet}$ and H_2O_2 has also been described (Garcia-Mata and Lamattina, 2010; Honda *et al.*, 2015; Jin *et al.*, 2013; Lisjak *et al.*, 2010; Papanatsiou *et al.*, 2015; Scuffi *et al.*, 2014; Scuffi *et al.*, 2018; Wang

et al., 2016). Different interplays between hydrogen sulfide and other signaling molecules and phytohormones have been observed in the various processes by which sulfide exerts important physiological effects (Table 1).

Biosynthesis of hydrogen sulfide inside the cells

Hydrogen sulfide is endogenously produced in animal cells mainly by the action of enzymes involved in the metabolism of sulfur amino acids: cystathionine gamma-lyase, cystathionine beta-synthase and 3-mercaptopyruvate sulfurtransferase. These enzymes show differential tissue and subcellular localization and control the synthesis of H_2S with different efficiencies (Kabil and Banerjee, 2010; Kimura, 2011, 2015). In addition, other pathways of H_2S production are currently being identified (Olson, 2018).

The endogenous production of hydrogen sulfide by plant cells is also related to the biosynthesis and metabolism of cysteine (Fig. 1). Its main source is located in the chloroplast, where sulfite is reduced to sulfide by the action of sulfite reductase during the photosynthetic sulfate assimilation pathway (Garcia *et al.*, 2015; Takahashi *et al.*, 2011). Indeed, when subcellular metabolite concentrations were estimated, plastids contained the highest sulfide concentrations (Krueger *et al.*, 2009). It was proposed that chloroplastic H_2S could reach other cellular compartments by diffusion through membranes; however, other enzymatic processes have been demonstrated to be responsible for the sulfide synthesis in other subcellular compartments in plant cells, which is described below. Hydrogen sulfide is a weak acid with pK_{a1} and pK_{a2} of 6.9 and >12 (Kabil and Banerjee, 2010), and in aqueous solution it dissociates into the H^+ and HS^- ions; and the anionic forms are unable to cross the chloroplast envelope membrane. Under physiological, neutral pH conditions, two-thirds of hydrogen sulfide is in the form of HS^-, which can dissociate to H^+ and S^{2-} at higher pH (Kabil and Banerjee, 2010; Lowicka and Beltowski, 2007). The chloroplast stroma increases the pH from neutral to relatively basic (pH 8) upon illumination for the optimization of photosynthetic reactions (Höhner *et al.*, 2016; Shen *et al.*, 2013). Therefore, most sulfide present inside the chloroplast is dissociated into its ionic form HS^-, which is unable to freely permeate the membrane and requires a currently unknown active transporter. However, in bacteria a hydrosulfide ion channel has already been described (Czyzewski and Wang, 2012).

The last enzymatic step of the photosynthetic sulfate assimilation pathway consists of the synthesis of cysteine catalyzed by the *O*-acetylserine(thiol)lyase (OASTL) enzymes. Although different OASTLs are localized to the chloroplasts, mitochondria and cytosol, which produces diversity in subcellular cysteine pools, it is currently known that cysteine is synthesized mainly in the cytosol (Garcia *et al.*, 2015; Takahashi *et al.*, 2011) (Fig. 1). Accordingly, the cytosol is the compartment with the highest cysteine concentration, which is estimated to be over 300 μM, while in other compartments, the cysteine concentrations are less than 10 μM (Krueger *et al.*, 2009). Therefore, the cytosol is a source of hydrogen sulfide metabolically generated from cysteine, and several types of cysteine-degrading enzymes have been reported in plant systems (Papenbrock *et al.*, 2007) (Fig. 1). The L-cysteine desulfhydrase (L-CDES) enzymes catalyze the conversion of L-cysteine to sulfide, ammonia and pyruvate, and some L-CDES enzymes from Arabidopsis have been characterized in more detail (Alvarez *et al.*, 2010; Gotor *et al.*, 2010; Shen *et al.*, 2012). In addition to L-CDES, in different plant species, D-cysteine desulfhydrase (D-CDES) enzymes that are specific for D-cysteine as a substrate and are completely different proteins than the L-CDES enzymes have been described (Cui *et al.*, 2014; Riemenschneider *et al.*, 2005). Other enzymes that catalyze the desulfurization of cysteine are the NifS-like proteins, which catalyze the conversion of cysteine to alanine and elemental sulfur or sulfide. These proteins provide sulfur for the synthesis of biotin and thiamine, the formation of Fe-S clusters and the formation of molybdenum cofactors and are located in the cytosol, chloroplasts and mitochondria (Van Hoewyk *et al.*, 2008).

Mitochondria can also be a source of hydrogen sulfide that is generated during the detoxification of cyanide by the action of the β-cyanoalanine synthase (CAS), which catalyzes the formation of β-cyanoalanine (Hatzfeld *et al.*, 2000; Yamaguchi *et al.*, 2000) (Fig. 1). The hydrogen sulfide produced by CAS is incorporated by the mitochondrial isoform of OASTL into the synthesis of cysteine, which is used by CAS to detoxify cyanide, producing a cyclic pathway in the mitochondria (Alvarez *et al.*, 2012c). In any case, if an excess of hydrogen sulfide occurs, similar to chloroplasts, the relatively basic pH of the mitochondrial stroma in metabolically active cells would provoke the accumulation of the charged HS^- form, and its transport would be avoided (Shen *et al.*, 2013).

In addition, the endogenous production of hydrogen sulfide has been shown to be induced in response to various abiotic stress conditions, and different molecules related

to signaling pathways are involved. Increased activities of the H_2S-generating desulfhydrases correlate with the induction of sulfide levels under stress conditions (Guo *et al.*, 2017; Jin *et al.*, 2011; Kabala *et al.*, 2018; Lai *et al.*, 2014), and the involvement of ethylene (Jia *et al.*, 2018), or NO (da Silva *et al.*, 2017; Khan *et al.*, 2017) has been described. An interesting study has recently provided evidence for the regulatory mechanism of H_2S production in response to chromium stress, which is enhanced through the calcium/calmodulin 2-mediated pathway, involving the transcription factor TGA3 (Fang *et al.*, 2017).

Hydrogen sulfide mechanism of action

Despite the fact that the number of physiological processes known to be affected by H_2S in plants has been continuously increasing as well as the evidence of its biological function in other organisms, there is an important lack of understanding of the mechanism by which H_2S performs its function. Without a doubt, the mechanism of action of H_2S must be related to the characteristics of its chemical reactivity with other molecules such as its affinity for metal centers in metalloproteins, its reactivity with other small oxygen and nitrogen species (ROS and RNS), and its capacity to modify protein cysteine residues to form persulfides (Fig. 2).

H_2S can coordinate the metal center of metalloproteins (Filipovic *et al.*, 2018) and attach covalently to heme porphyrins, acting as a potent inhibitor of mitochondrial cytochrome c oxidase and inhibiting respiration in mitochondria where sulfide is detoxified (Birke *et al.*, 2015). H_2S can also react with leghemoglobin to reduce its iron center and form a complex in a process that can be reversed by oxidizing or reducing agents (Puppo and Davies, 1995). In mammals, the reduction of ferric cytochrome c by H_2S, cytochrome c release during apoptosis and stimulation of procaspase 9 persulfidation have also been demonstrated (Vitvitsky *et al.*, 2018).

The sulfur atom in H_2S is at its lowest oxidation state (-2) and can only be oxidized; therefore, it acts as a reductant (Zaffagnini *et al.*, 2019). The reaction of H_2S with O_2 is thermodynamically disfavored, but several biological oxidants such as hydroxyl radical ($HO^{\bullet}$), nitrogen dioxide ($NO_2^{\bullet}$), superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), peroxynitrite (ONOOH) and hypochlorite (HOCl) can support its oxidation (Li and Lancaster, 2013). $NO^{\bullet}$ molecules can also react with H_2S, which can lead to the formation

of various nitrogen (N_2O, HNO) and sulfur derivatives (S^0, $S^{\bullet-}$), including S-nitrosothiols (Filipovic *et al.*, 2018). Studies of the cellular crosstalk between H_2S and S-nitrosothiols suggest that sulfide species may play a role in modulating the profile of these molecules through the reaction of H_2S with small or protein S-nitrosothiol molecules to form nitropersulfide ($SSNO^-$), polysulfides (HS_n^-) and dinitrososulfite [$ONN(OH)SO_3^-$], three products with distinct bioactive profiles that can modulate biological processes (Cortese-Krott *et al.*, 2015; Filipovic *et al.*, 2012). Although the direct reactions of H_2S with ROS or RNS have not been described and quantified in plants cells, the role of H_2S in the activation of antioxidant systems has been described in several plants, as described in previous section. The level of $NO^{\bullet}$ is also elevated by H_2S treatment of salt-stressed cucumbers, or tomato under excess of nitrate (Guo *et al.*, 2018) but direct reactions between H_2S and $NO^{\bullet}$ have not been measured in plant systems.

A third mechanism of action of H_2S, based on its chemical reactivity, is the modification of proteins by the oxidation of cysteine residues to form the corresponding persulfides. The first described method for detection of persulfides consisted in a first blocking step of the protein thiol residues in which persulfides remain free, followed by a reaction of persulfides with a biotinylating agent. In this way, all persulfide groups presente in proteins are transformed to biotinylated residues, which allowed the purification and identification of modified proteins. Using this modified biotin switch assay, Snyder and colleagues described for the first time protein S-sulfhydration (now called persulfidation) in mouse liver and detected this modification in proteins such as glyceraldehyde-3-phosphate dehydrogenase, β-tubulin and actin (Mustafa *et al.*, 2009). The identification of persulfidated proteins in mammalian systems and the pathophysiological processes in which they are involved are numerous (Zhang *et al.*, 2017); however, the specific chemical reactions by which this modification takes place are not clearly established due to the chemical complexity of sulfur and because there are probably several chemical scenarios, depending on the environment, that can lead to this modification (Filipovic *et al.*, 2018; Mishanina *et al.*, 2015). H_2S, or its ionic forms, HS^- and S^{2-}, cannot react directly with protein thiols and requires the presence of an oxidant; thus, it can react with oxidized cysteine residues as sulfenic acids (R-SOH). Disulfides and S-nitrosylated cysteines can also react with H_2S, leading to the formation of persulfidated residues plus thiol and HNO, respectively. Finally, oxidized sulfide species as polysulfides can also react and transfer a sulfane sulfur atom (S^0) to cysteine thiols or

307 be the carrier of persulfides by displacement reaction (Zhang *et al.*, 2017). All of these
308 processes may lead to the persulfidation of proteins.
309 In plants, a proteomic analysis in Arabidopsis untreated leaf samples using the
310 modified biotin switch assay described the presence of 106 persulfidated proteins and,
311 similar to mammalian systems, glyceraldehyde-3-phosphate dehydrogenase, β-tubulin
312 and actin were also detected as posttranslationally modified proteins (Aroca *et al.*, 2015).
313 After increasing doubts about the specificity of the blocking reagent used in the modified
314 biotin switch assay, a second method to detect the persulfidated proteins was described
315 initially in animal system, the tag switch assay (Zhang *et al.*, 2014). In this method, a
316 different blocking reagent is used that reacts equally with thiols and persulfide groups in
317 the first step, however, the resulting derivatives show different reactivity to nucleophilic
318 attack. In the second step, a biotin-linked cyanoacetate reagent specifically reacts with
319 the persulfide derivatives. Thus, the number of proteins susceptible to persulfidation in
320 Arabidopsis was later updated to 3478 in untreated wild-type leaves (present in at least
321 one replica sample) by the use of the tag switch assay, which allowed labelling of cysteine
322 persulfides with greater specificity (Aroca *et al.*, 2017a). This number shows that 10% of
323 the Arabidopsis proteome is persulfidated under normal growth conditions and that this
324 modification could be involved in a great variety of biological processes. The major
325 sulfide source in leaf tissue must come from chloroplast sulfate assimilation, and up to
326 22% of persulfidated proteins are localized to the plastid and function in the
327 photosynthetic light reactions in thylakoids and in the Calvin-Benson cycle in the stroma
328 (Fig. 3); with most of them with reactive cysteines reported to be redox regulated (Aroca
329 *et al.*, 2017a; Buchanan and Balmer, 2005). However, almost 50% of persulfidated
330 proteins are localized in the cytosol. This observation is not strange, since the cytosol is
331 where cysteine is mainly synthesized and several types of cysteine-degrading and sulfide-
332 releasing enzymes are located (Fig. 1) (Heeg *et al.*, 2008; Watanabe *et al.*, 2008).
333 As mentioned, autophagy and ABA signaling in guard cells are two of the
334 physiological processes demonstrated to be regulated by H_2S in plants, and proteomic
335 analysis also shows that some proteins involved in the ABA signaling pathway can be
336 persulfidated. Among them are the hormone receptors PYRABACTIN RESISTANCE 1
337 (PYR1) and PYR1-LIKE PROTEIN 1(PYL1), the SNF1-RELATED PROTEIN
338 KINASE 2.2 (SnRK2.2) and 2.6 (OST1), and several potassium channels (KAB1,
339 AKT2), and therefore this proteomic analysis points them out as putative targeted
340 candidates for H_2S-dependent stomatal closure regulation (Scuffi *et al.*, 2014). The

presence of several autophagy-related proteins such as ATG3, ATG5 and ATG18a also highlights them as possible candidates for the regulation of autophagy by H_2S (Alvarez *et al.*, 2012b; Laureano-Marín *et al.*, 2016). Further investigation is required to identify the specific persulfidated proteins responsible of the H_2S-regulation of autophagy and stomata movement.

Although the number of proteins susceptible to persulfidation in plants is high, the biological significance of this posttranslational modification on plant processes is still limited. The five glyceraldehyde-3-phosphate dehydrogenases from Arabidopsis can be persulfidated, and this modification can affect either its activity or its cytosolic/nuclear partitioning, as reported for the cytosolic GapC1 and GapC2 isoforms, which are persulfidated at Cys^{160} as analyzed by parallel reaction monitoring (Aroca *et al.*, 2017b; Aroca *et al.*, 2015). H_2S also signals and regulates the actin cytoskeleton and root hair growth; a higher level of H_2S thereby causes the depolymerization of F-actin bundles by the persulfidation of Arabidopsis ACTIN 2 (ACT2) at Cys^{287}, a conserved residue in actin sequences (Li *et al.*, 2018). In addition, in tomato plants under osmotic stress, ethylene regulates stomatal closure and induces the production of H_2S in guard cells, but H_2S feedback also regulates ethylene biosynthesis through inhibiting the enzymatic activity of 1-aminocyclopropane-1-carboxylic acid oxidase (LeACO1) by persulfidation at Cys^{60} (Jia *et al.*, 2018).

As mentioned before, H_2S is produced in plant cells from several sources, ranging from chloroplastic sulfate assimilation coupled to cysteine biosynthesis through *O*-acetylserine(thiol)lyases to the enzymatic production of H_2S in the cytosol and mitochondria from cysteine desulfhydrases or β-cyanoalanine synthase, among others (Fig. 1). Chloroplastic sulfide synthesis must occur mostly in the light coupled to photosynthesis because it requires reduced ferredoxin as an electron donor for sulfite reductase; however, enzymatically produced H_2S coupled to cysteine degradation or cyanide detoxification can occur either in the light or in the dark. Since the light reactions of photosynthesis constitute an important source of ROS, we can expect that part of the H_2S produced through sulfite reduction can be partially oxidized back to hydrogen disulfide or polysulfide as a stochastic event (Fig. 4). This reactive sulfur species can drive the persulfidation of proteins within the chloroplast. In fact, as mentioned, up to 22% of the proteins identified in the proteomic analysis of Arabidopsis leaf tissue are localized in the chloroplast (Aroca *et al.*, 2017a). Although plant cells have an extensive battery of enzymes that facilitate the reduction of proteins by cysteine thiol-disulfide

exchange such as thioredoxins, glutaredoxins, protein disulfide isomerases, along with different ROS scavenging systems in which glutathione is involved, and ROS detoxification enzymes, they are not enough to control the high level of ROS under stress conditions that can lead to the overoxidation of cysteine residues originating the irreversible sulfinic (P-SO_2H) or sulfonic (P-SO_3H) motif (Fig. 4). H_2S reacts with sulfenic acid to form persulfide, and in fact, oxidative conditions increase the level of persulfidation in culture cells treated with H_2O_2 (Cuevasanta *et al.*, 2015; Wedmann *et al.*, 2016). Persulfidated residues have lower pK_a values than their corresponding thiols, and the deprotonated forms (RSS^-) are more nucleophilic, enabling reactions with ROS. At pH 7.4, it has been measured that the reaction of peroxinitrite with albumin persulfide is an order of magnitude higher than reduced albumin (Cuevasanta *et al.*, 2015). Analogously to the reaction of thiol with H_2O_2, under persistent oxidation stress, persulfidated proteins can react with ROS to form perthiosulfenic acids (R-SSOH) as predicted by density functional theory calculation and observed in epidermal growth factor receptor (Heppner *et al.*, 2018), In the presence of excess oxidant, perthiosulfenic acid could be oxidized to perthiosulfinic and perthiosulfonic acid, species detected in papain, albumin and glutathione peroxidase (Benchoam *et al.*, 2019). Although sulfinic and sulfonic acids are generally considered irreversible modifications, perthiosulfinic and perthiosulfonic acid can be easily reduced back by reductants or by thioredoxin systems to restore the free thiols (Filipovic, 2015; Filipovic *et al.*, 2018; Millikin *et al.*, 2016). Human thioredoxins have been shown to have 10-fold higher reactivity towards cysteine persulfides than towards cystines (Wedmann *et al.*, 2016). Persulfidation can therefore serve to protect protein thiols from oxidative damage (Filipovic *et al.*, 2018).

Hydrogen cyanide action and signaling

Cyanide is a low-molecular-weight molecule that is highly reactive. It reacts with Schiff bases and keto radicals, producing cyanohydrins and nitrile derivatives, respectively. Its participation in the production of ribonucleotides, lipids and amino acids is likely due to this reactivity (Patel *et al.*, 2015). Cyanide is able to chelate di- and trivalent metallic ions in the prosthetic groups of some metalloproteins, affecting their function (Nagahara *et al.*, 1999). Its action is lethal in mitochondria, where it blocks electron transfer from cytochrome c to oxygen and interrupts mitochondrial oxygenic

409 respiration (Donato *et al.*, 2007), but it also affects photosynthetic enzymes in
410 chloroplasts (Berg and Krogmann, 1975).
411 Despite its toxicity, cyanide is produced naturally in organisms from all kingdoms,
412 including bacteria, fungi, arthropods, vertebrates and plants. In most bacteria and fungi,
413 cyanide is produced directly and stoichiometrically from the amino acid glycine in an
414 oxidative reaction catalyzed by the enzyme cyanide synthase (Blumer and Haas, 2000;
415 Knowles, 1976). In the case of some cyanide-producing algae such as *Chlorella vulgaris*,
416 the precursors for the synthesis of cyanide are D-histidine and other amino acids
417 (Pistorius *et al.*, 1977). In the animal kingdom, some arthropods produce cyanogenic
418 glucosides or accumulate the cyanogenic compounds produced by their host plants
419 (Zagrobelny *et al.*, 2008). Cyanide production has also been described in mammalian
420 cells, where glycine gives cyanide in a reaction catalyzed by peroxidases (Borowitz *et al.*,
421 1997; Stelmaszynska, 1986). In plants, cyanide biosynthesis is produced through two
422 different mechanisms, one associated with the production of ethylene and camalexin
423 (Bottcher *et al.*, 2009; Peiser *et al.*, 1984) and one associated with the degradation of
424 cyanogenic glucosides and cyanolipids (Moller, 2010; Poulton, 1990). Only plants
425 producing high concentrations of cyanide through the second mechanism are considered
426 cyanogenic, and they liberate cyanide from cyanogenic glucosides and lipids when they
427 are in contact with predatory herbivores (Conn, 2008; Miller and Conn, 1980).
428 Cyanogenic glucosides are widely distributed in all groups of plants, and since they have
429 been extensively studied and recently reviewed, we will not review them here (Gleadow
430 and Moller, 2014; Mithofer and Boland, 2012; Moller, 2010; Sun *et al.*, 2018; Zagrobelny
431 *et al.*, 2008). In noncyanogenic plants, cyanide is produced exclusively during the
432 biosynthesis of ethylene and the antipathogenic molecule camalexin (Glawischnig, 2007;
433 Wang *et al.*, 2002; Yip and Yang, 1988) (Fig. 5).
434 Cyanide functions are diverse and sometimes controversial or unknown (Borowitz *et*
435 *al.*, 1997; Knowles, 1976; Siegien and Bogatek, 2006; Zagrobelny *et al.*, 2008;
436 Zagrobelny *et al.*, 2018). In general, cyanide is associated with toxicity mechanisms for
437 defense towards detrimental organisms, but other roles have also been described or
438 suggested. In bacteria, cyanogenic glucosides and cyanide itself can serve as a nitrogen
439 source or reservoir ; likewise, they participate in the biocontrol mechanisms of certain
440 Pseudomonas strains (Kuzmanovic *et al.*, 2018) and cyanide functions as virulence factor
441 in some strains of the human opportunistic pathogen *P. aeruginosa* (Chowdhury and
442 Bagchi, 2017). In different cyanogenic arthropods, cyanide and cyanogenic glucosides

443 may act as pheromones for mating (Zagrobelny *et al.*, 2007; Zagrobelny *et al.*, 2018). In
444 neurons, cyanide production activates synaptic receptors and it is necessary for the
445 analgesic action of opioid compounds (Gunasekar *et al.*, 2000; Gunasekar *et al.*, 2004).
446 In blood, phagocytes are able to produce cyanide from thiocyanate when challenged by
447 bacteria or the T-cell stimulators (Stelmaszynska, 1986).

448 In plants, in addition to the protective role of cyanogenic compounds, cyanide itself
449 plays an important role in several essential biological processes that deserve special
450 attention in this review because they have driven the change in the perception of cyanide
451 from it being a poison to it being a signaling molecule (Fig. 6). It is well known that the
452 exogenous addition of cyanide breaks dormancy and thus stimulates seed germination.
453 Indeed, transient treatment with millimolar concentrations of cyanide stimulate the
454 germination of rice, barley, apple, sunflower and Arabidopsis seeds, among others
455 (Bethke *et al.*, 2004; Bogatek *et al.*, 1991; Cohn and Hughes, 1986; Oracz *et al.*, 2008),
456 and cyanide emission has been observed during the pregermination stage of many seeds
457 including those from noncyanogenic plants (Esashi *et al.*, 1991). Furthermore, the
458 germination burst observed in many species after a wildfire is due in part to cyanohydrins
459 present in the smoke that release cyanide (Flematti *et al.*, 2013; Nelson *et al.*, 2012). In
460 apple, the effect of cyanide in dormancy alleviation depends on the transient production
461 of ROS and indirect protein carbonylation and ethylene emission (Gniazdowska *et al.*,
462 2010; Krasuska *et al.*, 2014). However, although the alleviation of sunflower dormancy
463 by cyanide is also dependent on ROS production and protein carbonylation (Oracz *et al.*,
464 2007), it seems to be independent of ethylene production but needs the ethylene signaling
465 pathway, suggesting that it is required for both ethylene and cyanide action (Oracz *et al.*,
466 2008). Finally, sugar metabolism is increased by cyanide treatment in apple and walnut
467 kernel embryos during cyanide-induced alleviation of dormancy (Gerivani *et al.*, 2016;
468 Siegien and Bogatek, 2006). The understanding of the crosstalk between cyanide and
469 hormone signaling during germination is an open subject and requires further
470 investigation.

471 Exogenously applied cyanide also has an effect on the plant response to biotic stress.
472 It enhances the resistance of tobacco and Arabidopsis plants to viral attack independently
473 from the PATHOGENESIS-RELATED (PR) protein induction or signaling mediated by
474 NON-EXPRESSER OF PR GENES 1 (NPR1) but likely involves the alternative oxidase
475 (Chivasa and Carr, 1998; Wong *et al.*, 2002), and protects rice from blast fungus infection
476 (Iwai *et al.*, 2006; Seo *et al.*, 2011). Nevertheless, the effects of endogenously produced

cyanide have been relatively less studied thus far.

Plants have two enzymatic families for the detoxification of cyanide, β-cyanoalanine synthases (CAS, EC 4.4.1.9) and sulfurtransferases (STR, EC 2.8.1.1), which incorporate cyanide into cysteine and thiosulfate or mercaptopyruvate, respectively. In *A. thaliana*, cyanide remains at nontoxic levels mainly due to CAS activity, with the mitochondrion-localized CAS-C1 (formerly CYS-C1) being the main CAS (Arenas-Alfonseca *et al.*, 2018b; Hatzfeld *et al.*, 2000). *cas-c1* T-DNA insertion mutants that accumulate between 20 and 40% more cyanide in their tissues than in those of wild-type plants show a severe defect in root hair elongation (Garcia *et al.*, 2010). Through the measurement of cyanide in roots and treatment with exogenous cyanide, the ethylene donor 1-aminocyclopropane-1-carboxylic acid (ACC) and a cyanide antidote (hydroxocobalamin, COB), it has been shown that the inhibition of root hair elongation is due specifically to the cyanide produced by *cas-c1* mutants (Arenas-Alfonseca *et al.*, 2018b; Garcia *et al.*, 2010). The analysis of genetic crosses between *cas-c1* and root hair mutants concluded that cyanide action is exerted at the early steps of the root hair elongation pathway and that this is independent of ROS production or direct NADPH oxidase inhibition (Arenas-Alfonseca *et al.*, 2018a, b).

In addition, during compatible and incompatible plant-bacterium interactions, cyanide accumulation and CAS-C1 activity are regulated in opposite manners, resulting in an increase in cyanide concentration and a decrease in CAS-C1 expression in the case of incompatible interactions. Mutation of *CAS-C1* increases the tolerance to biotrophic pathogens, and this effect is reversed in the presence of COB, indicating that the endogenously produced cyanide might activate the pathogen response mediated by salicylic acid, hence influencing the plant immune system (Garcia *et al.*, 2013). The mechanisms that underlie cyanide modulation, the mode of action and the specific targets of this molecule are the subjects of recent investigation.

The results described here suggest that the cyanide molecule, which has a low molecular weight, a high solubility in water and a low melting point, could act as a signaling molecule in plants, similar to other molecules with widely accepted signaling roles such as $NO^{\bullet}$, H_2O_2, and H_2S (Siegien and Bogatek, 2006). The mode of action of these signaling molecules acts by provoking posttranslational modifications in proteins such as nitrosylation, oxidation and persulfidation specifically at the -SH groups of cysteines (Aroca *et al.*, 2018). Chemically, cyanide *per se* is capable of *S*-cyanylating oxidized cysteine residues by the nucleophilic displacement of one of the sulfur atoms of

511 the disulfide bridge to form a thiocyanate (Gawron, 1966). Although this protein
512 modification had never been described before in any organism, it has been shown that
513 cyanide itself could produce the *S*-cyanilation by the addition of SCN groups to cysteines
514 and thus alter or modulate the function of proteins with this new PTM. It is interesting to
515 note that cyanide can form covalent adducts with the cysteines of immunoglobulin G and
516 serum albumin in human plasma, which could serve as an indicator of cyanide poisoning
517 in patients (Fasco *et al.*, 2007). Very recently, it has been shown that *S*-cyanylation exists
518 naturally in plants and modifies the activity of some proteins *in vitro* (Garcia *et al.*, 2019)
519 (Fig. 6) . Indeed, a method has been adapted based on the hypersensitivity to hydrolysis
520 of the peptide bond adjacent to an *S*-cyanylated cysteine at basic pH, especially in the
521 presence of NH_4OH (Qi *et al.*, 2001; Wu and Watson, 1998). By directly treating extracts
522 of plant proteins with NH_4OH to induce the cleavage of the peptide bond adjacent to an
523 *S*-cyanylated cysteine, proteins that undergo hydrolysis have been identified, and their *S*-
524 cyano modification at cysteine residues has been verified by mass spectrometry (MS). In
525 addition, the massive analysis of protein extracts by LC/MS has enabled the identification
526 of other naturally *S*-cyanylated proteins in plant tissues, most of which involve glycolysis,
527 the Calvin cycle and the metabolism of *S*-adenosylmethionine. Moreover, the *in vitro*
528 analysis of selected target proteins has shown that treatment with cyanide and the
529 consequent *S*-cyanylation modifies their activity, either by activating or inactivating them
530 (Garcia *et al.*, 2019). The biochemistry, biological importance and prevalence of this new
531 posttranslational modification are as of yet unexplored and represent an important
532 challenge for future research.
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References

Abe K, Kimura H. 1996. The possible role of hydrogen sulfide as an endogenous neuromodulator. Journal of Neuroscience **16**, 1066-1071.

Alvarez C, Bermudez MA, Romero LC, Gotor C, Garcia I. 2012a. Cysteine homeostasis plays an essential role in plant immunity. New Phytologist **193**, 165-177.

Alvarez C, Calo L, Romero LC, Garcia I, Gotor C. 2010. An O-acetylserine(thiol)lyase homolog with L-cysteine desulfhydrase activity regulates cysteine homeostasis in Arabidopsis. Plant Physiology **152**, 656-669.

Alvarez C, Garcia I, Moreno I, Perez-Perez ME, Crespo JL, Romero LC, Gotor C. 2012b. Cysteine-generated sulfide in the cytosol negatively regulates autophagy and modulates the transcriptional profile in Arabidopsis. Plant Cell **24**, 4621-4634.

Alvarez C, García I, Romero LC, Gotor C. 2012c. Mitochondrial sulfide detoxification requires a functional isoform O-acetylserine(thiol)lyase C in Arabidopsis thaliana. Molecular Plant **5**, 1217-1226.

Arenas-Alfonseca L, Gotor C, Romero LC, Garcia I. 2018a. Role of mitochondrial cyanide detoxification in Arabidopsis root hair development. Plant Signaling & Behavior **13**, e1537699.

Arenas-Alfonseca L, Gotor C, Romero LC, Garcia I. 2018b. β-Cyanoalanine synthase action in root hair elongation is exerted at early steps of the root hair elongation pathway and is independent of direct cyanide inactivation of NADPH oxidase. Plant & Cell Physiology **59**, 1072-1083.

Aroca A, Benito JM, Gotor C, Romero LC. 2017a. Persulfidation proteome reveals the regulation of protein function by hydrogen sulfide in diverse biological processes in Arabidopsis. Journal of Experimental Botany **68**, 4915-4927.

Aroca A, Gotor C, Romero LC. 2018. Hydrogen sulfide signaling in plants: emerging roles of protein persulfidation. Frontiers in Plant Science **9**, 1369.

Aroca A, Schneider M, Scheibe R, Gotor C, Romero LC. 2017b. Hydrogen sulfide regulates the cytosolic/nuclear partitioning of glyceraldehyde-3-phosphate dehydrogenase by enhancing its nuclear localization. Plant and Cell Physiology **58**, 983-992.

Aroca Á, Serna A, Gotor C, Romero LC. 2015. S-Sulfhydration: a cysteine posttranslational modification in plant systems. Plant Physiology **168**, 334-342.

Balk J, Schaedler TA. 2014. Iron cofactor assembly in plants. Annual Review of Plant Biology **65**, 125-153.

Baudouin E, Poilevey A, Hewage NI, Cochet F, Puyaubert J, Bailly C. 2016. The significance of hydrogen sulfide for Arabidopsis seed germination. Frontiers in Plant Science **7**, 930.

Benchoam D, Cuevasanta E, Moller MN, Alvarez B. 2019. Hydrogen sulfide and persulfides oxidation by biologically relevant oxidizing species. Antioxidants (Basel) **8**.

Berg SP, Krogmann DW. 1975. Mechanism of KCN inhibition of photosystem I. Journal of Biological Chemistry **250**, 8957-8962.

Bethke PC, Gubler F, Jacobsen JV, Jones RL. 2004. Dormancy of Arabidopsis seeds and barley grains can be broken by nitric oxide. Planta **219**, 847-855.

Birke H, Hildebrandt TM, Wirtz M, Hell R. 2015. Sulfide detoxification in plant mitochondria. Methods in Enzymology **555**, 271-286.

Bloem E, Riemenschneider A, Volker J, Papenbrock J, Schmidt A, Salac I, Haneklaus S, Schnug E. 2004. Sulphur supply and infection with Pyrenopeziza brassicae influence L-cysteine desulphydrase activity in Brassica napus L. Journal of Experimental Botany **55**, 2305-2312.
Blumer C, Haas D. 2000. Mechanism, regulation, and ecological role of bacterial cyanide biosynthesis. Archives of Microbiology **173**, 170-177.
Bogatek R, Dziewanowska K, Lewak S. 1991. Hydrogen cyanide and embryonal dormancy in apple seeds. Physiologia Plantarum **83**, 417-421.
Borowitz JL, Gunasekar PG, Isom GE. 1997. Hydrogen cyanide generation by mu-opiate receptor activation: possible neuromodulatory role of endogenous cyanide. Brain Research **768**, 294-300.
Bottcher C, Westphal L, Schmotz C, Prade E, Scheel D, Glawischnig E. 2009. The multifunctional enzyme CYP71B15 (PHYTOALEXIN DEFICIENT3) converts cysteine-indole-3-acetonitrile to camalexin in the indole-3-acetonitrile metabolic network of Arabidopsis thaliana. Plant Cell **21**, 1830-1845.
Buchanan BB, Balmer Y. 2005. Redox regulation: a broadening horizon. Annual Review of Plant Biology **56**, 187-220.
Calderwood A, Kopriva S. 2014. Hydrogen sulfide in plants: From dissipation of excess sulfur to signaling molecule. Nitric Oxide **41C**, 72-78.
Chen J, Shang YT, Wang WH, Chen XY, He EM, Zheng HL, Shangguan Z. 2016a. Hydrogen sulfide-mediated polyamines and sugar changes are involved in hydrogen sulfide-induced drought tolerance in Spinacia oleracea seedlings. Frontiers in Plant Science **7**, 1173.
Chen J, Wang WH, Wu FH, He EM, Liu X, Shangguan ZP, Zheng HL. 2015a. Hydrogen sulfide enhances salt tolerance through nitric oxide-mediated maintenance of ion homeostasis in barley seedling roots. Scientific Reports **5**, 12516.
Chen J, Wu FH, Shang YT, Wang WH, Hu WJ, Simon M, Liu X, Shangguan ZP, Zheng HL. 2015b. Hydrogen sulphide improves adaptation of Zea mays seedlings to iron deficiency. Journal of Experimental Botany **66**, 6605-6622.
Chen J, Wu FH, Wang WH, Zheng CJ, Lin GH, Dong XJ, He JX, Pei ZM, Zheng HL. 2011. Hydrogen sulphide enhances photosynthesis through promoting chloroplast biogenesis, photosynthetic enzyme expression, and thiol redox modification in Spinacia oleracea seedlings. Journal of Experimental Botany **62**, 4481-4493.
Chen X, Chen Q, Zhang X, Li R, Jia Y, Ef AA, Jia A, Hu L, Hu X. 2016b. Hydrogen sulfide mediates nicotine biosynthesis in tobacco (Nicotiana tabacum) under high temperature conditions. Plant Physiology and Biochemistry **104**, 174-179.
Cheng W, Zhang L, Jiao C, Su M, Yang T, Zhou L, Peng R, Wang R, Wang C. 2013. Hydrogen sulfide alleviates hypoxia-induced root tip death in Pisum sativum. Plant Physiology and Biochemistry **70**, 278-286.
Chivasa S, Carr JP. 1998. Cyanide restores N gene-mediated resistance to tobacco mosaic virus in transgenic tobacco expressing salicylic acid hydroxylase. Plant Cell **10**, 1489-1498.
Chowdhury N, Bagchi A. 2017. Structural insight into the gene expression profiling of the hcn operon in Pseudomonas aeruginosa. Applied Biochemistry and Biotechnology **182**, 1144-1157.

Christou A, Filippou P, Manganaris GA, Fotopoulos V. 2014. Sodium hydrosulfide induces systemic thermotolerance to strawberry plants through transcriptional regulation of heat shock proteins and aquaporin. BMC Plant Biology **14**, 42.
Christou A, Manganaris GA, Papadopoulos I, Fotopoulos V. 2013. 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. Journal of Experimental Botany **64**, 1953-1966.
Chung HS, Wang S-B, Venkatraman V, Murray CI, Eyk JEV. 2013. Cysteine oxidative posttranslational modifications. Circulation Research **112**, 382-392.
Cohn MA, Hughes JA. 1986. Seed dormancy in red rice. 5. Response to azide, hydroxylamine and cyanide. Plant Physiology **80**, 531-533.
Conn EE. 2008. Our work with cyanogenic plants. Annual Review of Plant Biology **59**, 1-19.
Cortese-Krott MM, Kuhnle GG, Dyson A, Fernandez BO, Grman M, DuMond JF, Barrow MP, McLeod G, Nakagawa H, Ondrias K, Nagy P, King SB, Saavedra JE, Keefer LK, Singer M, Kelm M, Butler AR, Feelisch M. 2015. Key bioactive reaction products of the NO/H2S interaction are S/N-hybrid species, polysulfides, and nitroxyl. Proceedings of the National Academy of Sciences USA **112**, E4651-4660.
Cuevasanta E, Lange M, Bonanata J, Coitiño EL, Ferrer-Sueta G, Filipovic MR, Alvarez B. 2015. Reaction of hydrogen sulfide with disulfide and sulfenic acid to form the strongly nucleophilic persulfide. Journal of Biological Chemistry **290**, 26866-26880.
Cui W, Chen H, Zhu K, Jin Q, Xie Y, Cui J, Xia Y, Zhang J, Shen W. 2014. Cadmium-induced hydrogen sulfide synthesis is involved in cadmium tolerance in Medicago sativa by reestablishment of reduced (homo)glutathione and reactive oxygen species homeostases. PLoS ONE **9**, e109669.
Czyzewski BK, Wang DN. 2012. Identification and characterization of a bacterial hydrosulphide ion channel. Nature **483**, 494-497.
da Silva CJ, Batista Fontes EP, Modolo LV. 2017. Salinity-induced accumulation of endogenous H2S and NO is associated with modulation of the antioxidant and redox defense systems in Nicotiana tabacum L. cv. Havana. Plant Science **256**, 148-159.
Donato DB, Nichols O, Possingham H, Moore M, Ricci PF, Noller BN. 2007. A critical review of the effects of gold cyanide-bearing tailings solutions on wildlife. Environment International **33**, 974-984.
Dooley FD, Nair SP, Ward PD. 2013. Increased growth and germination success in plants following hydrogen sulfide administration. PLoS ONE **8**, e62048.
Esashi Y, Isuzugawa K, Matsuyama S, Ashino H, Hasegawa R. 1991. Endogenous evolution of HCN during pre-germination periods in many seed species. Physiologia Plantarum **83**, 27-33.
Fang H, Liu Z, Jin Z, Zhang L, Liu D, Pei Y. 2016. An emphasis of hydrogen sulfide-cysteine cycle on enhancing the tolerance to chromium stress in Arabidopsis. Environmental Pollution **213**, 870-877.
Fang H, Liu Z, Long Y, Liang Y, Jin Z, Zhang L, Liu D, Li H, Zhai J, Pei Y. 2017. The Ca2+/calmodulin2-binding transcription factor TGA3 elevates LCD expression and H2S production to bolster Cr6+ tolerance in Arabidopsis. The Plant Journal **91**, 1038-1050.
Fang T, Cao Z, Li J, Shen W, Huang L. 2014. Auxin-induced hydrogen sulfide generation is involved in lateral root formation in tomato. Plant Physiology and Biochemistry **76**, 44-51.

Fasco MJ, Iii CR, Stack RF, O'Hehir C, Barr JR, Eadon GA. 2007. Cyanide adducts with human plasma proteins: albumin as a potential exposure surrogate. Chemical Research in Toxicology **20**, 677-684.
Filipovic MR. 2015. Persulfidation (S-sulfhydration) and H_2S. Wiley Interdisciplinary Reviews: RNA **230**, 29-59.
Filipovic MR, Miljkovic J, Nauser T, Royzen M, Klos K, Shubina T, Koppenol WH, Lippard SJ, Ivanovic-Burmazovic I. 2012. Chemical characterization of the smallest S-nitrosothiol, HSNO; cellular cross-talk of H_2S and S-nitrosothiols. Journal of the American Chemical Society **134**, 12016-12027.
Filipovic MR, Zivanovic J, Alvarez B, Banerjee R. 2018. Chemical biology of H_2S signaling through persulfidation. Chemical Reviews **118**, 1253-1337.
Flematti GR, Waters MT, Scaffidi A, Merritt DJ, Ghisalberti EL, Dixon KW, Smith SM. 2013. Karrikin and cyanohydrin smoke signals provide clues to new endogenous plant signaling compounds. Molecular Plant **6**, 29-37.
Gadalla MM, Snyder SH. 2010. Hydrogen sulfide as a gasotransmitter. Journal of Neurochemistry **113**, 14-26.
Garcia I, Arenas-Alfonseca L, Moreno I, Gotor C, Romero LC. 2019. HCN regulates cellular processes through posttranslational modification of proteins by S-cyanylation. Plant Physiology **179**, 107-123.
Garcia I, Castellano JM, Vioque B, Solano R, Gotor C, Romero LC. 2010. Mitochondrial beta-cyanoalanine synthase is essential for root hair formation in Arabidopsis thaliana. Plant Cell **22**, 3268-3279.
Garcia I, Gotor C, Romero LC. 2015. Cysteine homeostasis. In: D'Mello JPF, ed. *Amino Acids in Higher Plants*. Wallingford, United Kingdom: CABI Publishing, 219-233.
Garcia I, Rosas T, Bejarano ER, Gotor C, Romero LC. 2013. Transient transcriptional regulation of the CYS-C1 gene and cyanide accumulation upon pathogen infection in the plant immune response. Plant Physiology **162**, 2015-2027.
Garcia-Mata C, Lamattina L. 2010. Hydrogen sulphide, a novel gasotransmitter involved in guard cell signalling. New Phytologist **188**, 977-984.
Garcia-Mata C, Lamattina L. 2013. Gasotransmitters are emerging as new guard cell signaling molecules and regulators of leaf gas exchange. Plant Science **201-202**, 66-73.
Gawron O. 1966. Chapter 14 - On the reaction of cyanide with cystine and cystine peptides. *The Chemistry of Organic Sulfur Compounds*: Pergamon, 351-365.
Gerivani Z, Vashaee E, Sadeghipour HR, Aghdasi M, Shobbar ZS, Azimmohseni M. 2016. Short versus long term effects of cyanide on sugar metabolism and transport in dormant walnut kernels. Plant Science **252**, 193-204.
Glawischnig E. 2007. Camalexin. Phytochemistry **68**, 401-406.
Gleadow RM, Moller BL. 2014. Cyanogenic glycosides: synthesis, physiology, and phenotypic plasticity. Annual Review of Plant Biology **65**, 155-185.
Gniazdowska A, Krasuska U, Bogatek R. 2010. Dormancy removal in apple embryos by nitric oxide or cyanide involves modifications in ethylene biosynthetic pathway. Planta **232**, 1397-1407.
Gotor C, Alvarez C, Bermudez MA, Moreno I, Garcia I, Romero LC. 2010. Low abundance does not mean less importance in cysteine metabolism. Plant Signaling & Behavior **5**, 1028-1030.
Gotor C, Laureano-Marín AM, Arenas-Alfonseca L, Moreno I, Aroca Á, García I, Romero LC. 2017. Advances in plant sulfur metabolism and signaling. In: Cánovas FM,

Lüttge U, Matyssek R, eds. *Progress in Botany Vol. 78*. Cham: Springer International Publishing, 45-66.
Gotor C, Laureano-Marin AM, Moreno I, Aroca A, Garcia I, Romero LC. 2015. Signaling in the plant cytosol: cysteine or sulfide? Amino Acids **47**, 2155-2164.
Gunasekar PG, Borowitz JL, Turek JJ, Van Horn DA, Isom GE. 2000. Endogenous generation of cyanide in neuronal tissue: Involvement of a peroxidase system. Journal of Neuroscience Research **61**, 570-575.
Gunasekar PG, Prabhakaran K, Li L, Zhang L, Isom GE, Borowitz JL. 2004. Receptor mechanisms mediating cyanide generation in PC12 cells and rat brain. Journal of Neuroscience Research **49**, 13-18.
Guo H, Xiao T, Zhou H, Xie Y, Shen W. 2015. Hydrogen sulfide: a versatile regulator of environmental stress in plants. Acta Physiologiae Plantarum **38**, 1-13.
Guo H, Zhou H, Zhang J, Guan W, Xu S, Shen W, Xu G, Xie Y, Foyer CH. 2017. L-cysteine desulfhydrase-related H_2S production is involved in OsSE5-promoted ammonium tolerance in roots of Oryza sativa. Plant, Cell & Environment **40**, 1777-1790.
Guo Z, Liang Y, Yan J, Yang E, Li K, Xu H. 2018. Physiological response and transcription profiling analysis reveals the role of H2S in alleviating excess nitrate stress tolerance in tomato roots. Plant Physiology and Biochemistry **124**, 59-69.
Hatzfeld Y, Maruyama A, Schmidt A, Noji M, Ishizawa K, Saito K. 2000. beta-Cyanoalanine synthase is a mitochondrial cysteine synthase-like protein in spinach and Arabidopsis. Plant Physiology **123**, 1163-1171.
Heeg C, Kruse C, Jost R, Gutensohn M, Ruppert T, Wirtz M, Hell R. 2008. Analysis of the Arabidopsis O-acetylserine(thiol)lyase gene family demonstrates compartment-specific differences in the regulation of cysteine synthesis. Plant Cell **20**, 168-185.
Heppner DE, Hristova M, Ida T, Mijuskovic A, Dustin CM, Bogdandi V, Fukuto JM, Dick TP, Nagy P, Li J, Akaike T, van der Vliet A. 2018. Cysteine perthiosulfenic acid (Cys-SSOH): A novel intermediate in thiol-based redox signaling? Redox Biology **14**, 379-385.
Höhner R, Aboukila A, Kunz H-H, Venema K. 2016. Proton gradients and proton-dependent transport processes in the chloroplast. Frontiers in Plant Science **7**.
Honda K, Yamada N, Yoshida R, Ihara H, Sawa T, Akaike T, Iwai S. 2015. 8-Mercapto-cyclic GMP mediates hydrogen sulfide-induced stomatal closure in Arabidopsis. Plant and Cell Physiology **56**, 1481-1489.
Huo J, Huang D, Zhang J, Fang H, Wang B, Wang C, Liao W. 2018. Hydrogen sulfide: a gaseous molecule in postharvest freshness. Frontiers in Plant Science **9**.
Iwai T, Miyasaka A, Seo S, Ohashi Y. 2006. Contribution of ethylene biosynthesis for resistance to blast fungus infection in young rice plants. Plant Physiology **142**, 1202-1215.
Jia H, Chen S, Liu D, Liesche J, Shi C, Wang J, Ren M, Wang X, Yang J, Shi W, Li J. 2018. Ethylene-induced hydrogen sulfide negatively regulates ethylene biosynthesis by persulfidation of ACO in tomato under osmotic stress. Frontiers in Plant Science **9**.
Jia H, Hu Y, Fan T, Li J. 2015. Hydrogen sulfide modulates actin-dependent auxin transport via regulating ABPs results in changing of root development in Arabidopsis. Science Report **5**, 8251.

Jin Z, Pei Y. 2015. Physiological implications of hydrogen sulfide in plants: pleasant exploration behind Its unpleasant odour. Oxidative Medicine and Cellular Longevity **2015**, 397502.
Jin Z, Shen J, Qiao Z, Yang G, Wang R, Pei Y. 2011. Hydrogen sulfide improves drought resistance in Arabidopsis thaliana. Biochemical and Biophysical Research Communications **414**, 481-486.
Jin Z, Xue S, Luo Y, Tian B, Fang H, Li H, Pei Y. 2013. Hydrogen sulfide interacting with abscisic acid in stomatal regulation responses to drought stress in Arabidopsis. Plant Physiology and Biochemistry **62**, 41-46.
Kabala K, Zboinska M, Glowiak D, Reda M, Jakubowska D, Janicka M. 2018. Interaction between the signaling molecules hydrogen sulfide and hydrogen peroxide and their role in vacuolar H(+) -ATPase regulation in cadmium-stressed cucumber roots. Physiologia Plantarum **0**.
Kabil O, Banerjee R. 2010. Redox biochemistry of hydrogen sulfide. Journal of Biological Chemistry **285**, 21903-21907.
Khan MN, Mobin M, Abbas ZK, Siddiqui MH. 2017. Nitric oxide-induced synthesis of hydrogen sulfide alleviates osmotic stress in wheat seedlings through sustaining antioxidant enzymes, osmolyte accumulation and cysteine homeostasis. Nitric Oxide **68**, 91-102.
Kharbech O, Houmani H, Chaoui A, Corpas FJ. 2017. Alleviation of Cr(VI)-induced oxidative stress in maize (Zea mays L.) seedlings by NO and H2S donors through differential organ-dependent regulation of ROS and NADPH-recycling metabolisms. Journal of Plant Physiology **219**, 71-80.
Kimura H. 2011. Hydrogen sulfide: its production and functions. Experimental Physiology **96**, 833-835.
Kimura H. 2015. Hydrogen sulfide and polysulfides as signaling molecules. Proceeding of the Japan Academy Series B Physical and Biological Sciences **91**, 131-159.
Knowles CJ. 1976. Microorganisms and cyanide. Bacteriological Reviews **40**, 652-680.
Koprivova A, Kopriva S. 2014. Molecular mechanisms of regulation of sulfate assimilation: first steps on a long road. Frontiers in Plant Science **5**, 589.
Krasuska U, Ciacka K, Debska K, Bogatek R, Gniazdowska A. 2014. Dormancy alleviation by NO or HCN leading to decline of protein carbonylation levels in apple (Malus domestica Borkh.) embryos. Journal of Plant Physiology **171**, 1132-1141.
Krueger S, Niehl A, Lopez Martin MC, Steinhauser D, Donath A, Hildebrandt T, Romero LC, Hoefgen R, Gotor C, Hesse H. 2009. Analysis of cytosolic and plastidic serine acetyltransferase mutants and subcellular metabolite distributions suggests interplay of the cellular compartments for cysteine biosynthesis in Arabidopsis. Plant Cell and Environment **32**, 349-367.
Kuzmanovic N, Eltlbany N, Ding G, Baklawa M, Min L, Wei L, Smalla K. 2018. Analysis of the genome sequence of plant beneficial strain Pseudomonas sp. RU47. Journal of Biotechnology **281**, 183-192.
Lai D, Mao Y, Zhou H, Li F, Wu M, Zhang J, He Z, Cui W, Xie Y. 2014. 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 Science **225**, 117-129.
Laureano-Marín AM, Moreno I, Aroca Á, García I, Romero LC, Gotor C. 2016. Regulation of autophagy by hydrogen sulfide. In: Lamattina L, García-Mata C, eds.

Gasotransmitters in Plants: The Rise of a New Paradigm in Cell Signaling. Cham: Springer International Publishing, 53-75.
Laureano-Marin AM, Moreno I, Romero LC, Gotor C. 2016. Negative regulation of autophagy by sulfide is independent of reactive oxygen species. Plant Physiology **171**, 1378-1391.
Li H, Gao MQ, Xue RL, Wang D, Zhao HJ. 2015. Effect of hydrogen sulfide on D1 protein in wheat under drought stress. Acta Physiologiae Plantarum **37**, 225.
Li J, Chen S, Wang X, Shi C, Liu H, Yang J, Shi W, Guo J, Jia H. 2018. Hydrogen sulfide disturbs actin polymerization via S-sulfhydration resulting in stunted root hair growth. Plant Physiology **178**, 936-949.
Li J, Jia H, Wang J, Cao Q, Wen Z. 2014a. 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 **251**, 899-912.
Li Q, Lancaster JR, Jr. 2013. Chemical foundations of hydrogen sulfide biology. Nitric Oxide **35C**, 21-34.
Li YJ, Chen J, Xian M, Zhou LG, Han FX, Gan LJ, Shi ZQ. 2014b. In site bioimaging of hydrogen sulfide uncovers its pivotal role in regulating nitric oxide-induced lateral root formation. PLoS ONE **9**, e90340.
Li ZG, Gong M, Xie H, Yang L, Li J. 2012. Hydrogen sulfide donor sodium hydrosulfide-induced heat tolerance in tobacco (Nicotiana tabacum L) suspension cultured cells and involvement of Ca(2+) and calmodulin. Plant Science **185-186**, 185-189.
Li ZG, Yang SZ, Long WB, Yang GX, Shen ZZ. 2013. Hydrogen sulphide may be a novel downstream signal molecule in nitric oxide-induced heat tolerance of maize (Zea mays L.) seedlings. Plant Cell and Environment **36**, 1564-1572.
Lisjak M, Srivastava N, Teklic T, Civale L, Lewandowski K, Wilson I, Wood ME, Whiteman M, Hancock JT. 2010. A novel hydrogen sulfide donor causes stomatal opening and reduces nitric oxide accumulation. Plant Physiology and Biochemistry **48**, 931-935.
Lisjak M, Teklic T, Wilson ID, Whiteman M, Hancock JT. 2013. Hydrogen sulfide: environmental factor or signalling molecule? Plant Cell and Environment **36**, 1607-1616.
Lowicka E, Beltowski J. 2007. Hydrogen sulfide (H_2S) - the third gas of interest for pharmacologists. Pharmacological Reports **59**, 4-24.
Lv W, Yang L, Xu C, Shi Z, Shao J, Xian M, Chen J. 2017. Cadmium disrupts the balance between hydrogen peroxide and superoxide radical by regulating endogenous hydrogen sulfide in the root tip of Brassica rapa. Frontiers in Plant Science **8**, 232.
Ma D, Ding H, Wang C, Qin H, Han Q, Hou J, Lu H, Xie Y, Guo T. 2016. Alleviation of drought stress by hydrogen sulfide is partially related to the abscisic acid signaling pPathway in wheat. PLoS ONE **11**, e0163082.
Miller JM, Conn EE. 1980. Metabolism of hydrogen cyanide by higher plants. Plant Physiology **65**, 1199-1202.
Millikin R, Bianco CL, White C, Saund SS, Henriquez S, Sosa V, Akaike T, Kumagai Y, Soeda S, Toscano JP, Lin J, Fukuto JM. 2016. The chemical biology of protein hydropersulfides: Studies of a possible protective function of biological hydropersulfide generation. Free Radical Biology and Medicine **97**, 136-147.

Mishanina TV, Libiad M, Banerjee R. 2015. Biogenesis of reactive sulfur species for signaling by hydrogen sulfide oxidation pathways. Nature Chemical Biology **11**, 457-464.
Mithofer A, Boland W. 2012. Plant defense against herbivores: chemical aspects. Annual Review of Plant Biology **63**, 431-450.
Moller BL. 2010. Functional diversifications of cyanogenic glucosides. Current Opinion in Plant Biology **13**, 338-347.
Mostofa MG, Saegusa D, Fujita M, Tran LS. 2015. Hydrogen sulfide regulates salt tolerance in rice by maintaining Na(+)/K(+) balance, mineral homeostasis and oxidative metabolism under excessive salt stress. Frontiers in Plant Science **6**, 1055.
Mustafa AK, Gadalla MM, Sen N, Kim S, Mu W, Gazi SK, Barrow RK, Yang G, Wang R, Snyder SH. 2009. H_2S signals through protein S-sulfhydration. Science Signaling **2**, ra72.
Nagahara N, Ito T, Minami M. 1999. Mercaptopyruvate sulfurtransferase as a defense against cyanide toxication: molecular properties and mode of detoxification. Histology and Histopathology **14**, 1277-1286.
Nelson DC, Flematti GR, Ghisalberti EL, Dixon KW, Smith SM. 2012. Regulation of seed germination and seedling growth by chemical signals from burning vegetation. Annual Review of Plant Biology **63**, 107-130.
Noctor G, Mhamdi A, Chaouch S, Han Y, Neukermans J, Marquez-Garcia B, Queval G, Foyer CH. 2012. Glutathione in plants: an integrated overview. Plant Cell and Environment **35**, 454-484.
Olas B. 2015. Hydrogen sulfide in signaling pathways. Clinica Chimica Acta **439**, 212-218.
Olson KR. 2018. H_2S and polysulfide metabolism: Conventional and unconventional pathways. Biochemical Pharmacology **149**, 77-90.
Oracz K, Bouteau HEM, Farrant JM, Cooper K, Belghazi M, Job C, Job D, Corbineau F, Bailly C. 2007. ROS production and protein oxidation as a novel mechanism for seed dormancy alleviation. Plant Journal **50**, 452-465.
Oracz K, El-Maarouf-Bouteau H, Bogatek R, Corbineau F, Bailly C. 2008. Release of sunflower seed dormancy by cyanide: cross-talk with ethylene signalling pathway. Journal of Experimental Botany **59**, 2241-2251.
Papanatsiou M, Scuffi D, Blatt MR, García-Mata C. 2015. Hydrogen sulfide regulates inward-rectifying K^+ channels in conjunction with stomatal closure. Plant Physiology **168**, 29-35.
Papenbrock J, Riemenschneider A, Kamp A, Schulz-Vogt HN, Schmidt A. 2007. Characterization of cysteine-degrading and H_2S-releasing enzymes of higher plants - from the field to the test tube and back. Plant Biology (Stuttg) **9**, 582-588.
Patel BH, Percivalle C, Ritson DJ, Duffy CD, Sutherland JD. 2015. Common origins of RNA, protein and lipid precursors in a cyanosulfidic protometabolism. Nature Chemistry **7**, 301-307.
Paul BD, Snyder SH. 2015. Modes of physiologic H_2S signaling in the brain and peripheral tissues. Antioxidants and Redox Signaling **22**, 411-423.
Peiser GD, Wang TT, Hoffman NE, Yang SF, Liu HW, Walsh CT. 1984. Formation of cyanide from carbon 1 of 1-aminocyclopropane-1-carboxylic acid during its conversion to ethylene. Proceedings of the National Academy of Sciences USA **81**, 3059-3063.
Pistorius EK, Gewitz HS, Voss H, Vennesland B. 1977. Cyanide formation from histidine in Chlorella. A general reaction of aromatic amino acids catalyzed by amino

acid oxidase systems. Biochimica et Biophysica Acta (BBA) - Reviews on Cancer **481**, 384-391.
Poulton JE. 1990. Cyanogenesis in plants. Plant Physiology **94**, 401-405.
Puppo A, Davies MJ. 1995. The reactivity of thiol compounds with different redox states of leghaemoglobin: evidence for competing reduction and addition pathways. Biochimica et Biophysica Acta (BBA) - Reviews on Cancer **1246**, 74-81.
Qi J, Isupov MN, Littlechild JA, Anderson LE. 2001. Chloroplast glyceraldehyde-3-phosphate dehydrogenase contains a single disulfide bond located in the C-terminal extension to the B subunit. Journal of Biological Chemistry **276**, 35247-35252.
Ravilious GE, Jez JM. 2012. Structural biology of plant sulfur metabolism: from assimilation to biosynthesis. Natural Product Reports **29**, 1138-1152.
Rennenberg H, Herschbach C. 2014. A detailed view on sulphur metabolism at the cellular and whole-plant level illustrates challenges in metabolite flux analyses. Journal of Experimental Botany **65**, 5711-5724.
Richau KH, Kaschani F, Verdoes M, Pansuriya TC, Niessen S, Stuber K, Colby T, Overkleeft HS, Bogyo M, Van der Hoorn RA. 2012. Subclassification and biochemical analysis of plant papain-like cysteine proteases displays subfamily-specific characteristics. Plant Physiology **158**, 1583-1599.
Riemenschneider A, Wegele R, Schmidt A, Papenbrock J. 2005. Isolation and characterization of a D-cysteine desulfhydrase protein from Arabidopsis thaliana. FEBS Journal **272**, 1291-1304.
Romero LC, Aroca MA, Laureano-Marin AM, Moreno I, Garcia I, Gotor C. 2014. Cysteine and cysteine-related signaling pathways in Arabidopsis thaliana. Molecular Plant **7**, 264-276.
Scuffi D, Álvarez C, Laspina N, Gotor C, Lamattina L, García-Mata C. 2014. Hydrogen sulfide generated by L-cysteine desulfhydrase acts upstream of nitric oxide to modulate abscisic acid-dependent stomatal closure. Plant Physiology **166**, 2065-2076.
Scuffi D, Nietzel T, Di Fino LM, Meyer AJ, Lamattina L, Schwarzländer M, Laxalt AM, García-Mata C. 2018. Hydrogen sulfide increases production of NADPH oxidase-dependent hydrogen peroxide and phospholipase D-derived phosphatidic acid in guard cell signaling. Plant Physiology **176**, 2532.
Seo S, Mitsuhara I, Feng J, Iwai T, Hasegawa M, Ohashi Y. 2011. Cyanide, a coproduct of plant hormone ethylene biosynthesis, contributes to the resistance of rice to blast fungus. Plant Physiology **155**, 502-514.
Shan C, Liu H, Zhao L, Wang X. 2014. Effects of exogenous hydrogen sulfide on the redox states of ascorbate and glutathione in maize leaves under salt stress. Biologia Plantarum **58**, 169-173.
Shan C, Zhang S, Ou X. 2018. The roles of H_2S and H_2O_2 in regulating AsA-GSH cycle in the leaves of wheat seedlings under drought stress. Protoplasma **255**, 1257-1262.
Shen J, Zeng Y, Zhuang X, Sun L, Yao X, Pimpl P, Jiang L. 2013. Organelle pH in the Arabidopsis endomembrane system. Molecular Plant **6**, 1419-1437.
Shen JJ, Qiao ZJ, Xing TJ, Zhang LP, Liang YL, Jin ZP, Yang GD, Wang R, Pei YX. 2012. Cadmium toxicity is alleviated by AtLCD and AtDCD in Escherichia coli. Journal of Applied Microbiology **113**, 1130-1138.
Shi H, Ye T, Han N, Bian H, Liu X, Chan Z. 2015. Hydrogen sulfide regulates abiotic stress tolerance and biotic stress resistance in Arabidopsis. Journal of Integrative Plant Biology **57**, 628-640.

Siegien I, Bogatek R. 2006. Cyanide action in plants - from toxic to regulatory. Acta Physiologiae Plantarum **28**, 483-497.
Singh VP, Singh S, Kumar J, Prasad SM. 2015. Hydrogen sulfide alleviates toxic effects of arsenate in pea seedlings through up-regulation of the ascorbate–glutathione cycle: Possible involvement of nitric oxide. Journal of Plant Physiology **181**, 20-29.
Stelmaszynska T. 1986. Formation of HCN and its chlorination to ClCN by stimulated human neutrophils--2. Oxidation of thiocyanate as a source of HCN. International Journal of Biochemistry **18**, 1107-1114.
Sun J, Wang R, Zhang X, Yu Y, Zhao R, Li Z, Chen S. 2013. Hydrogen sulfide alleviates cadmium toxicity through regulations of cadmium transport across the plasma and vacuolar membranes in Populus euphratica cells. Plant Physiology and Biochemistry **65**, 67-74.
Sun Z, Zhang K, Chen C, Wu Y, Tang Y, Georgiev MI, Zhang X, Lin M, Zhou M. 2018. Biosynthesis and regulation of cyanogenic glycoside production in forage plants. Applied Microbiology and Biotechnology **102**, 9-16.
Takahashi H, Kopriva S, Giordano M, Saito K, Hell R. 2011. Sulfur assimilation in photosynthetic organisms: molecular functions and regulations of transporters and assimilatory enzymes. Annual Review of Plant Biology **62**, 157-184.
Tian B, Qiao Z, Zhang L, Li H, Pei Y. 2016. Hydrogen sulfide and proline cooperate to alleviate cadmium stress in foxtail millet seedlings. Plant Physiology and Biochemistry **109**, 293-299.
Tridevi MV, Laurence JS, Siahaan TJ. 2009. The role of thiols and disulfides on protein stability. Current Protein & Peptide Science **10**, 614-625.
Van Hoewyk D, Pilon M, Pilon-Smits EAH. 2008. The functions of NifS-like proteins in plant sulfur and selenium metabolism. Plant Science **174**, 117-123.
Vitvitsky V, Miljkovic JL, Bostelaar T, Adhikari B, Yadav PK, Steiger AK, Torregrossa R, Pluth MD, Whiteman M, Banerjee R, Filipovic MR. 2018. Cytochrome c reduction by H_2S potentiates sulfide signaling. ACS Chemical Biology **13**, 2300-2307.
Wang BL, Shi L, Li YX, Zhang WH. 2010. Boron toxicity is alleviated by hydrogen sulfide in cucumber (Cucumis sativus L.) seedlings. Planta **231**, 1301-1309.
Wang KLC, Li H, Ecker JR. 2002. Ethylene biosynthesis and signaling networks. Plant Cell **14**, S131-S151.
Wang L, Wan R, Shi Y, Xue S. 2016. Hydrogen sulfide activates S-type anion channel via OST1 and Ca(2+) modules. Molecular Plant **9**, 489-491.
Wang R. 2002. Two's company, three's a crowd: can H2S be the third endogenous gaseous transmitter? The FASEB Journal **16**, 1792-1798.
Wang R. 2012. Physiological implications of hydrogen sulfide: A whiff exploration that blossomed. Physiological Reviews **92**, 791-896.
Wang R. 2014. Gasotransmitters: growing pains and joys. Trends in Biochemical Sciences **39**, 227-232.
Wang Y, Li L, Cui W, Xu S, Shen W, Wang RJP, Soil. 2012. Hydrogen sulfide enhances alfalfa (Medicago sativa) tolerance against salinity during seed germination by nitric oxide pathway. Plant and Soil **351**, 107-119.
Watanabe M, Kusano M, Oikawa A, Fukushima A, Noji M, Saito K. 2008. Physiological roles of the beta-substituted alanine synthase gene family in Arabidopsis. Plant Physiology **146**, 310-320.

Wedmann R, Onderka C, Wei S, Szijarto IA, Miljkovic JL, Mitrovic A, Lange M, Savitsky S, Yadav PK, Torregrossa R, Harrer EG, Harrer T, Ishii I, Gollasch M, Wood ME, Galardon E, Xian M, Whiteman M, Banerjee R, Filipovic MR. 2016. Improved tag-switch method reveals that thioredoxin acts as depersulfidase and controls the intracellular levels of protein persulfidation. Chemical Science **7**, 3414-3426.
Wong CE, Carson RA, Carr JP. 2002. Chemically induced virus resistance in Arabidopsis thaliana is independent of pathogenesis-related protein expression and the NPR1 gene. Molecular Plant Microbe Interaction **15**, 75-81.
Wu J, Watson JT. 1998. Optimization of the cleavage reaction for cyanylated cysteinyl proteins for efficient and simplified mass mapping. Analytical Biochemistry **258**, 268-276.
Xie Y, Zhang C, Lai D, Sun Y, Samma MK, Zhang J, Shen W. 2014. Hydrogen sulfide delays GA-triggered programmed cell death in wheat aleurone layers by the modulation of glutathione homeostasis and heme oxygenase-1 expression. Journal of Plant Physiology **171**, 53-62.
Yamaguchi Y, Nakamura T, Kusano T, Sano H. 2000. Three Arabidopsis genes encoding proteins with differential activities for cysteine synthase and beta-cyanoalanine synthase. Plant & Cell Physiology **41**, 465-476.
Yao GF, Wei ZZ, Li TT, Tang J, Huang ZQ, Yang F, Li YH, Han Z, Hu F, Hu LY, Hu KD, Zhang H. 2018. Modulation of enhanced antioxidant activity by hydrogen sulfide antagonization of ethylene in tomato fruit ripening. Journal of Agricultural and Food Chemistry **66**, 10380-10387.
Yip WK, Yang SF. 1988. Cyanide metabolism in relation to ethylene production in plant tissues. Plant Physiology **88**, 473-476.
Yu L-x, Zhang C-j, Shang H-q, Wang X-f, Wei M, Yang F-j, Shi Q-h. 2013. Exogenous hydrogen sulfide enhanced antioxidant capacity, amylase activities and salt tolerance of Cucumber hypocotyls and radicles. Journal of Integrative Agriculture **12**, 445-456.
Zaffagnini M, Fermani S, Marchand CH, Costa A, Sparla F, Rouhier N, Geigenberger P, Lemaire SD, Trost P. 2019. Redox homeostasis in photosynthetic organisms: novel and established thiol-based molecular mechanisms. Antioxidants and Redox Signaling **0**, null.
Zagrobelny M, Bak S, Moller BL. 2008. Cyanogenesis in plants and arthropods. Phytochemistry **69**, 1457-1468.
Zagrobelny M, Bak S, Olsen CE, Moller BL. 2007. Intimate roles for cyanogenic glucosides in the life cycle of Zygaena filipendulae (Lepidoptera, Zygaenidae). Insect Biochemistry and Molecular Biology **37**, 1189-1197.
Zagrobelny M, de Castro ECP, Moller BL, Bak S. 2018. Cyanogenesis in arthropods: from chemical warfare to nuptial gifts. Insects **9**.
Zhang D, Du J, Tang C, Huang Y, Jin H. 2017. H_2S-induced sulfhydration: biological function and detection methodology. Frontiers in Pharmacology **8**.
Zhang D, Macinkovic I, Devarie-Baez NO, Pan J, Park CM, Carroll KS, Filipovic MR, Xian M. 2014. Detection of protein S-sulfhydration by a tag-switch technique. Angewandte Chemie **53**, 575-581.
Zhang H, Hu LY, Hu KD, He YD, Wang SH, Luo JP. 2008. Hydrogen sulfide promotes wheat seed germination and alleviates oxidative damage against copper stress. Journal of Integrative Plant Biology **50**, 1518-1529.

Zhang H, Tan ZQ, Hu LY, Wang SH, Luo JP, Jones RL. 2010. Hydrogen sulfide alleviates aluminum toxicity in germinating wheat seedlings. Journal of Integrative Plant Biology **52**, 556-567.
Zhang L, Pei Y, Wang H, Jin Z, Liu Z, Qiao Z, Fang H, Zhang Y. 2015. Hydrogen sulfide alleviates cadmium-induced cell death through restraining ROS accumulation in roots of Brassica rapa L. ssp. pekinensis. Oxidative medicine and cellular longevity **2015**, 11.
Zhao N, Zhu H, Zhang H, Sun J, Zhou J, Deng C, Zhang Y, Zhao R, Zhou X, Lu C, Lin S, Chen S. 2018. Hydrogen sulfide mediates K^+ and Na^+ homeostasis in the roots of salt-resistant and salt-sensitive poplar species subjected to NaCl stress. Frontiers in Plant Science **9**.
Zhou Z-H, Wang Y, Ye X-Y, Li Z-G. 2018. 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. Frontiers in Plant Science **9**.
Ziogas V, Molassiotis A, Fotopoulos V, Tanou G. 2018. Hydrogen sulfide: A potent tool in postharvest fruit biology and possible mechanism of action. Frontiers in Plant Science **9**.

Table 1. *Effects of hydrogen sulfide in plant adaptation to abiotic stresses*

Stress/Hydrogen sulfide Treatment	**Consequences**	**References**
Aluminum stress/NaHS pretreatment	Promotion seed germination/alleviation oxidative damage	(Zhang *et al.*, 2010)
Arsenate stress/ NaHS addition	Induction of ascorbate-glutathione cycle	(Singh *et al.*, 2015)
Boron stress/ NaHS addition	Alleviation of inhibition of root elongation	(Wang *et al.*, 2010)
Cadmium stress/endogenous H_2S induction	Vacuolar H^+-ATPase alteration	(Kabala *et al.*, 2018)
Cadmium stress/endogenous H_2S induction	Balance between H_2O_2 and $O_2^{\cdot-}$	(Lv *et al.*, 2017)
Cadmium stress/ NaHS pretreatment	Proline increase	(Tian *et al.*, 2016)
Cadmium stress/endogenous H_2S induction	Glutathione and ROS homeostasis	(Cui *et al.*, 2014)
Cadmium stress/ NaHS addition	Alleviation cell death /oxidative damage	(Zhang *et al.*, 2015)
Cadmium stress/ NaHS pretreatment	Alleviation of oxidative stress/activation Cd transport	(Sun *et al.*, 2013)
Chromium stress/ NaHS addition	Alleviation of oxidative stress	(Kharbech *et al.*, 2017)
Chromium stress/ endogenous H_2S induction	Induction of cysteine accumulation	(Fang *et al.*, 2016)
Copper stress/ NaHS pretreatment	Promotion seed germination/alleviation oxidative damage	(Zhang *et al.*, 2008)
Drought/ endogenous H_2S induction	Reduction of stomatal aperture/induction of drought associated genes	(Jin *et al.*, 2011)
Drought/ NaHS addition	Increased level of polyamines and sugars	(Chen *et al.*, 2016a)
Drought/ NaHS addition	Induction of components of the ABA signaling pathway	(Ma *et al.*, 2016)
Drought/ NaHS addition	Alleviation of PSII damage through D1 protein level	(Li *et al.*, 2015)
Heat stress/ NaHS pretreatment	Involvement of Ca^{2+} and calmodulin	(Li *et al.*, 2012)
Heat stress/ endogenous H_2S induction	NO mediated tolerance/reduction electrolyte leakage	(Li *et al.*, 2013)
Heat stress/ NaHS pretreatment	Alleviation of oxidative stress/induction of heat shock proteins and aquaporin	(Christou *et al.*, 2014)
Heat stress/ NaHS pretreatment	Alleviation of oxidative stress/induction of osmolyte biosynthesis	(Zhou *et al.*, 2018)
Heat stress/ endogenous H_2S induction	Induction of nicotine biosynthesis	(Chen *et al.*, 2016b)
Hypoxia/ endogenous H_2S induction	Alleviation of oxidative stress	(Cheng *et al.*, 2013)
Osmotic stress/ endogenous H_2S induction	Alleviation of oxidative stress/osmolyte accumulation	(Khan *et al.*, 2017)
Osmotic stress/ endogenous H_2S induction	Ethylene mediated stomatal closure/persulfidation of ACC Oxidase	(Jia *et al.*, 2018)
Salinity/ endogenous H_2S induction	Alleviation of oxidative stress	(da Silva *et al.*, 2017)
Salinity/ NaHS pretreatment	Alleviation of oxidative stress/maintenance Na^+/K^+ balance	(Mostofa *et al.*, 2015)
Salinity/ endogenous H_2S induction	Alleviation of oxidative stress/maintenance Na^+/K^+ balance	(Lai *et al.*, 2014)

Salinity/ NaHS addition	Alleviation of oxidative stress/maintenance Na^+/K^+ balance	(Li *et al.*, 2014a)
Salinity/ NaHS addition	Maintenance Na^+/K^+ balance	(Zhao *et al.*, 2018)
Salinity/ NaHS addition	NO-dependent maintenance ion homeostasis	(Chen *et al.*, 2015a)
Salinity/ NaHS pretreatment	Alleviation of oxidative stress/affecting the SOS pathway	(Christou *et al.*, 2013)
Iron deficiency/ NaHS addition	Enhanced photosynthesis	(Chen *et al.*, 2015b)
Ammonium stress/ endogenous H_2S induction	Increased ammonium incorporation/alleviation of inhibition of root growth	(Guo *et al.*, 2017)

The table shows a representation of some of published data.

Figure legends

Fig. 1. Subcellular locations of hydrogen sulfide production in plant cells. The main source is located in the chloroplast, where sulfite is reduced to sulfide by the action of sulfite reductase (SiR) during the photosynthetic sulfate reduction pathway and at the chloroplast stromal basic pH most of hydrogen sulfide (H_2S) is dissociated into its ionic form (HS^-) that requires an unknown active transporter (shown as interrogation mark) to permeate the membrane. In the cytosol, cysteine is mainly synthesized by the action of the O-acetylserine(thiol)lyase (OSATL) and this cell compartment is other source of hydrogen sulfide that it is generated from cysteine by different cysteine-degrading enzymes, such as the L-cysteine (LCDES) and D-cysteine (D-CDES) desulfhydrases, and the L-cysteine desulfurases (NifS-like). NifS-like proteins are also located in chloroplasts and mitochondria. Mitochondria is also a source of hydrogen sulfide that is generated during the detoxification of cyanide by the action of the β-cyanoalanine synthase (CAS) which uses cysteine synthesized by mitochondrial OASTL. Mitochondrial hydrogen sulfide is also dissociated to its ionic form at basic pH.

Fig. 2. Schematic representation of the hydrogen sulfide action mechanism in biological processes. The mechanism of action of H_2S is related to its chemical reactivity with other molecules. It can coordinate the metal center of metalloproteins. It can act as a reductant reacting with biological oxidants, such as nitric oxide ($NO^{\bullet}$), hydrogen peroxide (H_2O_2), superoxide radical ($O_2^{\bullet-}$), peroxynitrite (ONOOH), hypochlorite (HOCl) and S-nitrosothiols. It can modify proteins by the oxidation of cysteine residues to form the corresponding persulfides (-SSH), process called persulfidation.

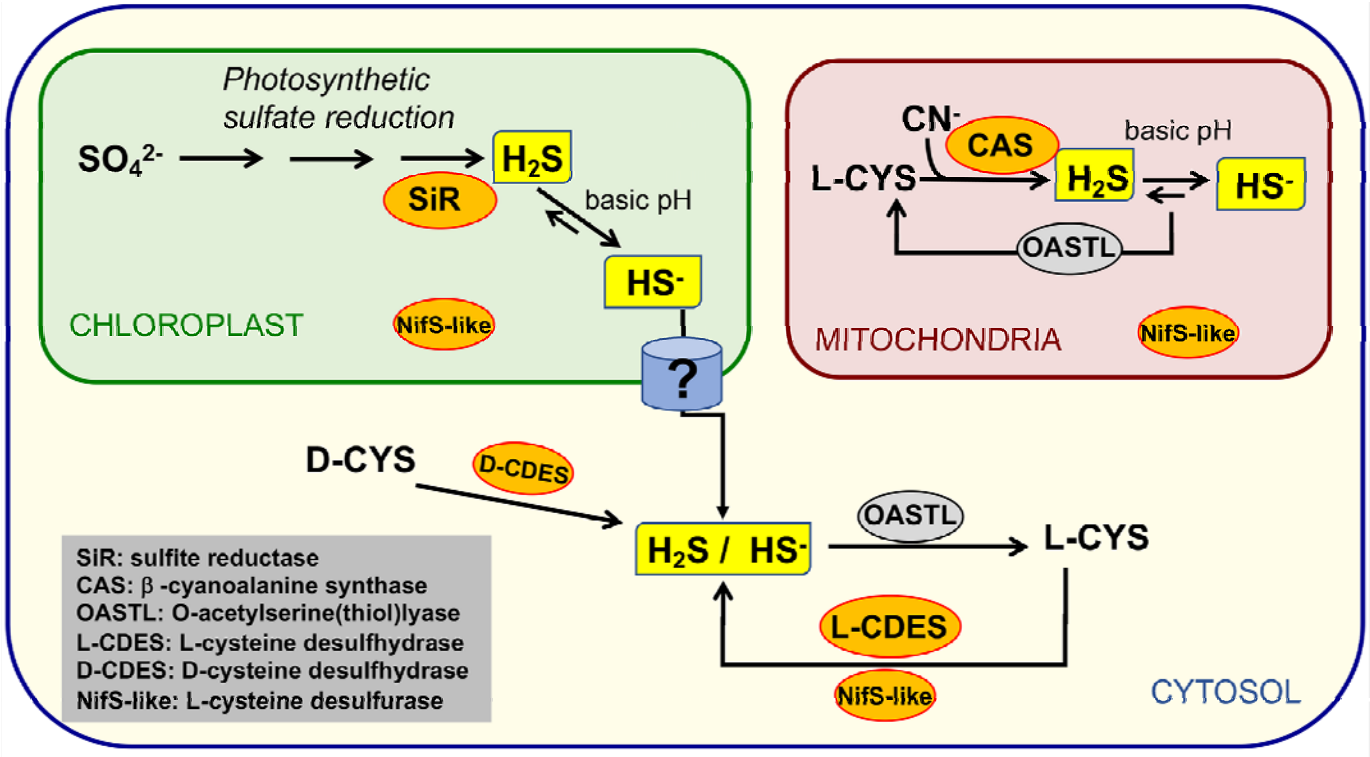
Fig. 3. Persulfidated proteins in the plant photosynthesis pathway. The persulfidated proteins involved in the photosynthetic light reactions located in chloroplast thylakoids and in the Calvin-Benson cycle located in chloroplast stroma are shown as blue squares.

Fig. 4. Schematic representation of the function of protein persulfidation in protection. The major source of sulfide in plant cells must proceed from photosynthetic sulfate assimilation by sulfite reductase (SiR) activity in chloroplasts that is coupled to the biosynthesis of cysteine within the chloroplast, the cytosol or the mitochondria by

OASTL enzymes. L/D-DES and HCN/CAS activity can also generate H_2S from cysteine within the cytosol or the mitochondria. Reactive oxygen species (ROS) generated by the light reaction of photosynthesis or stress processes can lead to sulfide oxidation to hydrogen disulfide (H_2S_2) or polysulfide (H_2S_n), which can react with thiol residues in proteins to form persulfides (-SSH). Thiolate residues within proteins (-SH) can be oxidized by ROS to form disulfide bridges (-SS-) or by persistent oxidizing conditions to form sulfenic (-SOH), sulfinic ($-SO_2H$) and sulfonic acid residues ($-SO_3H$). Free H_2S can react with sulfenic acid residues to form persulfidated proteins (-SSH). Either disulfide bridges or persulfidated proteins can be reduced back by the ferredoxin (Fd)-thioredoxin reductase (Fdx)-thioredoxin (Trx) system in the light or by the NADPH-thioredoxin reductase (Ntr)-thioredoxin (Trx) system in the dark or in nonphotosynthetic tissues. Similar to oxidized thiol residues (-SOH, $-SO_2H$, $-SO_3H$), persulfidated proteins residues can be also oxidized to perthiosulfenic, perthiosulfinic and perthiosulfonic acid ($-SSO_3H$, $-SSO_2H$, $-SSO_3H$), which can easily be reduced by reductants or thioredoxin systems.

Fig. 5. Pathways involved in the formation of hydrogen cyanide in non-cyanogenic plants. A) A conjugate of cysteine and the tryptophan derivative indole-3-acetonitrile (IAN), Cys(IAN), is converted either spontaneously or by the CYP71B15 (PAD3) enzymatic action in the intermediate dihydrocamalexic acid (DHCA) and giving hydrogen cyanide. DHCA is then converted to camalexin by the action of CYP71B15 (PAD3). B) Ethylene is synthesized from 1-Aminocyclopropane-1-carboxylic acid (ACC) by the ACC oxidase giving ethylene, carbon dioxide and hydrogen cyanide.

Fig. 6. Schematic representation of hydrogen cyanide action in plant biology and proposed mechanisms. Cyanide induces the germination process and inhibits root hair elongation and plant defense against bacterial pathogens (upper part). Several mechanisms of action have been proposed, including the S-cyanylation of cysteine residues, which modifies protein activity, and hormone and/or ROS signaling modulation (lower part). Arrows and blunt lines represent activation and repression by hydrogen cyanide, respectively. Solid lines indicate demonstrated functions and mechanism, and dashed lines indicate proposed mechanism.



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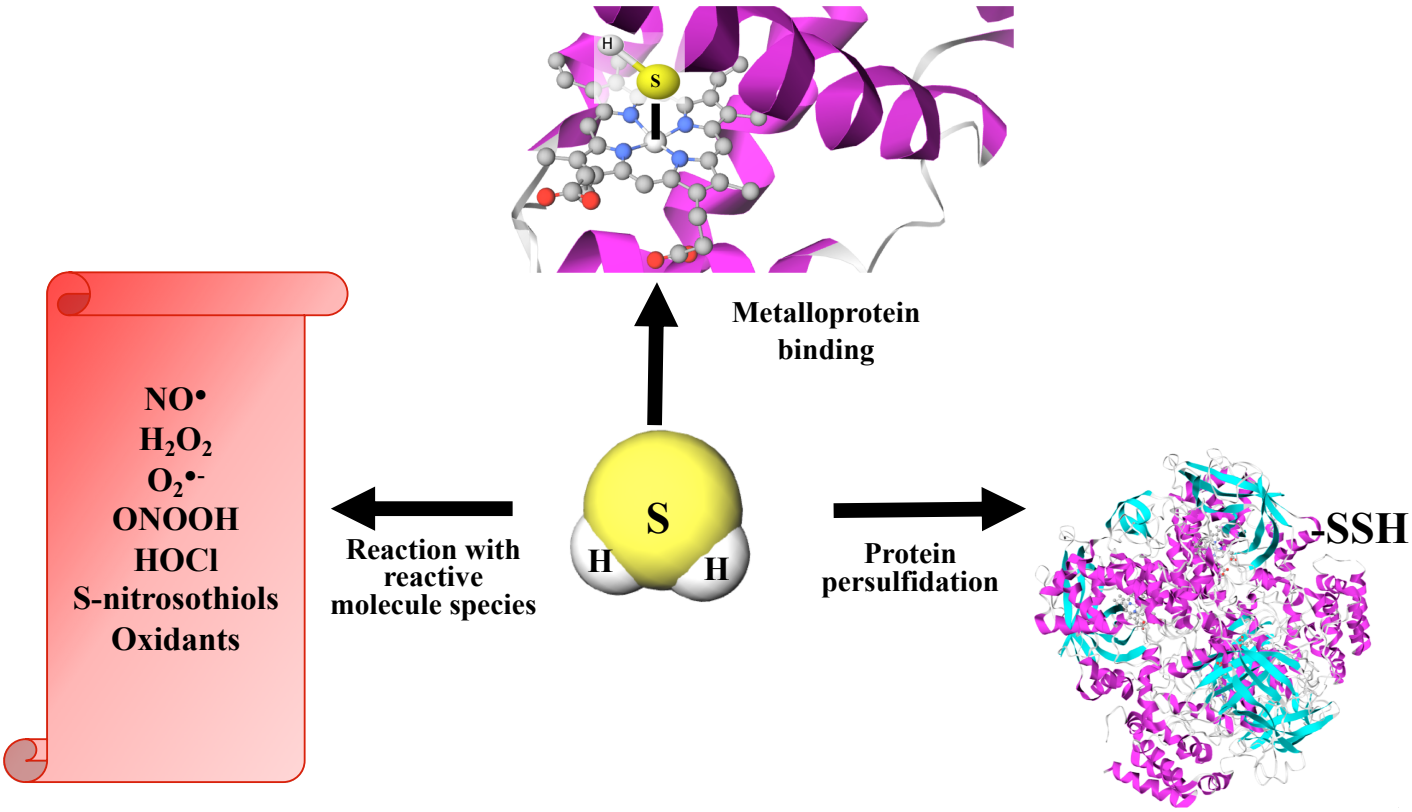

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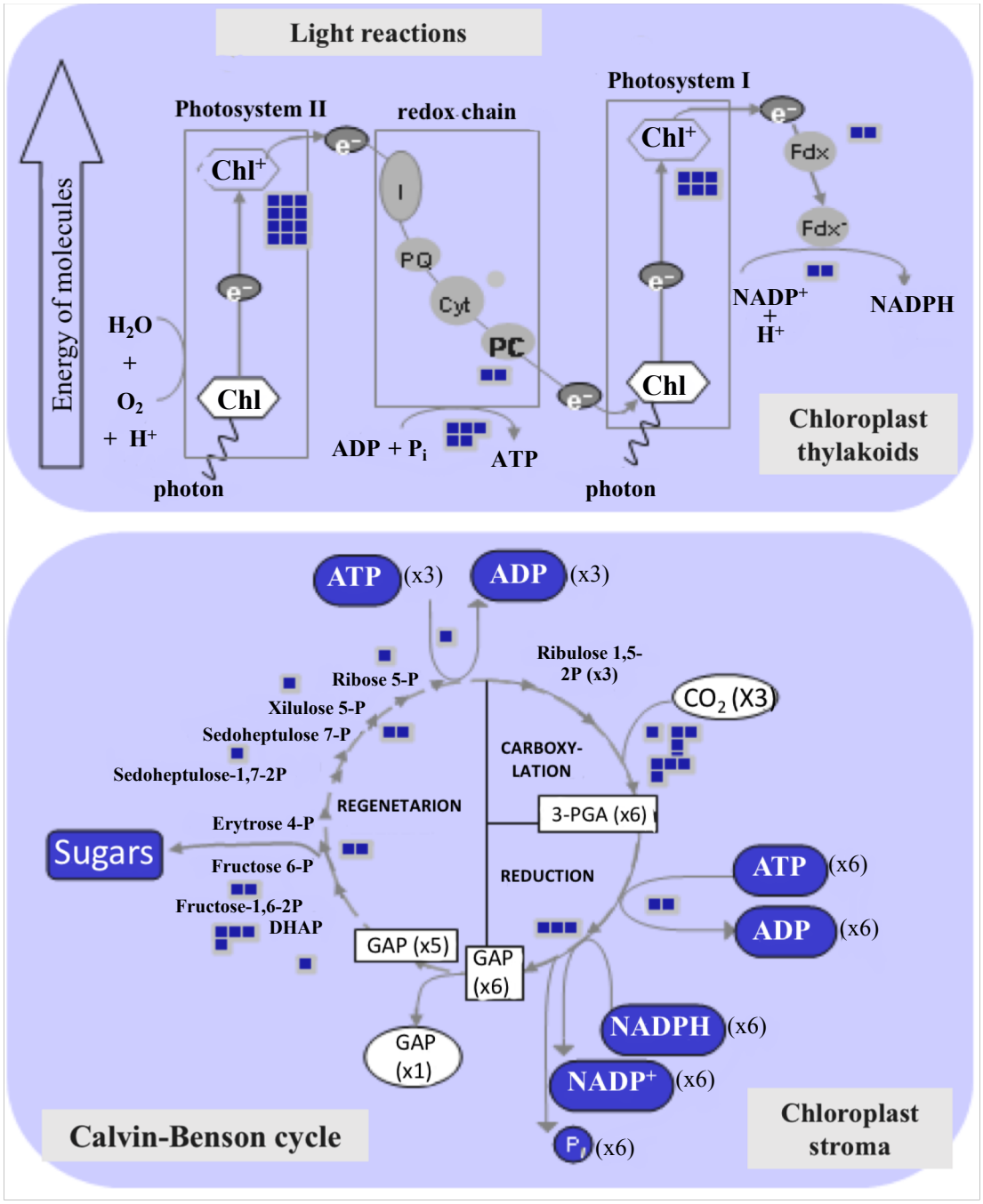

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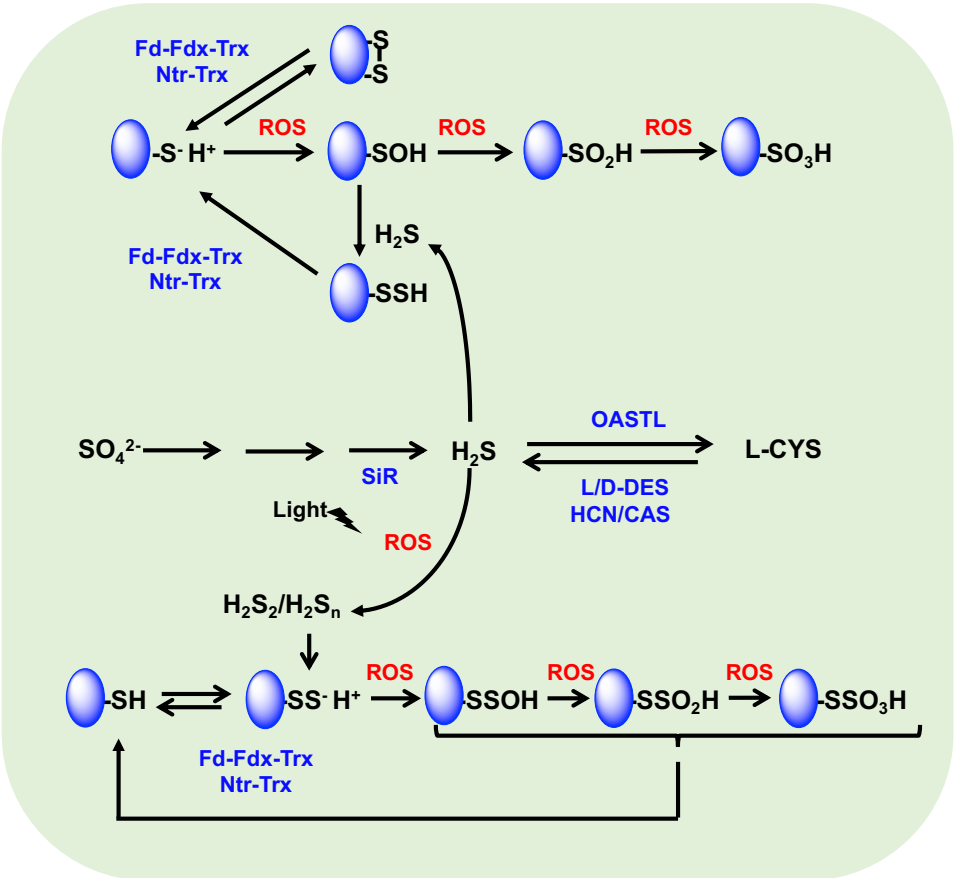

Fig. 4. Schematic representation of the function of protein persulfidation in protection. The major source of sulfide in plant cells must proceed from photosynthetic sulfate assimilation by sulfite reductase (SiR) activity in chloroplasts that is coupled to the biosynthesis of cysteine within the chloroplast, the cytosol or the mitochondria by OASTL enzymes. L/D-DES and HCN/CAS activity can also generate H_2S from cysteine within the cytosol or the mitochondria. Reactive oxygen species (ROS) generated by the light reaction of photosynthesis or stress processes can lead to sulfide oxidation to hydrogen disulfide (H_2S_2) or polysulfide (H_2S_n), which can react with thiol residues in proteins to form persulfides (-SSH). Thiolate residues within proteins (-SH) can be oxidized by ROS to form disulfide bridges (-SS-) or by persistent oxidizing conditions to form sulfenic (-SOH), sulfinic ($-SO_2H$) and sulfonic acid residues ($-SO_3H$). Free H_2S can react with sulfenic acid residues to form persulfidated proteins (-SSH). Either disulfide bridges or persulfidated proteins can be reduced back by the ferredoxin (Fd)-thioredoxin reductase (Fdx)-thioredoxin (Trx) system in the light or by the NADPH-thioredoxin reductase (Ntr)-thioredoxin (Trx) system in the dark or in nonphotosynthetic tissues. Similar to oxidized thiol residues (-SOH, $-SO_2H$, $-SO_3H$), persulfidated proteins residues can be also oxidized to perthiosulfenic, perthiosulfinic and perthiosulfonic acid ($-SSO_3H$, $-SSO_2H$, $-SSO_3H$), which can easily be reduced by reductants or thioredoxin systems.

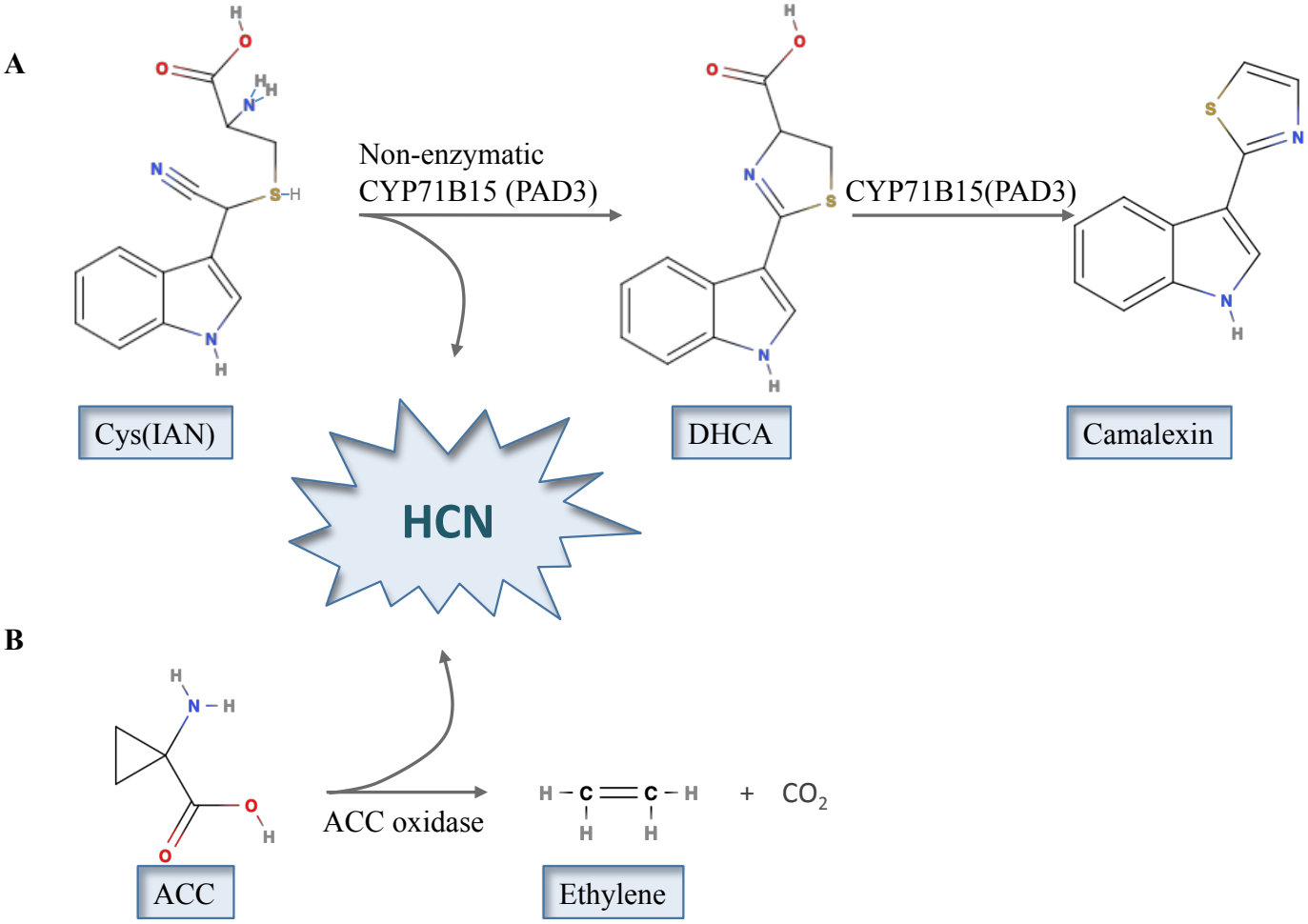

Fig. 5. Pathways involved in the formation of hydrogen cyanide in non-cyanogenic plants. (A) A conjugate of cysteine and the tryptophan derivative indole-3-acetonitrile (IAN), Cys(IAN), is converted either spontaneously or by the CYP71B15 (PAD3) enzymatic action in the intermediate dihydrocamalexic acid (DHCA) and giving hydrogen cyanide. DHCA is then converted to camalexin by the action of CYP71B15 (PAD3). (B) Ethylene is synthesized from 1-Aminocyclopropane-1-carboxylic acid (ACC) by the ACC oxidase giving ethylene, carbon dioxide and hydrogen cyanide.

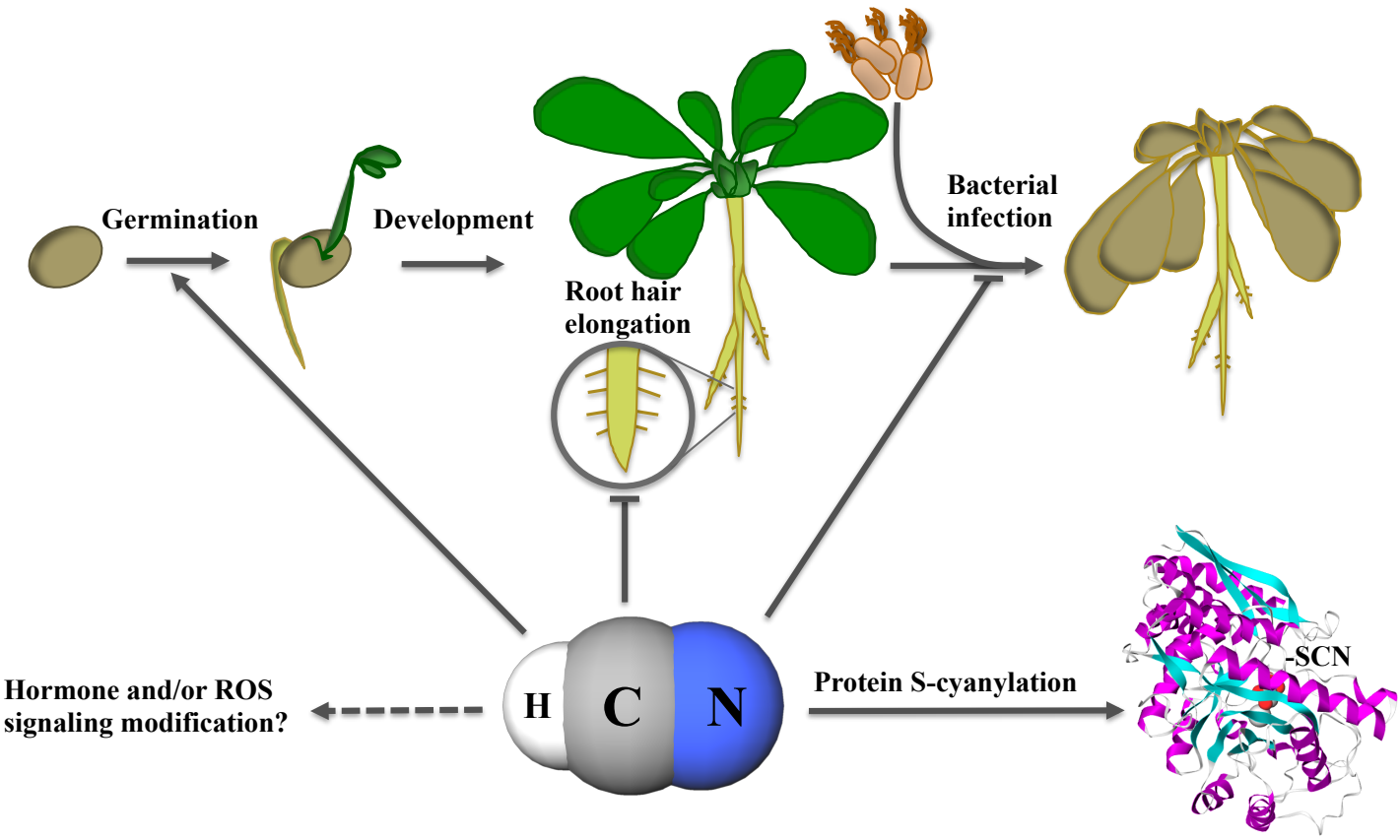

Fig. 6. Schematic representation of hydrogen cyanide action in plant biology and proposed mechanisms. Cyanide induces the germination process and inhibits root hair elongation and plant defense against bacterial pathogens (upper part). Several mechanisms of action have been proposed, including the S-cyanylation of cysteine residues, which modifies protein activity, and hormone and/or ROS signaling modulation (lower part). Arrows and blunt lines represent activation and repression by hydrogen cyanide, respectively. Solid lines indicate demonstrated functions and mechanism, and dashed lines indicate proposed mechanism.