Hydrogen sulfide and environmental stresses

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Abstract

Hydrogen sulfide (H_2S) is part of a suite of small reactive molecules which are known to be involved in cell signaling events in plants. It is produced by cells, can move around, including through membranes, and can be removed when no longer needed. It is perceived by cells, partly through persulfidation of proteins. Along with nitric oxide (NO) and reactive oxygen species (ROS) H_2S is involved in a range of stress responses, including following treatment with heavy metals, salt, temperature change and pathogen challenge. H_2S can lead to changes in the activity of antioxidants, cell signaling proteins such as mitogen activated protein kinases (MAPKs) and gene expression. Understanding how H_2S fits into cell signaling pathways may lead to advances in how treatment with H_2S or H_2S releasing donors may improve plant tolerance to stress, and hence plant growth and agricultural outputs.

Keywords: Antioxidants; cell signaling; drought; heavy metals; hydrogen peroxide; hydrogen sulfide; NaHS; nitric oxide; reactive oxygen species

Introduction

Despite its inherent toxicity hydrogen sulfide (H_2S) (Jiang *et al*., 2016) has now become recognized as an important part of the suite of small diffusible compounds used by organisms in cell signaling (Hancock, 2017; Filipovic and Jovanovic, 2017). It has been known for some time that reactive compounds such as hydrogen peroxide (H_2O_2), along with other reactive oxygen species (ROS), can partake in signaling. In 1987 it was reported that endothelial derived relaxing factor in mammals was in fact the gas nitric oxide (NO) (Palmer *et al*., 1987), and later the role of NO in plant signaling was recognized as being important (Neill *et al*., 2002; Wilson *et al.,* 2008). Therefore, suggesting that other gases were also involved was not such an off-piste idea and so H_2S was being dubbed as the third gaseous transmitter (Wang, 2002) along with NO and carbon monoxide (CO), and it has been accepted that H_2S can act in cell signaling in plants (Wang, 2003; Hancock *et al*., 2011; Wang 2012; Li, 2013; Lisjak *et al*., 2013).

H_2S is inherently toxic (Jiang *et al*., 2016). In *Escherichia coli* toxicity has been proposed to be through an oxidative damage mediated mechanism (Fu *et al*., 2018) but in eukaryotes H_2S can act as a mitochondrial inhibitor, reducing the activity of Complex IV, lowering the electrochemical potential across the inner mitochondrial membrane (IMM) and reducing ATP generation (Dorman, 2002), although there is different sensitivity in different plants (Martin and Maricle, 2015). However, H_2S can also be produced by cells and can be used as a regulatory molecule (Filipovic and Jovanovic, 2017), impinging on cell signaling processes, especially those involving ROS and RNS. Interestingly, in mammals, at low concentrations H_2S can be used as mitochondrial reductant, and therefore an electron source for mitochondria (Bouillaud *et al*., 2013; Módis *et al*., 2016). Here electrons are fed into ubiquinone and hence

contribute to the generation of the IMM protomotive force and hence ATP production. From an evolutionary point of view perhaps this is not too surprising (Hancock, 2017). Organisms are known to use sulfur metabolism for energy conversion and early life would have been associated with sulfur rich thermal vents (Martin *et al*., 2008). What is more surprising is the use of H_2S in cell signaling. It seems as though organisms have evolved to not only tolerate the presence of H_2S, but to use it as an integral part of a cell's control system (Hancock, 2017), in both animals and plants.

H_2S is small and therefore easy able to diffuse through cells, and being uncharged is likely to be able to traverse the hydrophobic nature of membranes: either the plasma membrane or those of organelles, not needing the assistance of proteins such as aquaporins (Mathai *et al*., 2009). However, using erythrocyte membranes it has also be suggested that H_2S/HS^- movements across the lipid bilayer may involve exchange for Cl^- and use of the anion exchange protein AE1 in what is called the Jacobs-Stewart cycle (Jennings, 2013). Whether facilitated or not, movement of H_2S across membranes would facilitate its role in signaling, carrying the message from the point of production to the point of perception. The diffusion distance would however be determined by its removal, either enzymatic (Youssefian *et al*., 1993; Tai and Cook, 2000) or by reacting with other cellular components.

Production of H_2S has been reported in cells. H_2S generating enzymes such as cystathione Ɣ-lyase (CSE), cystathione β-synthase (CBS) and 3-mercaptopyruvate sulfurtransferase (3-MST) have been reported in animals (Prabhakar, 2012) but of more relevance here, desulfhydrases in plants (Alvarez *et al*., 2010). DES1 in Arabidopsis has been shown to be a pyridoxal-5′-phosphate-dependent cysteine-degrading enzyme found in the cytosol (Alvarez *et al*., 2010). In mitochondria, β-cyanoalanine synthase can also generate H_2S. The action of this

enzyme converts cysteine and cyanide to hydrogen sulfide and β-cyanoalanine (Hatzfeld *et al*., 2000; Meyer *et al*., 2003). The involvement of such enzymes in H_2S action is important to ascertain, as recently demonstrated by a study on seed germination, where in fact H_2S was concluded to have a marginal role (Baudouin *et al*., 2016). Sulfide is also an intermediate in plant sulfur metabolism, being used along with *O*-acylserine to generate cysteine (Calderwood and Kopriva, 2014), while bacteria to which plants may be interacting may also have the capacity to generate H_2S (Clarke, 1953). Therefore plants cells will most likely be exposed to sulfide, whether it is being used in signaling or not. Removal of H_2S can be catalyzed by enzymes such as *O*-acetylserine (thiol) lyase (Youssefian *et al*., 1993; Tai and Cook, 2000), as well as by mitochondria in animals (Bouillaud *et al*., 2013; Módis *et al*., 2016), a type of activity which may also be relevant to plants. The oxidation of sulfide may also be important to the generation of further sulfur-based compounds which can also be involved in signaling, including persulfides, polysulfides and thiosulfate (Mishanina *et al*., 2015), which may be important for stress responses.

The production, movement and removal of H_2S all make the role of this gas in cell signaling possible. Of course there will be issues of local production, use and removal so discussions of compartmentalization, as had been discussed for redox, ROS and RNS (Go and Jones, 2008; Noctor and Foyer, 2016), would be relevant to H_2S signaling, but such fine details have not been reported to date. New H_2S probes (Lin *et al*., 2013; Chen *et al*., 2018) in conjunction with confocal microscopy will no doubt shed light on such matters in the future. How different redox-related components are compartmentalized, either in the same manner or differently, will determine the type of competition there will be between them and therefore the ultimate signaling response.

It may be worth at this point noting that experimentally H_2S is often supplied to a system under study using compounds which release H_2S into solution, rather than the use of the gas *per se*. The kinetics of release varies with different donors. Compounds such as NaHS tend to release H_2S very quickly, while newer donors have slower release kinetics (such as GYY3147), or are targeted to specific organelles (such as AP39). For a review of H_2S donors see Powell *et al*. (2018).

Intracellular effects of H_2S

As well as being able to inhibit mitochondrial activity (Dorman, 2002; Martin and Maricle, 2015) H_2S may also be used by mitochondria (Bouillaud *et al*., 2013) as an electron donor. In fact, the mechanisms of how H_2S may interact and regulate mitochondrial activity is quite complex, and may involve feeding electrons through sulfide:quinone oxidoreductase (SQR) and Complex II, interacting with free radicals, and controlling cell signaling pathways (Módis *et al*., 2016**).** However, for H_2S to be useful in cell signaling and stress responses H_2S needs to be perceived and its presence acted on. Normally such activity is triggered by a ligand (in this case H_2S) arriving at a receptor and initiating a signal transduction pathway. It has been suggested that H_2S is too small to dock to a receptor in the classical sense (Moore and Whiteman, 2015), but in animals the protein VEGFR2 has been suggested as a candidate as it can react directly with H_2S. It is likely that in plants a similar chemistry is involved in H_2S perception (as discussed below).

One of the direct influences of H_2S will be on the presence of the other stress signals, such as ROS and NO. H_2S can react with peroxynitrite ($ONOO^-$) for example (Carbellal *et al*., 2011), so removing the latter from solution, and so removing its signaling capacity. NO can also react with H_2S. This has two consequences. Firstly

the presence of both H_2S and NO will be reduced, and so removing the ability of both to cause further signaling. Secondly a new compound is produced, nitrosothiol, which itself may have a signaling property (Whiteman *et al*., 2006). The chemistry of H_2S was extensively explored by Li and Lancaster (2013): it will not be stable and unreactive in cells, which would limit its capacity to move and act as a distant signal. Here the notion of compartmentalization is needed to be considered once more.

A major target for many reactive molecules in signaling is the thiol group on proteins. The cysteine –SH group can undergo attack (often after removal of the proton) in a variety of ways (Hancock, 2009). It can be oxidized by H_2O_2 to the sulfenic acid group. This modification is akin to phosphorylation, in that the thiol can be toggled between two states: modified and un-modified. Therefore the activity of a protein can be toggled between two states by the action of H_2O_2, for example between active and inactive. With increased concentrations of H_2O_2 the thiol group may be further oxidized to the sulfinic acid and further more to the sulfonic acid, the latter two probably being irreversible changes. On the other hand NO can modify the thiol by *S*-nitrosation (often referred to as *S*-nitrosylation), which can be identified using the biotin switch assay (Jaffrey and Snyder, 2001; Grennan, 2007). Again, this is akin to phosphorylation, giving two states between which the protein may toggle. Other modifications include glutathionylation (Sun *et al.,* 2013), especially important when you consider the high concentration of glutathione in cells (Schafer and Buettner, 2001). In a similar manner H_2S can lead to the modification of the thiol group, by a process known as *S*-sulfhydration (or persulfidation) (Romero *et al*., 20113; Paul and Snyder, 2015, Aroca *et al*., 2017a). In each of these cases the modification of the thiol is different and there is no reason to assume that the final activity of the protein would be the same (Figure 1). Clearly, the proportion of the

protein which has been modified in each way will depend on the local concentrations of the reactive molecules, which can be altered by each other: that is, H_2S can remove NO, as can superoxide anions etc. Local concentrations will also be determined by compartmentalization. In plants persulfidation has been shown to be involved in the regulation of the Kreb's cycle, glycolysis and the Calvin cycle, showing that it has a central role in regulating metabolic processes (Aroca *et al*., 2017a), and not just a trivial chemical anomaly. However, further work is needed here to fully understand the role of H_2S and persulfidation in controlling metabolic processes in plants.

A good example of a protein that is covalently modified by a range of small reactive molecules is glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Holtgrede *et al*., 2008). This protein is central to glycolysis but isoforms are also found in the chloroplasts of cells (Martin and Cerff, 2017). GAPDH can be oxidized and *S*-nitrosated (Hancock *et al*., 2005; Lindermayr *et al*., 2005), and can be modified by H_2S (Aroca *et al.*, 2015; Aroca *et al*., 2017b). On modification this protein can move from the cytoplasm, where it is involved in glycolysis, to the nucleus where it can control transcription factors and hence gene expression. Therefore, thiol modification has a profound effect on the protein's function, bestowing on it a completely disparate activity.

Immensely important targets for covalent modification by reactive signals are transcription factors themselves. As well as often being phosphorylated, a process which can be enhanced by the presence of reactive signals – as discussed further below – transcription factors can be directly modified. Albeit in animals, a good example is NF-kB which can be modified by a range of mechanisms (Perkins, 2006),

including by NO and H_2S (Sen *et al*., 2012). Certainly H_2S has been shown to have large effects on the gene expression profiles of plants cells (Li *et al*., 2017b).

One of the influences of H_2S is that it can increase the levels of glutathione (De Kok *et al*., 1985). Glutathione is found in high concentrations in cells and has an immensely important role in maintaining the intracellular redox balance, and perhaps preventing cells from entering programmed cell death (apoptosis) or necrosis (Schafer and Buettner, 2001). More recently in a study on water stress in wheat seedlings following NaHS treatment there were effects on both glutathione and ascorbate metabolism (Shan *et al*., 2011), with levels of reduced glutathione and reduced ascorbate both being increased. Again, along with soluble thiols such as glutathione, ascorbate is important to control cellular redox (Noctor *et al*., 2018). If intracellular redox becomes oxidized levels of important reduced molecules such as NADH/NADPH are harder to maintain and apoptosis (programmed cell death) or necrosis can be initiated (Schafer and Buettner, 2001). Therefore, the presence of H_2S may help to maintain the GSH. Loss of glutathione would alter the redox status of the plant cell (Foyer *et al*., 2001) as the concentrations of oxidised and reduced glutathione feed into a squared relationship in the Nernst equation (Schafer and Buettner, 2001), meaning that the total glutathione present (i.e. GSH + GSSG) helps to dictate the overall redox state of the cell, not just the GSH/GSSG ratio. Concentration levels of reduced glutathione may decrease during oxidative stress, when ROS accumulation increases, and hence, by increasing intracellular glutathione, H_2S can have an important influence on cell function, particularly in times of stress. The importance of changes in antioxidants is highlighted, perhaps, by the work on postharvest. H_2S has been shown to increase the shelf life of strawberries, an effect mediated by antioxidant levels (Hu *et al*., 2012). Similar

studies and effects have also been reported for other fruit such as kiwifruit (Zhu *et al*. 2014) and grape (Ni *et al*., 2015), although effects of ethylene signaling have also been reported (Li *et al*., 2017a). Therefore, manipulation of H_2S may alter intracellular redox but may also be of commercial importance too.

In a similar manner, gaseous signal molecules such as H_2S can influence proline levels (He and He, 2018) which is immensely important for the maintenance of redox and protecting against oxidative stress, amongst other things (Kavi Kishor and Sreenivasulu, 2014). As an example of the importance of such redox modulators, Wang *et al*. (2012) suggested that the effects of H_2S on salinity tolerance were through effects on oxidative stress, and possibly NO. Therefore, overall the influence of H_2S on the intracellular redox status of cells is an important issue to be aware of.

Stress responses involving H_2S

Stress responses in cells often involve reactive signaling molecules. It has long been known that plant stress leads to an increase in ROS accumulation (Mittler, 2002; Baxter *et al*., 2014). In response to a wide range of stresses, such as heat, light, heavy metals and pathogen challenge plant cells will accumulate H_2O_2 which will lead to a range of downstream effects, mediated by signal transduction components such as mitogen activated protein kinases (MAPKs), and resulting in altered gene expression. In a similar way, NO is also integrally involved in plant stress responses (Arasimowicz and Floryszak-Wieczorek, 2007), again being accumulated in response to similar initiators and resulting in similar cellular alterations. Therefore, the involvement of other small gaseous signaling molecules such as H_2 (Cui *et al*., 2014) and H_2S would be expected. Previous reviews have discussed how H_2S may

mediate plant responses, as shown in Figure 2, and how there may be an interplay with other signaling molecules (Hancock and Whiteman, 2014; 2015), as illustrated in Figure 3. Below is a short discussion on some of the current reports of H_2S medicating plant stress responses.

Being sessile plants have to tolerate the temperature that their environment dictates and this is often not optimal (Bita and Gerats, 2013). H_2S has been implicated in the tolerance to both high and low temperatures by several research groups. Stuiver *et al*., (1992) investigated freeze tolerance in wheat seedlings and found that H_2S altered the cellular levels of amino acids and sugars. They also measured water-soluble sulfhydryl content (discussed in Hancock and Whiteman, 2018), which was primarily glutathione and found that this too increased following H_2S fumigation. At the other end of the temperature scale, heat tolerance is also improved by the treatment with H_2S or donors. It was found that H_2S – in the form of NaHS – increased germination of maize seeds under heat stress, and further enhanced tissue viability and lowered malondialdehyde (MDA) accumulation caused by heat treatment (Li *et al.,* 2013a). It was concluded that H_2S action was being mediated by an increase in proline levels in cells. Subsequently, NaHS, acting as a H_2S donor, enhanced salicyclic acid (SA)-induced heat tolerance in maize (Li *et al*., 2015), an effect blocked if a H_2S biosynthesis inhibitor or H_2S scavenger was used. The authors suggested that H_2S was acting downstream of SA. In tobacco, the H_2S donor NaHS was used to increase the survival of cell cultures under heat stress (Li *et al*., 2012b). Here it was suggested that the effects were mediated by the entry of extracellular calcium ions across the plasma membrane and that intracellularly the effects were reliant on the action of calmodulin, a ubiquitous calcium binding protein. In another study, strawberry roots (*Fragaria x ananassa* cv. ‘Camarosa’) were

exposed to an acute heat shock in the presence and absence of NaHS. MDA, H_2O_2 and NO were all lower in H_2S treated tissues. Ascorbate and glutathione metabolism were suggested to be involved in preserving tissues against heat-induced damage and gene expression of several antioxidants and heat shock proteins was induced, including catalase, superoxide dismutase, HSP70, HSP80 and HSP90. Aquaporin was also induced (Christou *et al*., 2014). More recently, the foliar application of NaHS on wheat seedlings showed an increase in heat tolerance. As would be expected, antioxidant levels were increased, signs of oxidative stress, such as MDA, were decreased and the authors suggested that foliar application of H_2S donors may be beneficial (Zhang *et al*., 2016).

Another environmental stress often needing to be tolerated by plants is the presence of heavy metals (Nikalje and Suprasanna, 2018). Previously the role of H_2S in plants in response to cadmium, aluminum, chromium, copper and lead have been discussed (Hancock and Whiteman, 2015). Li *et al*. (2012c) suggested that NO was involved in the alleviation of cadmium stress, while with chromium stress NaHS promoted plant growth and photosynthesis (Zhang *et al*., 2010a; Ali *et al.*, 2013). In wheat, NaHS could relieve the reduction of germination of seed caused by copper stress (Zhang *et al*., 2008), while antioxidant levels were increased and MDA and H_2O_2 accumulation was reduced. Lead stress in oilseed rape (*Brassica napus* L) was alleviated by NaHS, with improved growth, photosynthesis and cell structure (Ali *et al.*, 2014). Finally, in aluminum stress NaHS increased the activities of antioxidant enzymes and citrate secretion. The expression of the citrate transporter gene was enhanced as was that of the PM H^+-ATPase (Chen *et al*., 2012), while H_2S alleviated the toxicity of Al to germinating wheat seedlings (Zhang *et al*., 2010b).

The role in heavy metal stress by H_2S has also recently reviewed by others (He *et al.,* 2018), and such research continues. Recently chromium treatment of maize *(Zea mays* L.) led to oxidative stress symptoms in some tissues, but not all. However, the effects were alleviated by the addition of NO and H_2S donors (Kharbech *et al.*, 2017). ROS was found to be important in the H_2S effects seen on Zn treatment of pepper (*Capsicum annuum* L.). Exogenous NaHS enhanced plant growth and fruit yield, altered levels of antioxidants and suppressed MDA and H_2O_2 content (Kaya *et al*., 2018). In a study on roots, Lv *et al*. (2017) showed that 4μM Cd led to an increase in H_2S at the root tip, an effect blocked by a H_2S scavenger or an inhibitor of H_2S biosynthesis. It was concluded that part of the action of H_2S was through the modulation of the H_2O_2 and $O_2^{\cdot-}$ levels at the tip. Also recently, H_2S was shown to alleviate aluminum toxicity in rice (Zhu *et al*., 2018). Al content in the root tips was reduced after treatment with NaHS. NaHS pretreatment lowered the negative charge in cell walls and masked Al binding sites by upregulation of OsSTAR1 band OsSTAR2 in roots. The gene, OsFRDL4, which is involved in citrate exudation was over-expressed. Intracellular Al was altered, as there was an increase in the translocation of Al to the vacuole: OsALS1 was over-expressed. MDA and H_2O_2 content was decreased following changes in antioxidant levels. The authors also pointed out that H_2S exhibited crosstalk with NO signaling, emphasizing that H_2S was not working alone, as shown in Figure 3.

This latter point highlights the importance of considering the reactive molecules together (Hancock and Whiteman, 2014), as well as interactions with other signals. H_2S toxicity itself appears in some instances to require ROS and NO. The inhibition of growth caused by H_2S was reported to be auxin dependent but a further study showed that MAPKs were involved, specifically MPK6, but also was

accompanied by an increase in ROS accumulation and NO production (Zhang *et al*., 2017). In rice (*Zizania palustris*) sulfide toxicity was alleviated by the presence of iron (Fort *et al*., 2017), itself a metal often involved in ROS metabolism (Koskenkorva-Frank *et al*., 2013).

The control of water use in plants is extremely important. Plants may experience too much or too little water, a topic of increasing importance with the unset of global climate change (Jasechko, 2018). Under waterlogging stress the application of NaHS up to 0.1mM alleviated the symptoms of the stress and it was suggested that this was partly mediated by a reduction in ROS accumulation (Wei *et al*., 2017a). In a similar study of submerged macrophytes exposed to hypoxia and H_2S, in *Potamogeton crispus* and *Myriophyllum spicatum* there was an increase in oxidative stress, increased H_2O_2 and MDA when exposed to sulfide in the form of NaHS. However, this was not replicated in other species such as *Egeria densa* and *Potamogeton oxyphyllus* (Parveen *et al*., 2017).

Plants often are exposed to conditions where water is less than optimal. Drought stress has been studied for many years and the role of H_2S on the control of stomatal closure has been of some debate. H_2S may cause stomatal opening in some instances but closure under others (Garcia-Mata and Lamattina, 2010; 2013; Lisjak *et al*., 2010; 2011). More recently, this apparent anomaly has been further investigated (Honda *et al*., 2015). They showed that short-term exposure to a H_2S donor caused closure, but that prolonged exposure to the H_2S donor caused increased stomatal apertures. Of particular significance, they showed that the effects of H_2S were mediated by 8-mercapto-cGMP. Downstream of this signaling molecule it was found that Ca^{2+}, cADP ribose and slow anion channel 1 were involved. It has also been suggested that H_2S interacts with abscisic acid (ABA) to have effects (Jin *et al*., 2013)

and that H_2S is involved in ethylene signaling which leads to stomatal closure (Liu *et al*., 2011). Using a DES1 mutant in Arabidopsis the role of H_2S and its interactions with NO and ABA were further investigated (Scuffi *et al*., 2014). It was found that ABA induced DES1 expression, and that DES1 was needed for ABA-dependent NO generation, with NO being downstream of H_2S in the signaling pathway. The authors concluded that DES1, and hence H_2S accumulation, should be included as a component of the ABA pathway. The involvement of ABA has been studied further recently, showing cross-talk between ABA signaling and H_2S during drought responses (Ma *et al*., 2016). The expression levels of genes involved in ABA metabolism were altered, somewhat differently in roots and leaves, although expression levels of ABA receptors were increased in both roots and leaves following H_2S treatment during drought stress. More recently drought-responsive genes which may be regulated by H_2S were studied in wheat (*Triticum aestivum* L.). Over 7500 genes were identified as being worth further investigation (Li *et al*., 2017b). Genes involved in iron transport were of significance as were pathways for protein processing in the endoplasmic reticulum and fatty acid degradation. H_2S was also shown to influence plant hormone signaling pathways, including transcription factors and protein kinases. Such studies highlight the wide-ranging influences of H_2S on plant growth and survival.

Also looking at gene expression Wei *et al*. (2017b), suggested that H_2S positively enhances the expression of senescence associated genes (SAGs). It their system H_2S suppressed chlorophyll degradation of detached leaves and they suggested that S-nitrosoglutathione reductase was important, implicating NO metabolism in the mechanism.

354 H_2S alleviated the effects of salt stress of roots in Arabidopsis. Using NaHS
355 ion transport was shown to be important, but there was also a dependence on H_2O_2
356 (Li *et al*., 2013b). It appeared that H_2S increased intracellular H_2O_2 by regulating the
357 activities of two enzymes: glucose-6-phosphate dehydrogenase (G6PDH) and
358 plasma membrane (PM) NADPH oxidase. This seems to be the wrong way around,
359 as this would increase oxidative stress in the tissues unless the increases are
360 carefully controlled by antioxidants and/or compartmentalisation. In contrast, H_2S
361 was also found to alleviate oxidative stress in sweet potato undergoing osmotic
362 stress, induced with PEG-6000 (Zhang *et al*., 2009b). NaHS treatment alleviated
363 chlorophyll loss, while antioxidants such as catalase and superoxide dismutase
364 (SOD) were increased. Both H_2O_2 and MDA were decreased on NaHS treatment. In
365 a subsequent study NaHS was found to increase wheat seed germination during
366 osmotic stress with PEG-6000 (Zhang *et al*., 2010c). Again, H_2O_2 and MDA were
367 reduced, while catalase and ascorbate peroxidase activities were increased.
368 Interestingly, SOD was not affected in this study.

369 Both ROS and NO have been extensively studied in pathogen challenge of
370 plants (Bellin *et al*., 2013), but less is known about the involvement of H_2S. Some work
371 has been reported with fungi infections (Bloem *et al.*, 2012) while effects of H_2S on
372 plant pathogenic bacteria have be studied (Neale *et al*., 2017).

373 Other volatile compounds need to be considered too (Piechulla *et al*., 2017)
374 and interestingly H_2S was shown to be downstream of methane in the induction of
375 adventitious root development in cucumber (Kou *et al.,* 2018). One gas that is gaining
376 prominence in the literature due to its ability to ameliorate disease symptoms and to
377 alleviate stress is H_2 gas (Cui *et al*., 2014; Wilson *et al*., 2018). How cell signaling by
378 this gas intermeshes with that of H_2S needs to be determined in the future.

Fitting H_2S into signaling pathways

The placement of H_2S in signaling pathways is important: is it upstream or downstream of other effectors? In a study with tomato and high salt stress, DaSilva *et al.* (2018) concluded that H_2S was downstream of NO, mitigating oxidative stress and helping the plant to tolerance the stress it was exposed to. Others have also suggested that H_2S was downstream to NO (Li *et al*., 2013c) and also downstream to salicylic acid (Li *et al*., 2015). In banana H_2S alleviated post-harvest ripening and senescence by a reduction of oxidative stress but also an inhibition of ethylene signaling (Ge *et al*., 2017). The wider issue of the involvement of sulfur-based compounds on phytohormones was reviewed elsewhere (Hasanuzzaman *et al.*, 2018). Also in tomato H_2S was shown to be upstream of NADPH oxidase (RBOH1) and H_2O_2 accumulation (Mei *et al*., 2017). In Arabidopsis roots H_2S was also shown to regulate NADPH oxidase, as well as glucose-6-phosphate dehydrogenase (G6PDH) (Li *et al*., 2013b). In guard cells of Arabidopsis H_2S was shown to regulate NADPH oxidase activity, and hence ROS accumulation, while at the same time increased phospholipase Dδ-derived phosphatidic acid levels, and so alter further signaling pathways in the cells (Scuffi *et al*., 2018).

MAPKs have been been shown to be involved in some studies. In discussion above MAPK was implicated in the toxicity response to H_2S, but in a study on cold stress in *Arabidopsis thaliana* MPK4 was shown to be important. H_2S inhibited stomatal opening under cold stress, and it was concluded that H_2S was upstream of the MAPK pathway (Du *et al.,* 2017).

In has been suggested that H_2S has a modulating effect on ROS and RNS metabolism in some cases (Hancock and Whiteman, 2014). The over-accumulation of

ROS, or RNS, can lead to detrimental effects on cells and tissues, perhaps triggering programmed cell death. Such effects of ROS and RNS may be mediated by their influence on the intracellular redox poise of the cell (Schafer and Buettner, 2001). As H_2S can directly react with some ROS species and NO then the accumulation of these signals may be lowered. Further, H_2S can react with enzymes which generate ROS and RNS, and can influence the levels of antioxidants which lower ROS and RNS, and so restore the cellular redox poise. This has be previously discussed in more detail (Hancock and Whiteman, 2014; 2015), but such an influence of H_2S would account for many of the results of H_2S during cellular stress. Further, it has been argued recently that the maintenance of the cellular redox, perhaps with H_2S's influence, is essential to allow the correct functioning of redox-based signals such as ROS and RNS (Hancock and Whiteman, 2018). It is undoubtedly a fine balance between the levels of ROS, RNS, H_2S, antioxidants and redox poise that will allow a cell to mount the correct response to any stress put upon it.

Conclusions and future perspectives

H_2S has been implicated in a large range of plant cell functions, from germination (Zhang *et al*., 2008; Li *et al*., 2012a; Dooley *et al*., 2013), root development (Zhang *et al*., 2009a; Lin *et al*., 2012), stomatal aperture control (Garcia-Mata and Lamattina, 2010; Lisjak *et al*, 2010), to flower senescence (Zhang *et al*., 2011). It has also be shown to be involved in a myriad of stress responses, including heavy metal stress, freezing, heat, salt stress and oxidative stress.

It is known that H_2S has an important role in plant growth and stress responses but it is becoming clear that numerous volatile compounds may need to be considered (Piechulla *et al*., 2017), including H_2S and H_2 gas (Cui *et al*., 2014). It

is a holistic approach to the effects of such compounds that is needed to fully understand how plants can respond to their environment. Many redox-based compounds are clearly involved in the control of cellular function and it is the interactions between them that needs to be considered. Much work over many years has concentrated on ROS and RNS metabolism in plants, but the influence of H_2S needs to be understood in different tissues under different conditions, including a wide range of stresses. Furthermore, the effects on intracellular redox and thiol-controlled proteins are vital to understand so that redox-based metabolism, be it that of ROS, RNS or H_2S can be modulated to aid plant growth under stressful conditions. This is perhaps even more important now that world climate change is being perceived to be having tangible effects (Makuvaro *et al*., 2018).

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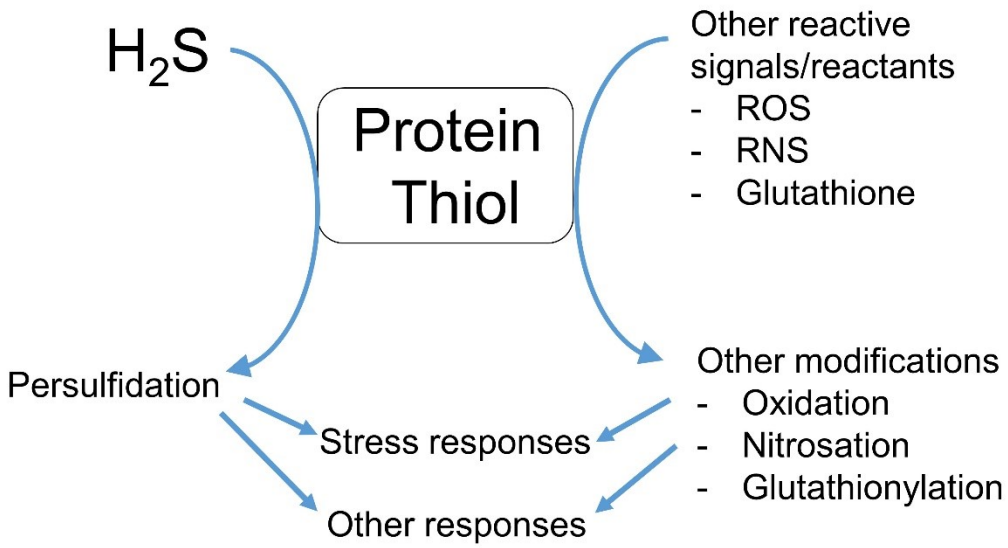
Figure Legends

Figure 1: Hydrogen sulfide may directly modify protein thiol groups.

The thiol group (-SH) on proteins may be modified by H_2S but thiol groups can be reacted with other reactive signaling compounds in the cell. Therefore, a competition may be set up in the cell, with the end result dependent on the prominence of the signaling molecules present.



839 Figure 2: Hydrogen sulfide is a central component in plant stress responses.

840 Many stresses may lead to the accumulation of H_2S in plants and this may lead to

841 the alleviation of cell stress.

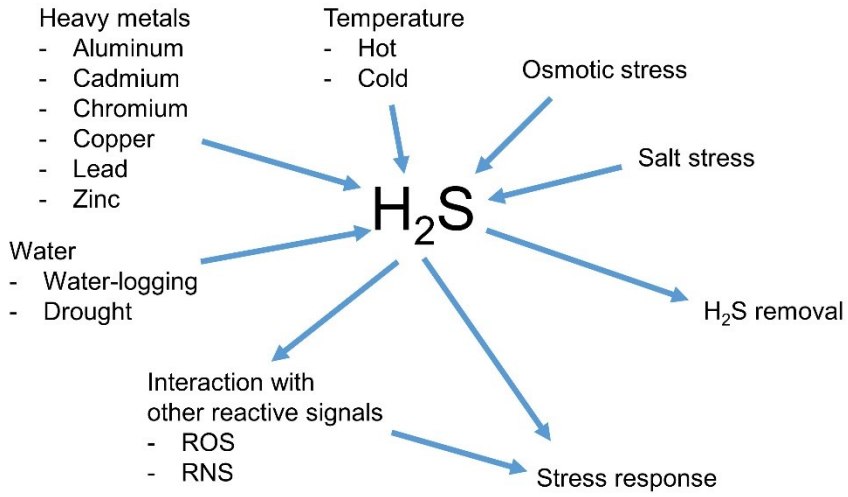


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Figure 3: Downstream effects of H_2S that may lead to a response.

There are many ways in which H_2S may influence cellular function: interaction with other reactive signals; modulation of enzyme activities; persulfidation; effects on antioxidants. These are not exclusive and a combination of downstream events may lead to the ultimate response.