Chapter 5

Alleviation of Plant Stress by Molecular Hydrogen

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Abstract Gasotransmitters and gaseous-signaling molecules are hugely important for controlling cell function and especially so during stress challenges. Past research has concentrated on molecules such as nitric oxide (NO) and hydrogen sulfide (H_2S), although others such as ethylene and carbon monoxide (CO) are also important. Here, molecular hydrogen (H_2) is added to the mix. H_2 has been shown to ameliorate responses to a range of stressors in plants, including exposure to heavy metals, salinity, extreme temperatures, and UV radiation. Clearly, H_2 is an important gas, which may be useful for enhancing plant growth and food security in the future. Exogenous treatments with H_2 are easy in the form of hydrogen-rich water (HRW), but there are still issues with its wide-spread use. Furthermore, the molecular basis of the action of H_2 in cells is still not clear. Here, aspects of the use and the action of H_2 in plants are discussed, along with what might be learnt from other species.

Keywords Heavy metals · Hydrogen sulfide · Molecular hydrogen · Nitric oxide · Salinity · Reactive oxygen species · Redox

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T. Aftab, F. J. Corpas (eds.), *Gasotransmitters Signaling in Plants under Challenging Environment*, Plant in Challenging Environments 5,
https://doi.org/10.1007/978-3-031-43029-9_5

5.1 Introduction

In 1987, work in animals showed that endothelial-derived relaxing factor (EDRF) was in fact the gas nitric oxide (NO) (Palmer et al. 1987). This opened the door to studies not only on reactive nitrogen species (RNS) in biological systems, but also observations on other physiological gasotransmitters. Such analyses also sparked work on other small reactive compounds which could be involved in cell signaling, including nongaseous reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) (Veal et al. 2007), and reactive sulfur compounds such as hydrogen sulfide (H_2S) (Aroca et al. 2018). The year 2019 marked the fortieth anniversary of NO studies in plants (Klepper 1979; Kolbert et al. 2019), but more recently a new player has been added to the list, molecular hydrogen (H_2) (Wilson et al. 2017), which can alter plant cell activity (for example, Chen et al. 2017a), and may play a role in stress responses. As plants are sessile, they require convoluted strategies to overcome a range of stress challenges, which include exposure to UV light (Hideg et al. 2013), heavy metals (Morkunas et al. 2018), extreme temperature, both high (Niu and Xiang 2018) and low (Lyons 2012), drought (Farooq et al. 2009), flooding (Loreti et al. 2016), and salinity (Fahad et al. 2015).

The strategy for plants when under stress is to induce signal transduction pathways, which often lead to altered gene expression, and hence the complement of cellular proteins, enabling enhanced or new activities. Such actions are allowing the cells to manage the current stress, or even future stress challenges. The signaling invoked in plants involves a range of phytohormones (Khan et al. 2012), but it also involves numerous gasotransmitters, which are important mediators in other organisms as well. These include NO (Nabi et al. 2019), H_2S (Pandey and Gautam 2020), carbon monoxide (CO; Cui et al. 2012) and ethylene (Debbarma et al. 2019). Plant stress has a major impact on plant growth and productivity, and gasotransmitters are instrumental in the responses mounted by plants. Often there is an interaction and/or co-ordination of the signaling mediated by such molecules (Hancock and Whiteman 2016; Singh et al. 2020; Bhuyan et al. 2020). Here, the interactions of H_2 with other gaseous signaling molecules are discussed, with the focus on how H_2 alleviates plant stress.

5.2 H_2 Treatment of Plants

Hydrogen gas is hard to administer to plants. In mammals, hydrogen gas mixtures can be inhaled, and there are many examples of its use (Ge et al. 2017; Wu et al. 2019a, b), including in the treatment for COVID-19 (Chen et al. 2021a; Russell et al. 2021). However, the gas is highly flammable, raising safety issues, and is lighter than air, so H_2 will rapidly disperse into the upper atmosphere, making treatment of ground-level plants unpragmatic. Therefore, treatment of plants often involves the creation and diluting of a saturated solution of hydrogen in what is referred to as hydrogen-rich water (HRW). However, H_2 is not very soluble (Molecular Hydrogen Institute n.d.; Wilhelm et al. 1977) and will rapidly revert to the gaseous phase and be lost. This then may necessitate a frequent re-application of HRW to the plant tissues, either directly onto the leaves, or into the root feed water, to illicit an effect. However, as can be seen in Table 5.1, there are many examples of

Table 5.1 Examples of the use of hydrogen-rich water (HRW) in alleviating plant stress

Stress agent/ Conditions	Species used	Effects seen/comment	Reference
Aluminum	Maize seedlings	Alleviation of stress	Zhao et al. (2017)
	Alfalfa	Alleviated effects on root growth	Chen et al. (2014)
Cadmium	*Medicago sativa*	Alleviation of toxicity	Cui et al. (2013)
	Brassica chinensis L.	Reduced cadmium uptake	Wu et al. (2019b)
	Chinese cabbage	Reduced cadmium uptake	Wu et al. (2015)
	Brassica campestris ssp. chinensis	Reduced cadmium uptake	Wu et al. (2020a)
	Brassica chinensis and *Arabidopsis thaliana*	Mediated by iron-regulated transporter 1 (IRT1) and zinc-regulated transporter protein 2 (ZIP2)	Wu et al. (2021)
Mercury	Alfalfa	Tolerance to toxicity	Cui et al. (2014)
Salinity	Barley	Alleviation of stress	Wu et al. (2020b)
	Rice	Alleviates stress during germination	Xu et al. (2013)
UV-B	*Medicago sativa*	Alleviated stress	Xie et al. (2015)
UV-A	Radish sprouts	Anthocyanin biosynthesis	Zhang et al. (2018)
	Radish sprouts	Anthocyanin biosynthesis and ROS metabolism	Su et al. (2014)
Heat	Cucumber	Several parameters altered, including gas exchange, chlorophyll fluorescence, and antioxidant activities	Chen et al. (2017a)
Paraquat induced oxidative stress	*Medicago sativa*	Mediated by heme oxygenase (HO-1)	Jin et al. (2013)
Post-harvest	Lilly and rose	Increased vase life	Ren et al. (2017b)
	Kiwifruit	Delayed ripening	Hu et al. (2014)
	Mushroom *Hypsizygus marmoreus*	Enhanced antioxidant capacity and reduced postharvest senescence	Chen et al. (2017b)

the use of HRW in plants. With such a range of responses, including to heavy metals, temperature stress and light stress it is clear that plants can perceive and react to the presence of H_2 or HRW. Interestingly, one of the potentially significant uses of H_2 application is in post-harvest, where it may be useful to prolong storage of crops, particularly fruits (Hu et al. 2014) and flowers (Ren et al. 2017b). A new twist on the use of HRW is the formation of hydrogen nanobubble water (HNW) (Li et al. 2021b). This is suggested to increase the solubility of H_2 and prolong H_2 delivery.

For H_2 usage to be useful in practice, new and easier-to-use applications for the delivery of H_2 may need to be developed. These may come from disparate industries (Mayorga et al. 2020), for example, one potential donor is magnesium hydride (MgH_2) (Li et al. 2020b), a compound proposed for use in the solar-energy sector (Mathew et al. 2021). The kinetics of release of H_2 are slower and more sustained than just using HRW, but it was found to be more efficient when used in a citrate buffer. Another recently used compound for releasing H_2 in plants is AB@hMSN, an ammonia borane-loaded hollow mesoporous silica nanoparticle (Wang et al. 2021). However, there is a caveat here. If donor molecules are used, they are likely to leave behind by-products, and this could severely compromise the biologically safe use of H_2.

5.3 Molecular Targets of H_2

The hydrogen molecule is extremely small (relative to other signaling molecules) and relatively inert. Therefore, it is difficult to envisage how it is perceived by cells and acted on. Classical hormone-type signaling, for example with chemokines (D'Ambrosio et al. 2003), would use a protein receptor, but this is unlikely with a molecule such as H_2. Some signaling molecules, such as NO, will react with proteins, either through the prosthetic groups or via reacting with thiol groups (Feng et al. 2019). However, again, it is hard to see how this type of reaction would apply to H_2 because unlike NO, which is polar and a reactive free radical, H_2 is non-polar and not reactive. Therefore, other mechanisms must exist to account for the biological effects seen with H_2 administration.

One of the main thrusts of the argument regarding H_2 action is that it affects the antioxidant levels in cells. Many of these effects are indirect, with expression or accumulation of enzymes involved in the antioxidant capacity of the cell being altered (for example Zhao et al. 2017; Chen et al. 2017b). However, this can only happen if there is a direct perception of the H_2 molecule, and usually that is the aspect that is skirted in the literature.

It was reported that H_2 does have direct effects as an antioxidant by reacting with hydroxyl radicals ($\cdot OH$) but not with other ROS, such as the superoxide anion ($O_2\cdot^-$) or H_2O_2 (Ohsawa et al. 2007). $\cdot OH$ are known to be involved in plant stress responses, such as during heavy metal challenge (Cuypers et al. 2016), paraquat treatment (Babbs et al. 1989), and chilling and drought stresses (Shen et al. 1997). Therefore, the removal of $\cdot OH$ by a radical scavenger, suggested here to be H_2, could account for some of the effects seen. This being said, a later paper has suggested that a close investigation of the kinetics of this reaction does not support this notion (Penders et al. 2014), and in fact, it was suggested that the ·OH would react with other biomolecules before H_2, so that the effects of ·OH would not be mitigated against by H_2 addition. In a similar way, a second direct target was suggested to be the peroxynitrite molecule ($ONOO^-$). This would be produced by the reaction of superoxide ($O_2\cdot^-$) with NO, and as both are temporally and spatially produced together during stress responses, the presence of $ONOO^-$ is very likely. If H_2 removes $ONOO^-$, this could account for the effects seen. However, a close examination of the kinetics again, seems to rule out $ONOO^-$ as a direct H_2 target (Penders et al. 2014; LeBaron et al. 2019a).

With both ·OH and $ONOO^-$ being ruled out, it was suggested that a possible target could be the ferric (Fe^{3+}) ion (Penders et al. 2014). This would not be out of kilter with what has been reported for other gasotransmitters. One of the main actions of NO is the activation of soluble guanylyl cyclase (sGC) by a direct interaction of the NO with the heme prosthetic group of the enzyme (Xiao et al. 2019). With a foray into this area (Penders et al. 2014), the reduction of the iron by H_2 in myoglobin, cytochrome P450 and putidaredoxin was investigated, but it was concluded that there was no reduction of heme or iron-sulfur (Fe/S) clusters in these proteins. However, with a redox midpoint potential of −414 mV [relative to the Standard Hydrogen Electrode (SHE)], H_2 could thermodynamically reduce a range of heme groups in a variety of enzymes, and this is suggested as a focus of future investigation (Hancock et al. 2021). As discussed, enzymes such as the NAPDH oxidase homologues would be particularly interesting as they are known to be involved in a range of stress responses (for example, He et al. 2017). It is not inconceivable that sGC may be an H_2 target too. Clearly much more work is needed here, using a wide range of plant proteins which contain heme or Fe/S prosthetic groups, before such a mechanism can be ruled out. Nevertheless, there may need to be some caution here, as it cannot always be assumed that signaling pathways determined in animal systems are the same in plants. For example, the action of NO on a sCG has been thrown into doubt in plants (Astier et al. 2019). Therefore, the action of H_2 may be different too, although the use of other biological systems to advance plant science is a powerful tool, as discussed below.

Several effects of H_2 have been reported to be mediated by the enzyme heme-oxygenase (HO-1) (Jin et al. 2013; Lin et al. 2014). This enzyme catalyzes the breakdown of heme in a reaction which (1) involves oxygen, (2) uses NADPH as a cofactor and (3) produces biliverdin, CO and iron ions (Wilks 2002). However, the exact reaction with H_2 has yet to be reported, so it may be a consequence of downstream signaling which is yet to be determined. Another enzyme thought to mediate H_2 effects is glutathione peroxidase, an enzyme instrumental in the maintenance of intracellular redox. By the use of genetically deficient strains and inhibitors, it was shown that glutathione peroxidase was needed to mediate H_2 action in the *Ganoderma lucidum* fungus (Ren et al. 2017a). The enzyme is a selenium containing protein, making this an interesting potential H_2 target, unless the direct action of H_2 is upstream of the enzyme itself.

Table 5.2 Possible molecular targets and action of H_2

Molecular target proposed	Comment(s)	Reference(s)
Hydroxyl radical (·OH)	Kinetics do not support this mechanism	Ohsawa et al. (2007) and Penders et al. (2014)
Peroxynitrite ($ONOO^-$)	Kinetics do not support this mechanism	Ohsawa et al. (2007) and Penders et al. (2014)
Fe^{3+} ion	A range of heme groups could potentially be targets	Penders et al. (2014) and Hancock et al. (2021)
Heme oxygenase (HO-1)	No direct interaction reported	Jin et al. (2013) and Lin et al. (2014)
Glutathione peroxidase	Mediated effects in fungus	Ren et al. (2017a)
Spin states	Possible direct interaction, but not experimentally substantiated	Hancock and Hancock (2018)

Lastly, it has been suggested that because H_2 has two spin states that this could be a way for H_2 to influence other biomolecules (Hancock and Hancock 2018). However, to date, there is no experimental evidence of this.

As yet, no definitive mechanism of how H_2 interacts directly with biological systems has been identified, although several mechanisms have been suggested (Table 5.2). Therefore, much more work needs to be undertaken in this area. Despite this there clearly are effects in plants (Table 5.1), and this phenomenon can be exploited in the absence of a molecular mechanism, particularly as there appear to be no reports that H_2 application is harmful to neither plants nor animals. No H_2 mechanisms seem to leave by-products and so there seems to be no ramifications for food safety.

5.4 Signaling and Effects of H_2

Cell signaling events in plants, as with all species, is crucial for the organism to thrive and to survive stress challenges. The perception of an external signal, perhaps a biotic or abiotic stress, and the signal transduction pathway, leading to a response, involves a range of proteins and small molecules, and instrumental in many of these pathways are the small relatively reactive gasotransmitters, such as NO (Nabi et al. 2019) and H_2S (Pandey and Gautam 2020). Although, as discussed, it is hard to envisage how H_2 may have a direct interaction and effect on polypeptides, there is a body of evidence that shows that H_2 interacts, or has effects on, signaling events that involve other gasotransmitters and small redox compounds. Some of the evidence is discussed below.

5.4.1 Nitric Oxide, Stress and Hydrogen Gas Treatment

It has been known for several decades that NO is produced by plants and has a profound effect on controlling plant function (Kolbert et al. 2019). There is no doubt that NO has a central role in controlling cell function (Kumar and Pathak 2018), whilst more recently, it has been found that H_2 interacts in the NO pathways.

Decreased NO generation was reported when HRW was used to alleviate aluminum stress in alfafa (Chen et al. 2014). Fifty percent saturated HRW reduced the effects of a NO donor, suggesting that NO may mediate H_2 effects. In contrast, H_2 increased the NO production in tomato seedlings when root growth was being investigated. This was reduced by the NO scavenger 2-4-carboxyphenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO), which suggests that H_2 was not directly scavenging NO. The conclusion was that auxin-induced H_2 generation was then mediated by NO production from the enzyme nitrate reductase (NR) (Cao et al. 2017). Similar results were reported with cucumber, where HRW increased root growth and NO accumulated. Both HRW and NO increased the expression of cell cycle genes: *CycA* (A-type cyclin); *CycB* (B-type cyclin); *CDKA* (cyclin-dependent kinase A); and *CDKB* (cyclin-dependent kinase B). The effects were reduced by inhibitors of NR and nitric oxide synthase (NOS)-like enzymes, and NO scavengers (Zhu et al. 2016). NO also mediated root growth induced by H_2 in cucumber, where downstream proteins were identified as a plasma membrane H^+-ATPase and 14-3-3 proteins (Fu et al. 2000; Mhawech 2005; Li et al. 2020a, b). The latter being key regulatory proteins of such intracellular signaling cascades as mitogen activated protein kinase (MAPK) and p53. The enzyme NR was also found to be involved in NO generation when root formation was induced by a H_2 releasing donor AB@hMSN (Wang et al. 2021).

H_2 has the potential to be useful for postharvest storage of plant materials. One percent HRW [2.2 μM H_2] (calculated from the authors' information) and sodium nitroprusside (SNP: 150 μM) improved vase-life of cut lilies and these effects were reduced when NO was removed. It was also found, in a study of the genes expressed, that the chloroplast ATP synthase CF1 alpha subunit (AtpA) may be important in mediating these effects (Huo et al. 2018). Furthermore, nitrate accumulation was reduced in tomatoes by H_2, and this may have implications for the way fruits are stored (Zhang et al. 2019).

It is clear therefore, that H_2 has effects on, and is mediated, by NO metabolism, and it appears that this is not due to a direct scavenging of NO by H_2, which would be in line with what was previously reported (Ohsawa et al. 2007). However, H_2 may have effects through ROS too, which may also impinge on NO metabolism. To exemplify, abscisic acid (ABA) induced the accumulation of H_2 in *Arabidopsis thaliana*, which led to better drought tolerance. However, the effects also involved ROS and NO accumulation, with the enzymes NR and NADPH oxidase being used. In fact, it was found that the promotion of NO accumulation by H_2 was dependent on ROS production, showing what a complex and interdependent system H_2 is involved in (Xie et al. 2014).

5.4.2 Reactive Oxygen Species, Antioxidants and Hydrogen Gas

It is clear that ROS metabolism needs to be considered when the effects of H_2 are in question, especially as H_2 may have antioxidant and pro-oxidant effects (LeBaron et al. 2019b).

Even though the direct scavenging of $O_2\cdot^-$ and H_2O_2 were ruled out (Ohsawa et al. 2007), and the scavenging of $\cdot OH$ was also cast into doubt (Penders et al. 2014), many reports suggest that H_2 has affects in plants through the modulation of the antioxidant capacity of cells. The postharvest treatment of Chinese chive with H_2 reduced oxidative damage and increased the activity of several antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POD) and ascorbate peroxidase (APX) (Jiang et al. 2021), resulting in an increased shelf life of the chives. Oxidative stress in *Medicago sativa* was also alleviated by HRW following UV-B exposure, and this too was mediated by changes in antioxidants, particularly flavonoids (Xie et al. 2015). HRW also allowed better tolerance to light stress in *Zea mays*, again mediated by antioxidant enzymes (Zhang et al. 2015). These included SOD, CAT and APX, which reduced the accumulation of $O_2\cdot^-$ and H_2O_2.

The tolerance bestowed on plants by H_2 administration to other stress challenges is also mediated by antioxidants. This includes aluminum tolerance in maize, where HRW altered the cellular levels of CAT, APX, SOD, and POD (Zhao et al. 2017). In rice seedlings exposed to cold stress, the SOD levels were altered, which appeared to be mediated by changes in the miRNA levels, in particular miR-398 transcripts. The authors suggested that this was imperative to maintaining the redox homeostasis of the cells (Xu et al. 2017b).

The changes in antioxidant activity observed in cells will not only relieve the tangible aspects of oxidative stress, as seen with less lipid peroxidation and protein oxidation, but it will also be part of the system which maintains the redox poise of the cell, which will be part of the complex interplay used in signaling (Shao et al. 2008). Lowering ROS will mean that reactions with NO will potentially be reduced, and so reducing the production of $ONOO^-$, which acts as a downstream signaling molecule of NO (Speckmann et al. 2016). It is known that ROS will also act on glutathione, a molecule instrumental in maintaining the cellular redox balance. It has been suggested that the redox of a cell is carefully kept in a “Goldilocks zone” (Alleman et al. 2014), and therefore any changes in intracellular redox molecules will feed into this. A good example of how such redox active molecules interact to give the effects in plants is seen with the legume–*Rhizobium* symbiosis system (Pauly et al. 2006), where GSH, NO and ROS were studied.

5.4.3 Hydrogen Gas and Ethylene Signaling

One of the most well-known gasotransmittters is ethylene (C_2H_4; $H_2C{=}CH_2$). It is involved in a range of physiological systems in plants, such as plant growth (Dubois et al. 2018), but is probably best known for its role in fruit ripening (Barry and Giovannoni 2007).

The interaction of ethylene with other gasotransmitters is not novel. For example, NO and ethylene has been reported to work together in the root development of cucumber (Xu et al. 2017a). Therefore, an interaction of H_2 and ethylene is no surprise. Postharvest senescence of rose flowers was reduced by H_2 application and this was mediated by changes in ethylene signaling. There was a reduction of substrates and biosynthetic enzymes: 1-aminocyclopropene-1-carboxylate (ACC); ACC synthase (ACS); and ACC oxidase (ACO). Gene expression of *Rh-ACS3* and *Rh-ACO1* transcripts, encoding biosynthesis enzymes, was also reduced. Interestingly, expression of the ethylene receptor, *Rh-ETR1*, was increased (Wang et al. 2020). These data clearly show that there is an influence of H_2 on ethylene metabolism and concomitant signaling.

A proteomic study also showed that H_2 and ethylene cooperated in signaling (Huang et al. 2020). Using cucumber roots as the model system, it was shown that inhibitors of ethylene signaling, $AgNO_3$ and aminoethoxyvinylglycine (AVG), reduced the adventitious root development induced by H_2 treatment. The proteomic analysis, using 2D-gel electrophoresis coupled with mass spectroscopic analysis, showed that HRW induced the up-regulation of nine proteins and the down-regulation of fifteen. The authors concluded that ethylene was downstream of H_2 and that six proteins were worthy of note and were probably mediating H_2 effects. These were RuBisCO, oxygen-evolving enhancer protein (OEE1), sedoheptulose-1,7-bisphosphatase (SBPase), threonine dehydratase (TDH), cytosolic ascorbate peroxidase (cAPX), and protein disulfide-isomerase (PDI).

5.4.4 Hydrogen Gas and Hydrogen Sulfide Signaling

H_2S is recognized as being toxic (Truong et al. 2006), but it is also now accepted as being a therapeutic gasotransmitter controlling key events in physiology and cell function (Wang 2003; Gadalla and Snyder 2010). However, as with the other small reactive compounds, H_2S does not act alone but is part of the complex interaction in which these molecules partake. It has been suggested that H_2S may act as a brake on some of the other signaling pathways (Hancock and Whiteman 2014). Alongside this, H_2S has also reported to be part of the H_2 signaling taking place in cells.

With the expression in Arabidopsis of a hydrogenase gene from *Chlamydomonas reinhardtii* (*CrHYD1*), which leads to H_2 biosynthesis, it was shown that endogenous H_2 was needed for osmotic stress tolerance in plants. Exposure to H_2 stimulated the production of H_2S and it was suggested that, to cause the modulation of the stomatal apertures, leading to the tolerance observed, H_2S was downstream of H_2 (Zhang et al. 2020a). A similar result was found with cut flowers. In a study of cut carnations, it was shown that a MgH_2 and citrate solution increased H_2S generation. The redox homeostasis was maintained whilst the expression of senescence genes was repressed (Li et al. 2020b). Hypotaurine, a H_2S scavenger, reversed the effects and it was suggested that the downstream effects of H_2 were mediated by H_2S, which is in line with the study on stomata (Zhang et al. 2020a).

It can be seen therefore, that H_2 is involved in the signaling pathways of a range of gasotransmitters, including NO, ethylene and H_2S.

5.5 What Might Be Learnt from Other Species

Working across the kingdoms of organisms can be rewarding, but it does come with some caveats. To exemplify, the characterization of the NAPDH oxidases from humans (Schröder 2020) has greatly helped advance the research on homologues of these enzymes in plants (Qu et al. 2017). Indeed, oxidase proteins from plants and animals could be combined to reconstitute activity *in vitro* (Desikan et al. 1996). On the other hand, the discovery of a NOS in animals (Bredt and Snyder 1990) has only led to controversy in plant science (Astier et al. 2018). Furthermore, the lack of a sGC signaling pathway in plants, so well characterized in animals, further emphasizes the caution that may need to be used (Astier et al. 2019). Having said that, deliberated below is how much can be learnt about the role of H_2 in biological systems by taking a broad approach.

If H_2 is able to enhance stress responses in plants, there needs to be an increase in the H_2 concentration in the relevant cells. This can be achieved via two mechanisms: either the endogenous production of H_2 can be increased, or the H_2 can be supplied exogenously.

Probably one of the most well-known endogenous biological systems for the production of H_2 is in the algae *Chlamydomonas* (Vargas et al. 2018). This organism is so good at generating H_2 that it has been suggested to be used as a biofuel (Scranton et al. 2015). Generation of H_2 is via a hydrogenase enzyme, and such mechanisms have been recently reviewed (Russell et al. 2020). If enzyme-based H_2 production can be increased in plants, either by the manipulation of the control of such enzymes, or by increasing the expression and relevant polypeptide accumulation, then targeted H_2 signaling can be used to enhance plant growth and survival. Model organisms such as *Chlamydomonas,* and then higher plant models such as *Arabidopsis*, will be instrumental in such work.

Alternatively, H_2 can be supplied exogenously. As discussed above, this might be from anthropogenic activity such as the application of HRW. However, plants, like many organisms, are likely to be in synergy or symbiotically with prokaryotes and fungi, which themselves can produce H_2. In humans, it has been suggested that increased H_2 production by gut microflora may enhance health (Ostojic 2020). Therefore, an increase in the prokaryotic production of H_2 around the root system of plants may have beneficial effects. On the other hand, H_2 oxidizing soil bacteria have also been shown to be beneficial (Zhang et al. 2020b). Manipulation of the soil bacterial flora therefore may be complicated but changing the H_2 metabolisms in the vicinity of the root system might have future benefits.

A study of bacteria may also help unravel how H_2 works. As the H_2 couple has a very reducing mid-point potential (−414 mV relative to SHE), then reduction of many protein prosthetic groups may be thermodynamically possible. This principle is exemplified by the reports on the reduction of cytochrome c_3 in *Desulfovibrio desulfuricans* (Peck 1959). Interestingly, following the redox reactions which may proceed downstream of this reduction, it was suggested that H_2S could be produced, which is known to be an important gasotransmitter in plants (Aroca et al. 2018), including under stress conditions (Singh et al. 2020), relevant to the discussion here. The study by Peck (1959) shows two important things. Firstly, the reduction of a heme group by H_2 is possible in biological systems. Secondly, once the heme is reduced there are possible downstream reactions which could potentially yield signaling molecules. As already mooted (Hancock et al. 2021), this needs to be explored further in plants and animals, not just in prokaryotes.

One of the biggest areas where other species can be useful to study is in the biomedical arena. Here, H_2 has been shown to have a benefit in a variety of diseases, including those listed in Table 5.3. H_2 has been found to relieve symptoms of COVID-19 and has been used for clinical trials (Guan et al. 2020). It has also been found to be of benefit in neurodegenerative disease (Chen et al. 2021b), rheumatoid arthritis (Yang et al. 2020) and diabetes (Yang et al. 2020). Therefore, it is clear that H_2 has a range of benefits for human health and for alleviating disease symptoms. Moreover, if mechanisms are known for H_2 action in the biomedical arena, can this be translated across and used in plant science?

It is not only the support that data such as that in Table 5.3 gives to the argument that H_2 has profound effects in biological systems, but it is the manner in which H_2 has its effects that is relevant here. Clearly, some of the effects and proposed mechanisms in animals are not directly relevant to plants. For example, a reduction in IL-6 levels or a dampening of a cytokine storm is not a mechanism which would be seen in plants. However, H_2 may have effects on analogous intercellular signaling molecules in plants, such as ethylene (Wang et al. 2020). Other effects may be much more relevant. As previously mentioned, H_2 may work through the action it has on antioxidants, an effect which has already been seen in plants. Accordingly, changes in antioxidants and a dampening of oxidative stress are a common feature in neurogenerative disease alleviation, the reductive effects on diabetes, cancer therapies, in mood alterations and in Hepatitis B (Ichihara et al. 2015). This is also a common feature of how H_2 alleviates plant stress (e.g., Zhang et al. 2015; Jiang et al. 2021). The biochemistry of animal and plant cells differs in detail but remains the same in principle. Therefore, a close study of the research on H_2 from the animal kingdom may be very beneficial to plant science in the future and vice versa. With the list of conditions for which H_2 may benefit human health, it is no surprise that H_2 has been mooted as a future therapy for humans (Ge et al. 2017; Wu et al. 2019a). With a focus on respiratory diseases at the present time because of the COVID-19

Table 5.3 Human diseases for which symptoms are alleviated by H_2 treatment

Disease/condition	Effect of H_2	Proposed molecular mechanism (if known)	Reference(s)
COVID-19	Severe symptoms alleviated	Dampens cytokine storm.	Russell et al. (2021), Hirano et al. (2021), Chen et al. (2021a), and Guan et al. (2020)
Neurodegenerative (e.g., Parkinson's disease, Alzheimer's disease)	Significantly improved scores assessed by the Unified Parkinson's Disease Rating Scale (UPDRS), or Alzheimer's Disease Assessment Scale-cognition cub-scale (ADAS-cog)	Reduces the loss of dopaminergic neurons and inhibits oxidative stress.	Chen et al. (2021b), Yang et al. (2020), Nishimaki et al. (2018), Ge et al. (2017), and Yoritaka et al. (2013)
Rheumatoid Arthritis	Reduced symptom severity	Relieves inflammation, possibly through reduction of IL-6-mediated responses.	Yang et al. (2020) and Ishibashi et al. (2014)
Ischaemia/reperfusion injury (e.g., stroke, brain trauma, cerebral infarction, cardiac arrest)	Significant increase in neurological improvement	Antioxidant, anti-inflammatory and anti-apoptotic effects. Inhibition of endoplasmic reticulum stress. Preservation of the blood-brain barrier and mitochondrial function.	Chen et al. (2021b) and Ono et al. (2017)
Metabolic syndrome and Type 2 Diabetes Melllitus	Improvement in urinary oxidative stress markers and cholesterol profile. Normalised oral glucose tolerance test	Decreases glucose and insulin levels. Stimulates energy metabolism. Increased urinary antioxidant superoxide dismutase enzyme. Reduced low-density-lipoprotein-mediated inflammation. Suppression of chemical modifications of serum lipoproteins in the plasma membrane	Yang et al. (2020), Ge et al. (2017), Song et al. (2013), Nakao et al. (2010), Suzuki et al. (2009), Kajiyama et al. (2008), and LeBaron et al. (2020)
Aiding anti-cancer therapy	Improving Quality-of-Life scores for radiotherapy patients	Radioprotection via antioxidant increase.	Ge et al. (2017) and Kang et al. (2011)
Mood disorders	Improved mood, anxiety and autonomic nerve function	Reduced accumulation of oxidative stress.	Chen et al. (2021b) and Mizuno et al. (2017)
Hepatitis B	May have potential to improve liver function and reduce viral DNA level	Reduction of oxidative stress.	Xia et al. (2013)

pandemic, the research and application of H_2 is likely to be of continued interest in the biomedical field (Russell et al. 2021). On the other hand, H_2 application has already been suggested to be hugely beneficial to agriculture (Zeng et al. 2014; Li et al. 2021a). The responses to H_2 are likely to be supported by common molecular mechanisms in plants and animals, be that through antioxidants or Fe^{3+} reduction, or other means. Plant science might have a lot to learn from the work being carried out on prokaryotes and higher animals, and vice versa.

5.6 Conclusions and Future Perspectives

It seems clear now that H_2 is a useful treatment for plants, alleviating a range of stress challenges (Table 5.1), as well as a potential regimen for the post-harvest storage of fruits and flowers, where it evidently delays senescence (Hu et al. 2014; Ren et al. 2017b). However, there are many aspects of the biochemistry of H_2 which are simply not clear. Firstly, it is not known what the direct targets of H_2 are in cells, even though several mechanisms have been suggested (Table 5.2), including scavenging radicals and other reactive signals, or acting through HO-1. Secondly, the full range of effects are not known, even though there are numerous reports of H_2 application being beneficial (Table 5.1).

The redox mid-point potential of H_2 is relatively low when compared to other biomolecules. Thermodynamically, it would be possible for H_2 to reduce Fe^{3+} to Fe^{2+} and this would have ramifications for many enzymes, suggesting the reduction of prosthetic groups, particularly many heme groups, is theoretically possible, although not widely reported. Additionally, selenium-containing enzymes may be targets. However, such reactions are likely not to be kinetically feasible without the certain environments that could lower the activation energy for such a reduction to take place. However, clearly, a comprehensive study of the proteins controlled by H_2 is required, even if it is simply to rule them out as being involved. Unlike the work with NO and H_2S (Baty et al. 2005; Hawkins and Davies 2019), a proteomic approach would seem to not be feasible with H_2 as no direct covalent post-translational modification of proteins have yet been reported for this molecule. On the other hand, downstream post-translational protein modification will occur, and a full compendium of such effects would be useful to know.

Although endogenous generation of H_2 in some plants is possible and may be able to be manipulated, manipulation of exogenous sources of H_2 would be a better approach as it would be easier. The presence of H_2 may be dictated by the surrounding microflora, but H_2 may be applied to plants as a treatment. Using H_2 as a gas is unlikely to be of use, but the generation of HRW or HNW may allow application to either foliage or roots, or both. Clearly, there are safety aspects from a physical point-of-view, as H_2 is extremely flammable, but from a biological viewpoint H_2 appears to be safe to use, both for plants and animals. As with other similar molecules, for example H_2S (Song et al. 2014), donor molecules may open up the better use of H_2 in the future, and the use of some are already being reported, such as MgH_2 (Li et al. 2020b) and AB@hMSN (Wang et al. 2021).

H_2 use in agriculture and horticulture has yet to be widely adopted, but there is a growing interest in this biologically safe treatment. As more is known about how it works, and the significance of any effects are more widely reported, H_2 may become an accepted way to enhance plant growth and crop storage in the future.

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