**Hydrogen sulfide and environmental stresses**

John T. Hancock

Department of Applied Sciences, University of the West of England, Bristol, UK.

\*Correspondence:

Prof. John T. Hancock

Faculty of Health and Applied Sciences,

University of the West of England, Bristol, BS16 1QY, UK.

[john.hancock@uwe.ac.uk](mailto:john.hancock@uwe.ac.uk)

Short title: Hydrogen sulfide and plant stress

**Abstract**

Hydrogen sulfide (H2S) 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) H2S is involved in a range of stress responses, including following treatment with heavy metals, salt, temperature change and pathogen challenge. H2S can lead to changes in the activity of antioxidants, cell signaling proteins such as mitogen activated protein kinases (MAPKs) and gene expression. Understanding how H2S fits into cell signaling pathways may lead to advances in how treatment with H2S or H2S 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 (H2S) (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 (H2O2), 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 H2S was being dubbed as the third gaseous transmitter (Wang, 2002) along with NO and carbon monoxide (CO), and it has been accepted that H2S can act in cell signaling in plants (Wang, 2003; Hancock *et al*., 2011; Wang 2012; Li, 2013; Lisjak *et al*., 2013).

H2S 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 H2S 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, H2S 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 H2S 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 H2S in cell signaling. It seems as though organisms have evolved to not only tolerate the presence of H2S, but to use it as an integral part of a cell’s control system (Hancock, 2017), in both animals and plants.

H2S 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 H2S/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 H2S 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 H2S has been reported in cells. H2S generating enzymes such as cystathione ϒ-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 H2S. 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 H2S action is important to ascertain, as recently demonstrated by a study on seed germination, where in fact H2S 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 H2S (Clarke, 1953). Therefore plants cells will most likely be exposed to sulfide, whether it is being used in signaling or not. Removal of H2S 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 H2S 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 H2S signaling, but such fine details have not been reported to date. New H2S 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 H2S is often supplied to a system under study using compounds which release H2S 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 H2S 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 H2S donors see Powell *et al*. (2018).

**Intracellular effects of H2S**

As well as being able to inhibit mitochondrial activity (Dorman, 2002; Martin and Maricle, 2015) H2S may also be used by mitochondria (Bouillaud *et al*., 2013) as an electron donor. In fact, the mechanisms of how H2S 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 H2S to be useful in cell signaling and stress responses H2S needs to be perceived and its presence acted on. Normally such activity is triggered by a ligand (in this case H2S) arriving at a receptor and initiating a signal transduction pathway. It has been suggested that H2S 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 H2S. It is likely that in plants a similar chemistry is involved in H2S perception (as discussed below).

One of the direct influences of H2S will be on the presence of the other stress signals, such as ROS and NO. H2S 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 H2S. This has two consequences. Firstly the presence of both H2S 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 H2S 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 H2O2 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 H2O2, for example between active and inactive. With increased concentrations of H2O2 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 H2S 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, H2S 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 H2S 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 H2S (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 H2S (Sen *et al*., 2012). Certainly H2S has been shown to have large effects on the gene expression profiles of plants cells (Li *et al*., 2017b).

One of the influences of H2S 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 H2S 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, H2S 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. H2S 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 H2S may alter intracellular redox but may also be of commercial importance too.

In a similar manner, gaseous signal molecules such as H2S 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 H2S on salinity tolerance were through effects on oxidative stress, and possibly NO. Therefore, overall the influence of H2S on the intracellular redox status of cells is an important issue to be aware of.

**Stress responses involving H2S**

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 H2O2 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 H2 (Cui *et al*., 2014) and H2S would be expected. Previous reviews have discussed how H2S 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 H2S 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). H2S 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 H2S 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 H2S fumigation. At the other end of the temperature scale, heat tolerance is also improved by the treatment with H2S or donors. It was found that H2S – 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 H2S action was being mediated by an increase in proline levels in cells. Subsequently, NaHS, acting as a H2S donor, enhanced salicyclic acid (SA)-induced heat tolerance in maize (Li *et al*., 2015), an effect blocked if a H2S biosynthesis inhibitor or H2S scavenger was used. The authors suggested that H2S was acting downstream of SA. In tobacco, the H2S 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, H2O2 and NO were all lower in H2S 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 H2S 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 H2S 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 H2O2 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 H2S alleviated the toxicity of Al to germinating wheat seedlings (Zhang *et al*., 2010b).

The role in heavy metal stress by H2S 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 H2S donors (Kharbech *et al.*, 2017). ROS was found to be important in the H2S 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 H2O2 content (Kaya *et al*., 2018). In a study on roots, Lv *et al*. (2017) showed that 4µM Cd led to an increase in H2S at the root tip, an effect blocked by a H2S scavenger or an inhibitor of H2S biosynthesis. It was concluded that part of the action of H2S was through the modulation of the H2O2 and O2·- levels at the tip. Also recently, H2S 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 H2O2 content was decreased following changes in antioxidant levels. The authors also pointed out that H2S exhibited crosstalk with NO signaling, emphasizing that H2S 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. H2S toxicity itself appears in some instances to require ROS and NO. The inhibition of growth caused by H2S 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 H2S, in *Potamogeton crispus* and *Myriophyllum spicatum* there was an increase in oxidative stress, increased H2O2 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 H2S on the control of stomatal closure has been of some debate. H2S 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 H2S donor caused closure, but that prolonged exposure to the H2S donor caused increased stomatal apertures. Of particular significance, they showed that the effects of H2S were mediated by 8-mercapto-cGMP. Downstream of this signaling molecule it was found that Ca2+, cADP ribose and slow anion channel 1 were involved. It has also been suggested that H2S interacts with abscisic acid (ABA) to have effects (Jin *et al*., 2013) and that H2S is involved in ethylene signaling which leads to stomatal closure (Liu *et al*., 2011). Using a DES1 mutant in Arabidopsis the role of H2S 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 H2S in the signaling pathway. The authors concluded that DES1, and hence H2S 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 H2S 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 H2S treatment during drought stress. More recently drought-responsive genes which may be regulated by H2S 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. H2S was also shown to influence plant hormone signaling pathways, including transcription factors and protein kinases. Such studies highlight the wide-ranging influences of H2S on plant growth and survival.

Also looking at gene expression Wei *et al*. (2017b), suggested that H2S positively enhances the expression of senescence associated genes (SAGs). It their system H2S suppressed chlorophyll degradation of detached leaves and they suggested that S-nitrosoglutathione reductase was important, implicating NO metabolism in the mechanism.

H2S alleviated the effects of salt stress of roots in Arabidopsis. Using NaHS ion transport was shown to be important, but there was also a dependence on H2O2 (Li *et al*., 2013b). It appeared that H2S increased intracellular H2O2 by regulating the activities of two enzymes: glucose-6-phosphate dehydrogenase (G6PDH) and plasma membrane (PM) NADPH oxidase. This seems to be the wrong way around, as this would increase oxidative stress in the tissues unless the increases are carefully controlled by antioxidants and/or compartmentalisation. In contrast, H2S was also found to alleviate oxidative stress in sweet potato undergoing osmotic stress, induced with PEG-6000 (Zhang *et al*., 2009b). NaHS treatment alleviated chlorophyll loss, while antioxidants such as catalase and superoxide dismutase (SOD) were increased. Both H2O2 and MDA were decreased on NaHS treatment. In a subsequent study NaHS was found to increase wheat seed germination during osmotic stress with PEG-6000 (Zhang *et al*., 2010c). Again, H2O2 and MDA were reduced, while catalase and ascorbate peroxidase activities were increased. Interestingly, SOD was not affected in this study.

Both ROS and NO have been extensively studied in pathogen challenge of plants (Bellin *et al*., 2013), but less is known about the involvement of H2S. Some work has been reported with fungi infections (Bloem *et al.*, 2012) while effects of H2S on plant pathogenic bacteria have be studied (Neale *et al*., 2017).

Other volatile compounds need to be considered too (Piechulla *et al*., 2017) and interestingly H2S was shown to be downstream of methane in the induction of adventitious root development in cucumber (Kou *et al.,* 2018). One gas that is gaining prominence in the literature due to its ability to ameliorate disease symptoms and to alleviate stress is H2 gas (Cui *et al*., 2014; Wilson *et al*., 2018). How cell signaling by this gas intermeshes with that of H2S needs to be determined in the future.

**Fitting H2S into signaling pathways**

The placement of H2S 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 H2S was downstream of NO, mitigating oxidative stress and helping the plant to tolerance the stress it was exposed to. Others have also suggested that H2S was downstream to NO (Li *et al*., 2013c) and also downstream to salicylic acid (Li *et al*., 2015). In banana H2S 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 H2S was shown to be upstream of NADPH oxidase (RBOH1) and H2O2 accumulation (Mei *et al*., 2017). In Arabidopsis roots H2S was also shown to regulate NADPH oxidase, as well as glucose-6-phosphate dehydrogenase (G6PDH) (Li *et al*., 2013b). In guard cells of Arabidopsis H2S was shown to regulate NADPH oxidase activity, and hence ROS accumulation, while at the same time increased phospholipase D-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 H2S, but in a study on cold stress in *Arabidopsis thaliana* MPK4 was shown to be important. H2S inhibited stomatal opening under cold stress, and it was concluded that H2S was upstream of the MAPK pathway (Du *et al.,* 2017).

In has been suggested that H2S 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 H2S can directly react with some ROS species and NO then the accumulation of these signals may be lowered. Further, H2S 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 H2S would account for many of the results of H2S during cellular stress. Further, it has been argued recently that the maintenance of the cellular redox, perhaps with H2S’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, H2S, antioxidants and redox poise that will allow a cell to mount the correct response to any stress put upon it.

**Conclusions and future perspectives**

H2S 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 H2S 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 H2S and H2 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 H2S 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 H2S 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).

**References**

# Ali, S., Farooq, M.A., Hussain, S., Yasmeen, T., Abbasi, G.H., Zhang, G., 2013. Alleviation of chromium toxicity by hydrogen sulfide in barley. Environ. Toxicol. Chem. 32, 2234-2239.

# Ali, B., Song, W.J., Hu, W.Z., Luo, X.N., Gill, R.A., Wang, J., Zhou, W.J., 2014. Hydrogen sulfide alleviates lead-induced photosynthetic and ultrastructural changes in oilseed rape. Ecotox. Environ. Safe 102, 25-33.

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

# Arasimowicz, M., Floryszak-Wieczorek, J., 2007. Nitric oxide as a bioactive signalling molecule in plant stress responses. Plant Science 172, 876-887.

# Aroca, A., Benito, J.M., Gotor, C., Romero, L.C., 2017a. Persulfidation proteome reveals the regulation of protein function by hydrogen sulfide in diverse biological processes in Arabidopsis. J. Exp. Bot. 13, 4915-4927.

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

# Aroca, Á., Serna, A., Gotor, C., Romero, L.C., 2015. *S* -sulfhydration: A cysteine posttranslational modification in plant systems. Plant Physiol. 168, 334–342.

# Baxter, A., Mittler, R., Suzuki, N., 2014. ROS as key players in plant stress signalling Journal of Experimental Botany 65, 1229–1240.

# Bellin, D., Asai, S., Delledonne, M., Yoshioka, H., 2013. Nitric oxide as a mediator for defense responses. MPMI 26, 271–277.

# Bita, C.E., Gerats, T., 2013. Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. Front. Plant Sci. 4, 273.

# Bloem, E., Haneklaus, S., Kesselmeier, J., Schung, E., 2012. Sulfur fertilization and fungal infections affect the exchange of H2S and COS from agricultural crops. J. Agric. Food Chem.60, 7588-7596.

# Bouillaud, F., Ransy, C., Andriamihaja, M., 2013. Sulfide and mitochondrial bioenergetics. Nitric Oxide 31, S15.

# Baudouin, E., Poilevey, A., Indiketi Hewage, N., Cochet, F., Puyaubert, J., Bailly, C., 2016. The significance of hydrogen sulfide for Arabidopsis seed germination. Front. Plant Sci., *7, 930.*

# Calderwood, A., Kopriva, S., 2014. Hydrogen sulfide in plants: From dissipation of excess sulfur to signaling molecule. Nitric Oxide 41, 72-78.

# Carballal, S., Trujillo, M., Cuevasanta, E., Bartesaghi, S., Möller, M.N., Folkes, L.K., García-Bereguiaín, M.A., Gutiérrez-Merino, C., Wardman, P., Denicola, A., Radi, R., Alvarez, B., 2011. Reactivity of hydrogen sulfide with peroxynitrite and other oxidants of biological interest. Free Radic. Biol. Med. 50, 196-205.

# Chen, F., Han, D., Liu, H., Wang, S., Li, K.B., Zhang, S., Shi, W., 2018. A tri-site fluorescent probe for simultaneous sensing of hydrogen sulfide and glutathione and its bioimaging applications. Analyst 143, 440-448.

# Chen, J., Wang, W.-H., Wu ,F.-H., You, C.-Y., Liu, T.-W., Dong, X.-J., He, J.-X., Zheng, H.-L., 2012. Hydrogen sulfide alleviates aluminum toxicity in barley seedlings. Plant Soil 362, 301-318.

# Christou, A., Filippou, P., Manganaris, G.A., Fotopoulos, V., 2014. Sodium hydrosulfide induces systemic thermotolerance to strawberry plants through transcriptional regulation of heat shock proteins and aquaporin. BMC Plant Biol. 14, 42.

# Clarke, P.H., 1953. Hydrogen sulphide production by bacteria. J. Gen. Microbiol. 8, 397-407.

# Cui, W., Fang, P., Zhu, K., Mao, Y., Gao, C., Xie, Y., Wang, J., Shen, W., (2014) Hydrogen-rich water confers plant tolerance to mercury toxicity in alfalfa seedlings. Ecotoxicol Environ Saf. 105, 103-11

# DaSilva, C.J., Mollica, D.C.F., Vicente, M.H., Peres, L.E.P., Modolo, L.V., 2018. NO, hydrogen sulfide does not come first during tomato response to high salinity. Nitric Oxide 76,164-173.

# De Kok, J.L., Bosma, W., Maas, F.M., Kuiper, P.J.C., 1985. The effect of short-term H2S fumigation on water-soluble sulphydryl and glutathione levels in spinach. Plant Cell Environ. 8, 189-194.

# Dooley, F.D., Nair, S.P., Ward, P.D., 2013. Increased growth and germination success in plants following hydrogen sulfide administration. PLoS One 8, e62048.

# Dorman, D.C., Moulin, F.J., McManus, B.E., Mahle, K.C., James, R.A., Struve, M.F., 2002. Cytochrome oxidase inhibition induced by acute hydrogen sulfide inhalation: correlation with tissue sulfide concentrations in the rat brain, liver, lung, and nasal epithelium. Toxicol. Sci. 65, 18-25.

# Du, X., Jin, Z., Liu, D., Yang, G., Pei, Y., 2017. Hydrogen sulfide alleviates the cold stress through MPK4 in *Arabidopsis thaliana*. Plant Physiol. Biochem. 120, 112-119.

# Filipovic, M.R., Jovanovic, V.M., 2017, More than just an intermediate: hydrogen sulfide signalling in plants. J. Exp. Bot. 68, 4733-4736.

# Fort, D.J., Todhunter, K., Fort, T.D., Mathis, M.B., Walker, R., Hansel, M., Hall, S., Richards, R., Anderson, K., 2017. Toxicity of sulfide to early life stages of wild rice (*Zizania palustris*). Environ. Toxicol. Chem. 36, 2217-2226.

# Foyer, C.H., Theodoulou, F.L., Delrot, S., 2001. The functions of inter- and intracellular glutathione transport systems in plants. Trends in Plant Science 6, 486-492.

# Fu, L.H., Wei, Z.Z., Hu, K.D., Hu, L.Y., Li, Y.H., Chen, X.Y., Han, Z., Yao, G.F., Zhang, H., 2018. Hydrogen sulfide inhibits the growth of *Escherichia coli* through oxidative damage. J. Microbiol. 56, 238-245.

# García-Mata, C., Lamattina, L., 2010. Hydrogen sulfide, a novel gasotransmitter involved in guard cell signalling. NewPhytol. 188, 977-984.

# García-Mata, C., Lamattina, L., 2013. Gasotransmitters are emerging as new guard cell signaling molecules and regulators of leaf gas exchange. Plant Sci. 201/202, 66–73.

Ge, Y., Hu, K.D., Wang, S.S., Hu, L.Y., Chen, X.Y., Li, Y.H., Yang, Y., Yang, F., Zhang, H., 2017. Hydrogen sulfide alleviates postharvest ripening and senescence of banana by antagonizing the effect of ethylene. PLoS One 12, e0180113.

Go, Y.-M., Jones, D.P., 2008. Redox compartmentalization in eukaryotic cells. Biochim. Biophys. Acta 1780, 1273–1290.

Grennan, A.K., 2007. Protein S-nitrosylation: Potential targets and roles in signal transduction. Plant Physiol. 144, 1237-1239.

Hancock, J.T., 2009. The role of redox mechanisms in cell signaling. Mol. Biotechnol. 43, 162-166.

Hancock, J. T., 2017. Harnessing evolutionary toxins for signaling: Reactive oxygen species, nitric oxide and hydrogen sulfide in plant cell regulation. Frontiers in Plant Science 8, 189.

Hancock, J.T., Henson, D., Nyirenda, M., Desikan, R., Harrison, J., Lewis, M., Hughes, J., Neill, S.J., 2005. Proteomic identification of glyceraldehyde 3-phosphate dehydrogenase as an inhibitory target of hydrogen peroxide in *Arabidopsis*. Plant Physiol. Biochem. 43, 828-835.

Hancock, J.T., Lisjak, M., Teklic, T., Wilson, I.D., Whiteman, M., 2011. Hydrogen sulfide and signaling in plants. CAB Reviews: Perspectives in Agriculture, Veterinary Science. Nutrition and Natural Resources 6, 1-7.

Hancock, J. T., Whiteman, M., 2014. Hydrogen sulfide and cell signaling: Team player or referee? Plant Physiology and Biochemistry 78, 37-42.

Hancock, J. T., Whiteman, M., 2015. Hydrogen sulfide and reactive friends: The interplay with reactive oxygen species and nitric oxide signalling pathways. In: de Kok, L., Hawkesford, M., Rennenberg, H. and Saito, K., eds. (2015) Molecular Physiology and Ecophysiology of Sulfur. Springer, pp. 153-168. ISBN 9783319201368.

Hancock, J. T., Whiteman, M., 2018. Cellular redox environment and its influence on redox signalling molecules. Reactive Oxygen Species, 5 (14).

# Hasanuzzaman, M., Bhuyan, M.H.M.B., Mahmud, J.A., Nahar, K., Mohsin, S.M. Parvin, K., Fujita, M., 2018. Interaction of sulfur with phytohormones and signaling molecules in conferring abiotic stress tolerance to plants. Plant Signal. Behav. 25, 1-5.

# Hatzfeld, Y., Maruyama, A., Schmidt, A., Noji, M., Ishizawa, K., Saito, K., 2000. β-cyanoalanine synthase is a mitochondrial cysteine synthase-like protein in spinach and Arabidopsis. Plant Physiol. 123, 1163–1172.

# He, H., He, L.F., 2018. Regulation of gaseous signaling molecules on proline metabolism in plants. Plant Cell Rep. 37, 387-392.

# He, H., Li, Y., He, L.F., 2018. The central role of hydrogen sulfide in plant responses to toxic metal stress. Ecotoxicol Environ Saf. 157, 403-408.

# Holtgrede, S., Godlke, J., Starmann, J., Druce, S., Klocke, S., Altmann, B., Wcjtera, J., Lindermayr, C., Scheibe, R., 2008. Regulation of plant cytosolic glyceraldehyde 3-phosphate dehydrogenase isoforms by thiol modifications. Physiol. Plantarum 133, 211-228.

# 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 Cell Physiol. 56, 1481–1489.

# Hu, L.-Y., Hu, S.-L., Wu, J., Li, Y.-H., Zheng, J.-L., Wei, Z.-J., Liu, J., Wang, H.-L., Liu, Y.-S., Zhang, H., 2012. Hydrogen sulfide prolongs postharvest shelf life of strawberry and plays an antioxidative role in fruits. J Agric Food Chem 60, 8684-8693.

# Jaffrey, S.R., Snyder, S.H., 2001. The biotin switch method for the detection of *S*-nitrosylated proteins. Sci. STKE 2001, pl1.

# Jasechko, S., 2018. Plant turn on the tap. Nature Climate Change 8, 562-583.

# Jennings, M.L., 2013. Transport of H2S and HS− across the human red blood cell membrane: rapid H2S diffusion and AE1-mediated Cl−/HS− exchange. Am. J. Physiol. - Cell Physiol. Cell Physiol. 305, C941–C950.

# Jiang, J., Chan, A., Ali, S., Saha, A., Haushalter, K.J., Lam, W.-L. M., Glasheen, M., Parker, J., Brenner, M., Mahin, S.B., Patel, H., Ambasudhan, R., Lipton, S.A. Pilz, R.B., Boss, G.R., 2016. Hydrogen sulfide - mechanisms of toxicity and development of an antidote. Scientific Reports 6, 20831.

# Jin, Z.P., Xue, S.W., Luo, Y.N., Fang, B.H., Tian, H.H., Li, H., Pei, Y.X., 2013. Hydrogen sulﬁde interacting with abscisic acid in stomatal regulation responses to drought stress in *Arabidopsis*. Plant Physiol. Biochem. 62, 41–46.

# Kavi Kishor, P.B., Sreenivasulu, N., 2014. Is proline accumulation per se correlated with stress tolerance or is proline homeostasis a more critical issue? Plant Cell Environ. 37, 300-311.

# Kaya, C., Ashraf, M., Akram, N.A., 2018. Hydrogen sulfide regulates the levels of key metabolites and antioxidant defense system to counteract oxidative stress in pepper (*Capsicum annuum* L.) plants exposed to high zinc regime. Environ. Sci. Pollut. Res. Int. 25, 12612-12618.

# Kharbech, O., Houmani, H., Chaoui, A., Corpas, F.J., 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.

# Koskenkorva-Frank, T.S., Weiss, G., Koppenol, W.H., Burckhardt, S., 2013. The complex interplay of iron metabolism, reactive oxygen species, and reactive nitrogen species: insights into the potential of various iron therapies to induce oxidative and nitrosative stress. Free Radic. Biol. Med. 65, 1174-1194.

# Kou, N., Xiang, Z., Cui, W., Li, L., Shen, W., 2018. Hydrogen sulfide acts downstream of methane to induce cucumber adventitious root development. J. Plant Physiol. 228, 113-120.

# Li, Z.G., 2013. Hydrogen sulfide: a multifunctional gaseous molecule in plants. Russ. J. Plant Physiol.60, 733-740.

# Li, Z.G., Ding, X.J., Du, P.F., 2013a. Hydrogen sulfide donor sodium hydrosulfide-improved heat tolerance in maize and involvement of proline. J. Plant Physiol. 170, 741–747.

# Li, Z.G., Gong, M., Liu, P., 2012a. Hydrogen sulfide is a mediator in H2O2-induced seed germination in *Jatropha Curcas*. Acta Physiol. Plant 34, 2207–2213.

# Li, Z.G., Gong, M., Xie, H., Yang, L., Li, J., 2012b. Hydrogen sulﬁde donor sodium hydrosulﬁde-induced heat tolerance in tobacco (*Nicotiana tabacum* L.) suspension cultured cells and involvement of Ca2+ and calmodulin. Plant Sci. 185/186, 185–189.

# Li, J., Jia, H., Wang, J., Cao, Q., Wen, Z., 2013b. 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, J.R., 2013. Chemical foundations of hydrogen sulfide biology. Nitric Oxide 35, 21-34.

# Li, T.-T., Li, Z.-R., Hu, K.-D., Hu, L.-Y., Chen, X.-Y., Li, Y.-H., Yang, Y., Yang, F., Zhang, H., 2017a. Hydrogen sulfide alleviates Kiwifruit ripening and senescence by antagonizing effect of ethylene. HortScience 52, 1556-1562.

# Li, H., Li, M., Wei, X., Zhang, X., Xue, R., Zhao, Y., Zhao, H., 2017b. Transcriptome analysis of drought-responsive genes regulated by hydrogen sulfide in wheat (*Triticum aestivum* L.) leaves. Mol. Genet. Genomics. 292, 1091-1110.

# Li, L., Wang, Y., Shen, W., 2012c. Roles of hydrogen sulfide and nitric oxide in the alleviation of cadmium-induced oxidative damage in alfalfa seedling roots. BioMetals 25, 617-631.

# Li, Z.G., Xie, L.R. and Li, X.J., 2015. Hydrogen sulfide acts as a downstream signal molecule in salicylic acid-induced heat tolerance in maize (*Zea mays* L.) seedlings. J. Plant Physiol. 177, 121-127.

# Li, Z.G., Yang, S.Z., Long, W.B., Yang, G.X., Shen, Z.Z., 2013c. Hydrogen sulfide may be a novel downstream signal molecule in nitric oxide-induced heat tolerance of maize (*Zea mays* L.) seedlings. Plant Cell Environ. 36, 1564–1572.

# Lin, Y.-T., Li, M.-Y., Cui, W.-T., Lu, W., Shen, W.-B., 2012. Haem oxygenase-1 in involved in hydrogen sulfide induced cucumber adventitious root formation. J. Plant Growth Regul. 31, 519-528.

# Lin, V.S., Lippert, A.R., Chang, C.J., 2013. Cell-trappable fluorescent probes for endogenous hydrogen sulfide signalling and imaging H2O2-dependent H2S production. Proc. Natl. Acad. Sci. USA 110l, 7131-7135.

# Lindermayr, C., Sallbach, G., Durner, J., 2005. Proteomic identification of *S*-nitrosylated proteins in Arabidopsis. Plant Physiol. 137, 921-930.

# Lisjak, M., Srivastava, N., Teklic, T., Civale, L., Lewandowski, K., Wilson, I., Wood, M.E., Whiteman, M., Hancock, J.T., 2010. A novel hydrogen sulfide donor causes stomatal opening and reduces nitric oxide accumulation. Plant Physiol. Biochem. 48, 931-935.

# Lisjak, M., Teklic, T., Wilson, I.D., Whiteman, M., Hancock, J.T., 2013. Hydrogen sulfide: Environmental factor or signaling molecule? Plant Cell Environ. 36, 1607-1616/.

# Lisjak, M., Teklic, T., Wilson, I.D., Wood, M., Whiteman, M., Hancock, J.T., 2011. Hydrogen sulfide effects on stomatal apertures. Plant Signaling and Behavior 6, 1444-1446.

# Liu, J., Hou, L.-X., Liu, G.-H., Liu, X., Wang, X.-C., 2011. Hydrogen sulfide induced by nitric oxide mediates ethylene-induced stomatal closure of *Arabidopsis thaliana*. Chinese Sci. Bull. 56, 3547-3553.

# 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*. Front. Plant Sci. 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 pathway in wheat. PLoS One 11, e0163082.

# Makuvaro, V., Walker, S., Masere, T.P., Dimes, J., 2018. Smallholder farmer perceived effects of climate change on agricultural productivity and adaptation strategies. Journal of Arid Environments 152, 75-82.

# Martin, W., Baross, J., Kelley, D., Russell, M.J., 2008. Hydrothermal vents and the origin of life. Nat. Rev. Microbiol. 6, 805-814.

# Martin, W.F., Cerff, R., 2017. Physiology, phylogeny, early evolution, and GAPDH. Protoplasma 254, 1823-1834.

# Martin, N.M., Maricle, B. R., 2015. Species-specific enzymatic tolerance of sulfide toxicity in plant roots. Plant Physiology and Biochemistry 88, 36-41.

# Mathai, J.C., Missner, A., Kügler, P., Saparov, S.M., Zeidel, M.L., Lee, J.K., Pohl, P., 2009. No facilitator required for membrane transport of hydrogen sulfide. Proc. Natl. Acad. Sci. U.S.A. 106, 16633-16638.

# Mei, Y., Chen, H., Shen, W., Shen, W., Huang, L., 2017. Hydrogen peroxide is involved in hydrogen sulfide-induced lateral root formation in tomato seedlings. BMC Plant Biol. 17, 162.

# Meyer, T., Burow, M., Bauer, M., Papenbrock, J., 2003. Arabidopsis sulfurtransferases: investigation of their function during senescence and in cyanide detoxification. Planta 217, 1-10.

# Mishanina, T.V., Libiad, M., Banerjee, R., 2015. Biogenesis of reactive sulfur species for signaling by hydrogen sulfide oxidation pathways. Nature Chemical Biology 11, 457-464.

# Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science 7, 405-410.

# Módis, K., Ju, Y., Ahmad, A., Untereiner, A.A., Altaany, Z., Wu, L., Szabo, C., Wang, R., 2016. S-sulfhydration of ATP synthase by hydrogen sulfide stimulates mitochondrial bioenergetics. Pharmacol. Res. 113, 116–124.

# Moore, P.K., Whiteman, M., 2015. Chemistry, Biochemistry and Pharmacology of Hydrogen Sulfide. Springer. ISBN: 9783319181448

# Neale, H., Deshappriya, N., Arnold, D. L., Wood, M. E., Whiteman, M., Hancock, J. T., 2017. Hydrogen sulfide causes excision of a genomic island in *Pseudomonas syringae pv. phaseolicola*. European Journal of Plant Pathology 149, 911-921.

# Neill, S.J., Desikan, R., Clarke, A., Hurst, R.D., Hancock, J.T., 2002. Hydrogen peroxide and nitric oxide as signalling molecules in plants. J. Exp. Bot. 53, 1237-1247.

# Ni, Z.J., Hu, K.D., Song, C.B., Ma, R.H., Li, Z.R., Zheng, J.L., Fu, L.H., Wei, Z.J., Zhang, H., 2016. Hydrogen sulfide alleviates postharvest senescence of grape by modulating the antioxidant defenses. Oxid. Med. Cell Longev. 2016, 4715651.

# Nikalje, G.C., Suprasanna, P., 2018. Coping With Metal Toxicity - Cues From Halophytes. Front Plant Sci. 9, 777.

# Noctor, G., Foyer, C.H., 2016. Intracellular redox compartmentation and ROS-related communication in regulation and signaling. Plant Physiol. 171, 1581-1592.

# Noctor, G., Reichheld, J.P., Foyer, C.H., 2018. ROS-related redox regulation and signaling in plants. Semin. Cell Dev. Biol. 80, 3-12.

# Palmer, R.M.J., Ferrige, A.G., Moncada, S., 1987. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 327, 524-526.

# Parveen, M., Asaeda, T., Rashid, M.H., 2017. Biochemical adaptations of four submerged macrophytes under combined exposure to hypoxia and hydrogen sulphide. PLoS One 12, e0182691.

# Paul, B.D., Snyder, S.H., 2015. Protein sulfhydration. Methods Enzymology 555, 79-90.

# Perkins, N.D., 2006. Post-translational modifications regulating the activity and function of the nuclear factor kappa B pathway. Oncogene 25, 6717-6730.

# Piechulla, B., Lemfack, M.C., Kai, M., 2017. Effects of discrete bioactive microbial volatiles on plants and fungi. Plant Cell Environ. 40, 2042-2067.

# Powell, C.R., Dillon, K.M., Matson, J.B., 2018. A review of hydrogen sulfide (H2S) donors: Chemistry and potential therapeutic applications. Biochemical Pharmacology 149, 110-123.

# Prabhakar, N.R., 2012. Carbon monoxide (CO) & hydrogen sulfide (H2S) in hypoxic sensing by the carotid body. Respiratory Physiology and Neurobiology 184,165-169.

# Romero, L.C., Aroca, M.A., Serna, A., Gotor, C., 2013. Proteomic analysis of endogenous S-sulfhydration in *Arabidopsis thaliana*. Nitric Oxide 31, S23.

# Schafer, F.Q., Buettner, G.R., 2001. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic. Biol. Med. 30, 1191-1212.

# 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 Physiol. 166, 2065–76.

# Scuffi, D., Nietzel, T., Di Fino, L.M., Meyer, A.J., Lamattina, L., Schwarzländer, M., Laxalt, A.M., 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 Physiol. 176, 2532-2542.

# Sen, N., Paul, B.D., Gadalla, M.M., Mustafa, A.K., Sen, T., Xu, R., Kim, S., Snyder, S.H., 2012. Hydrogen sulfide-linked sulfhydration of NF-κB mediates its antiapoptotic actions. Mol. Cell 45, 13-24.

# Shan, C.J., Zhang, S.L., Li, D.F., Zhao, Y.Z., Tian, X.L., Zhao, X.L., Wu, Y.X., Wei, X.Y., Liu, R.Q., 2011. Effects of exogenous hydrogen sulﬁde on the ascorbate and glutathione metabolism in wheat seedlings leaves under water stress. Acta Physiol. Plant 33, 2533–2540.

# Stuiver, C.E.E., De Kok, L.J., Kuiper, P.J.C., 1992. Freezing tolerance and biochemical changes in wheat shoots as affected by H2S fumigation. Plant Physiol. Biochem. 30, 47–55.

# Sun, C., Shi, Z.-Z., Zhou, X., Chen, L., Zhao, X.-M., 2013. Prediction of *S*-glutathionylation sites based on protein sequences. PLoS One 8, e55512.

# Tai, C.H., Cook, P.F., 2000. O-acetylserine sulfhydrylase. Adv. Enzymol. RAMB 74, 185-234.

# Wang, R., 2002. Two's company, three's a crowd: can H2S be the third endogenous gaseous transmitter? FASEB J. 16, 1792-8.

# Wang, R., 2003. The gasotransmitter role of hydrogen sulfide. Antioxid. RedoxSign. 5, 493-501.

# Wang, R., 2012. Physiological implications of hydrogen sulfide: a whiff exploration that blossomed. Physiol. Rev. 92, 791–896.

# Wang, Y., Li, L., Ciu, W., Xu, S., Shen, W., Wang, R., 2012. Hydrogen sulfide enhances alfalfa (*Medicago sativa*) tolerance against salinity during seed germination by nitric oxide pathway. Plant Soil 351, 107-119.

# Wei, G.Q., Cao, H., Sun, Y.G., Deng, B., Zhang, W.W., Yang, H.Q., 2017a. Effects of hydrogen sulfide on root architecture, leaf reactive oxygen and photosynthetic characteristics of *Malus hupehensis* under waterlogging. Ying Yong Sheng Tai Xue Bao 28, 3267-3273.

# Wei, B., Zhang, W., Chao, J., Zhang, T., Zhao, T., Noctor, G., Liu, Y., Han, Y., 2017b. Functional analysis of the role of hydrogen sulfide in the regulation of dark-induced leaf senescence in Arabidopsis. Sci. Rep. 7, 2615.

# Whiteman, M., Li, L., Kostetski, I., Chu, S.H., Siau, J.L., Bhatia, M., Moore, P.K., 2006. Evidence for the formation of a novel nitrosothiol from the gaseous mediators nitric oxide and hydrogen sulphide. Biochem. Biophys. Res. Comm. 343, 303-310.

# Wilson, I.D., Neill, S.J., Hancock, J.T., 2008. Nitric oxide synthesis and signalling in plants. Plant Cell Environ. 31, 622-631.

# Wilson, H. R., Veal, D., Whiteman, M., Hancock, J. T., 2017. Hydrogen gas and its role in cell signaling. CAB Reviews 12, 1-3.

# Youssefian, S., Nakamura, M., Sano, H., 1993. Tobacco plants transformed with the O-acetylserine (thiol) lyase gene of wheat are resistant to toxic levels of hydrogen sulphide gas. Plant J. 4, 759-769.

# Zhang, H., Hu, L.Y., Hu, K.D., He, Y.D., Wang, S.H., Luo, J.P., 2008. Hydrogen sulfide promotes wheat seed germination and alleviates the oxidative damage against copper stress. J. Integrat. Plant Biol. 50, 1518-1529.

# Zhang, H., Hu, L.Y., Li, P., Hu, K.D., Jiang, C.X., Luo, J.P., 2010a. Hydrogen sulfide alleviated chromium toxicity in wheat. Biol. Plant 54, 743–747.

# Zhang, H., Hua, S.L., Zhang, Z.J., Hua, L.Y., Jiang, C.X., Wei, Z.J., Liu, J., Wang, H.L., Jiang, S.T., 2011. Hydrogen sulﬁde acts as a regulator of ﬂower senescence in plants. Postharv. Biol. Technol. 60, 251–257.

# Zhang, P., Luo, Q., Wang, R., Xu, J., 2017. Hydrogen sulfide toxicity inhibits primary root growth through the ROS-NO pathway. Sci. Rep. 13, 868.

# Zhang, M., Qin, B.-p., Ma, X.-l., Wang, P., Li, M,-l., Chen, L.-l., Chen, L.-t., Sun, A.-q, Wang, Z.-l., Yin, Y.-p., 2016. Foliar application of sodium hydrosulfide (NaHS), a hydrogen sulfide (H2S) donor, can protect seedlings against heat stress in wheat (Triticum aestivum L.). Journal of Integrative Agriculture 15, 2745-2758.

# Zhang, H., Tan, Z.Q., Hu, L.Y., Wang, S.H., Luo, J.P., Jones, R.L., 2010b. Hydrogen sulfide alleviates aluminum toxicity in germinating wheat seedlings. Journal of Integrative Plant Biol. 52, 556-567.

# Zhang, H., Tang, J., Liu, X.P., Wang, Y., Yu, W., Peng, W.P., Fang, F., Ma, D.F., Wei, Z.J., Hu, L.Y., 2009a. Hydrogen sulﬁde promotes root organogenesis in *Ipomoea batatas, Salix matsudana and Glycine max*. J. Integr. Plant Biol. 51, 1084–1092.

# Zhang, H., Wang, M.F., Hua, L.Y., Wang, S.H., Hua, K.D., Bao, L.J., Luo, J.P., 2010c. Hydrogen sulfide promotes wheat seed germination under osmotic stress. Russ. J. Plant Physiol. 57, 532–539.

# Zhang, H., Ye, Y.K., Wang, S.H., Luo, J.P., Tang, J., Ma, D.F., 2009b. Hydrogen sulﬁde counteracts chlorophyll loss in sweet potato seedling leaves and alleviates oxidative damage against osmotic stress. Plant Growth Regul. 58, 243-250.

# Zhu, L., Wang, W., Zhang, W., Shen, Y., Du, H., Wu, S., 2014. Hydrogen sulfide extends the postharvest life and enhances antioxidant activity of kiwifruit during storage. J. Sci. Food Agric. 94, 2699-2704.

# Zhu, C.Q., Zhang, J.H., Sun, L.M., Zhu, L.F., Abliz, B., Hu, W.J., Zhong, C., Bai. Z.G., Sajid, H., Cao, X.C., Jin, Q.Y., 2018. Hydrogen sulfide alleviates aluminum toxicity via decreasing apoplast and symplast Al contents in rice. Front. Plant Sci. 9, 294.

# Figure Legends

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

# The thiol group (-SH) on proteins may be modified by H2S 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.

# 

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

# Many stresses may lead to the accumulation of H2S in plants and this may lead to the alleviation of cell stress.

# 

# Figure 3: Downstream effects of H2S that may lead to a response.

# There are many ways in which H2S 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.

# 