Review

The Effects of Molecular Hydrogen on Plant Physiology and Metabolism: An Overview

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Abstract: Gaseous hydrogen (H_2) has emerged as a molecule that has a significant influence on the growth and development of plants, particularly if they are under stress. Often research has shown the ameliorating effects of H_2 during drought, in the presence of heavy metals or salt, or during UV light irradiation. Postharvest, H_2 has been shown to increase the quality of fruits and vegetables during storage, and slows the senescence of flowers. In molecular terms, H_2 has been shown to scavenge hydroxyl radicals and remove peroxynitrite, but not react with other reactive signalling molecules such as nitric oxide. However, not all the molecular actions of H_2 have yet been unravelled. This is not a totally comprehensive review of the topic, but hopefully gives an overview of the influence of H_2 on some of the molecular events in cells and how this can influence plant physiology. There is no doubt that H_2 has significant effects in plants, and there is potential scope for its wide adoption throughout the agricultural sector.

Keywords: antioxidants; flowers; fruits; hydrogen gas; hydrogen-rich water; molecular hydrogen; molecular mechanisms; postharvest

1. Introduction

Molecular hydrogen (H_2) has now become a molecule of interest across a range of biological disciplines, including plant sciences. H_2 was originally isolated by Henry Cavendish in 1766, and relatively soon after its effects on biological systems were being investigated by a number of researchers, including Humphry Davy, Antoine-Laurent de Lavoisier (usually referred to as Antoine Lavoisier), Joseph Priestley and Tiberius Cavallo [1], mainly while they were working on a range of other gases, such as oxygen and nitrous oxide. However, the research on H_2 seemed to fade away for decades [2]. The recent resurgence of interest probably followed the publication of work on animal cells [3], which in particular mooted a possible mechanism of action of H_2 in cells. The proposal, which has borne further investigations, was that H_2 selectively interacts with small reactive signalling molecules, in particular, the hydroxyl radical ($^{\cdot}OH$). It was found that other related signalling molecules such as superoxide anions ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and nitric oxide (NO) were not affected by H_2 (Figure 1). Therefore, here was a defined effect, and a possible mechanism which could be translated across a range of biological cells, including plants. The interest in H_2 steadily increased, and a Pubmed [4] search using the term "molecular hydrogen" in September 2024 yielded 3912 results, and of course there are other search terms which would have revealed research on H_2 in the literature. It is now well studied in the biomedical arena, being suggested for mitigation of a range of diseases, including neurodegenerative disease [5], diabetes [6], and cardiovascular disease [7]. A recent systematic review on the topic of H_2 in medicine gives further examples and details [8].

The interest is not limited to animal systems, and certainly many aspects of plant growth, physiology and metabolism have been investigated, and the use of H_2 in agriculture has been suggested in several recent papers [9–11]. This paper will give an overview of the effects of H_2 on plants, and how it may be of beneficial use in the future.

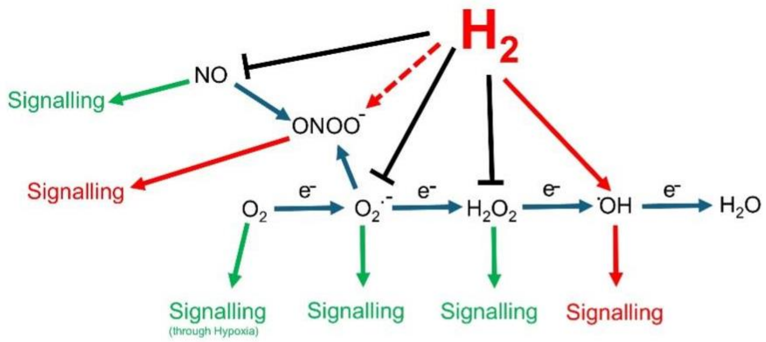

Figure 1. A scheme of how H_2 must initiate short- and long-term responses. Red arrows indicate possible signalling initiated by the presence of H_2. Black blunt arrows indicate no interaction. Blue arrows indicate chemical changes. Green arrows indicate signalling which is not affected by H_2.

2. Is H_2 Safe to Use?

If H_2 is to be used in agriculture and food sciences it needs to be safe. There are inherent risks with the handling and use of H_2 [12], with the most prominent being its flammability [13]. However, in biological systems it is inherently safe, showing no toxicity traits. In fact, it has been used as high concentrations in diving gas since the 1940s, with no apparent deleterious effects [14].

The easiest way to use H_2 is a gas (Figure 2). Plants can simply be fumigated with H_2 and the effects measured. As long as there is the cognisance of its flammability in the presence of oxygen it is relatively easy to do. However, being light and less dense than air H_2 will not stay at ground level and will be lost to the upper atmosphere relatively quickly. Alternatively, many researchers create a H_2 enriched solution, usually referred to as hydrogen-rich water (HRW). The same can be used with a saline solution, as hydrogen-rich saline (HRS), but many plants are not salt tolerant so this has limited use in plant science. Such solutions are easy to create, simply by bubbling the media of interest with H_2 gas, and letting it dissolve. However, the solubility in water is poor, and H_2 will rapidly revert to the gas phase and be lost to the atmosphere. In the biomedical arena HRW is often produced using magnesium-based tablets, which react with the water, with the H_2 gas bubbling and partially dissolving. Electrolytic cleavage of water can be used to produce oxy-hydrogen (66% H_2: 33% O_2), and if a membrane is included the H_2 can be collected as a pure gas, with the O_2 being exhausted to the atmosphere.

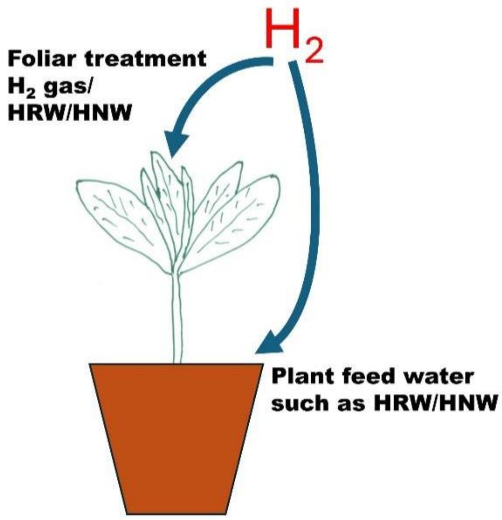

Figure 2. Plants can be treated with H_2 in a variety of ways. For example, foliar treatment may be by gas or with a solution enriched for H_2 such as hydrogen rich water (HRW) or hydrogen nanobubble water (HNW), or alternatively feedwater can be fed directly to the soil as HRW or HNW.

Of course, plant cells will be exposed to endogenous (e.g., by the action of hydrogenases) and exogenous (e.g., from bacterial activity) sources of H_2 naturally too. This was relatively recently discussed [15], and details will not be given here.

Therefore, H_2 is easy and cheap to administer to plants. The treatment with hydrogen as a biomedicine as been reviewed previously [16], while some of the issues with the use of hydrogen have also been discussed [17].

3. Effects of Molecular Hydrogen on Plant Metabolism and Molecular Processes

The effects of H_2 in biological systems are often short term, as the hydrogen will soon dissipate from the treatment. However, also quite often, research shows that H_2 is added for a short period of time and the effects are measured much later, perhaps hours or days after the H_2 treatment has been stopped. A good example here would be the use of H_2 in sports science [18]. For example, in a recent paper H_2 was given 30 mins before exercise, with measurements at 3 minutes before exercise and 5 mins after [19]. Similar regimes are often used in the treatment of plants too, such as the use of H_2 as a pre-treatment for studying high temperature stress [20]. Here, seedlings were treated for 7 days, but measurements were 3 days later following the heat stress period. It must be assumed therefore that the H_2 was still there after 3 days, which is unlikely, or that the plant cells had a long-term response that lasted beyond the period that H_2 was present. Hence, any molecular mechanism must account for such changes taking place, as summarised in the scheme in Figure 3, and discussed below.

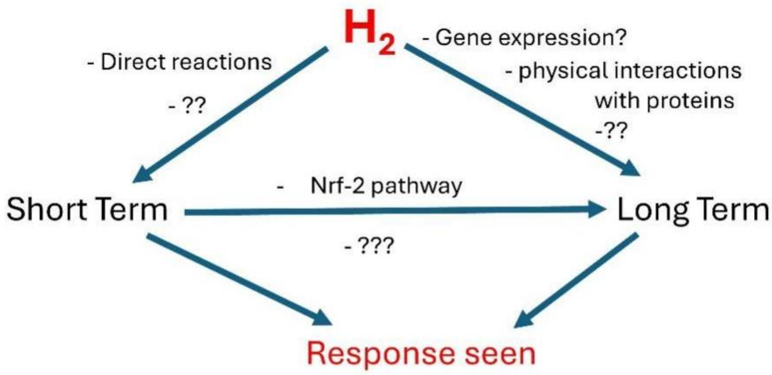

Figure 3. H_2 appears to have to have short- and long-term effects which need to be accounted for.

The original mechanisms of the action of H_2 on cells was carried out on mammalian cell cultures and rat models [3]. It was found that removal of hydroxyl radicals was the main effect seen. Cells, both plant and animal, will undergo oxidative stress when challenged with a range of conditions [21]. This involves the increase in the redox intracellular state towards oxidation. Cells are normally held a relatively low redox, being maintained by a high glutathione (GSH) level in cells. A shift of the GSH/GSSG balance, or an alteration of total cellular GSH+GSSG, can alter the cells redox poise and lead to oxidative stress, resulting in cell proliferation (if the change is relatively small) to programmed cell death (PCD) or even necrosis. Therefore, keeping the GSH/SGG metabolism regulated is vitally important in cells [22]. In plants, glutathione is in the low mM range in cells [23], and is vitally important. Under oxidative stress, the accumulation of reactive oxygen species (ROS) is increased, which can react with the GSH and drive the redox poise towards oxidation. ROS are a group of molecules, often initially being the $O_2^{\cdot-}$anion, which dismutes to H_2O_2, either naturally in the presence of proton, or catalysed by members of the superoxide dismutase (SOD) enzymes [24]. The accumulation of ROS is also often accompanied by an increase in reactive nitrogen species, in particular NO [25], which can react with the $O_2^{\cdot-}$anion to generate peroxynitrite ($ONOO^-$). All these ROS and RNS have important signalling roles in plants, particularly NO [26] and H_2O_2 [27]. However, according to Ohsawa et al. [3], H_2 reacts with $^{\cdot}OH$, but not with NO and H_2O_2, or other ROS. There is some reaction with $ONOO^-$, and Ohsawa et al. [3] say that the lack of reaction with signalling ROS and RNS is an advantage as only the deleterious reactive species are removed. However, can this mechanism account for all the changes seen?

In many reports of the effects of H_2 there is an increase in antioxidant in cells. For example, in kiwifruit HRW treatment postharvest resulted in an increase in SOD activity and decreased lipid peroxidation [28]. Under chilling stress, HRW treatment of kiwifruit had a similar effect, with ascorbate peroxidase (APX), ascorbic acid (AsA) and glutathione reductase (GR) all being increased in the presence of H_2 [29]. In Chinese cabbage HRW increased antioxidants under cadmium stress [30], including the activities of SOD, catalase and guaiacol peroxidase. In maize seedlings under aluminium stress, HRW enhanced the activities of APX, SOD and catalase, leading to better aluminium tolerance [31]. In *Medicago sativa*, HRW increased the antioxidant cell capacity under UV-B stress [32], and although not plants, similar results have been found in fungi, specifically *Hypsizygus marmoreus* [33].

There seems little doubt, therefore, that the presence of H_2 in cells leads to an increase in antioxidants, a decreased in ROS and therefore a lessening of oxidative stress. But what is the mechanism?

One mechanism which may account for some of the long-term effects is a change in gene expression. In rats, in a study of hyperoxic lung injury, it was found that HRW led to the induction of NF-E2-related factor-2 (Nrf-2)-dependent genes [34]. This included haem-oxygenase-1 (HO-1), an enzyme which is known to be affected by the presence of H_2, as reported for RAW 264-7 macrophages. [35]. HO-1 has also been implicated in the mediation of H_2 responses in plants. For example, Jin et al. [36] showed that HO-1 mediated the increased tolerance to paraquat-induced oxidative stress following H_2 treatment. In a similar manner, HO-1 was implicated in the regulation of adventitious root development in cucumber [37]. Here, several genes were also induced by HRW, including those involved in auxin signalling and root development. It was also found that there is an association between H_2 and HO-1 in plants during oxidative stress in alfalfa [38]. Interestingly, methane-rich water also induced adventitious root development through HO-1 mediated route [39] showing that this signalling pathway is not unique to H_2.

Associated with HO-1 activity is the action of carbon monoxide (CO) [40]. CO has effects on respiratory metabolism, being known to be an inhibitor of cytochrome oxidase (Complex IV). This will reduce the electron flow through the electron transport chain (ETC), decreasing the electrochemical potential across the inner mitochondrial membrane and therefore decreased ATP production. However, impeded electron flow at the terminal end of the ETC also leads to an increase in mitochondrial generated ROS, with possible signalling, leading to potential cell death. Therefore, if H_2 influences mitochondrial respiratory activity this can have a significant effect in the cell, although there seems to be no evidence of H_2 directly effecting proton gradients in plants. However, C_3 and C_4 plants have a differential sensitivity to CO. Using an assay of nitrate reductase (NR) as a measure of cytochrome a_3 (a component of Complex IV), it was found that C_3 plants were relatively insensitive whereas C_4 plants were much more sensitive [41]. Plant mitochondria contain an alternative oxidase (AO) as well [42], so the inhibition of Complex IV may not have same effect on electron flow as seen in animal cells.

It is known that the accumulation of ROS in cells, including plant cells. H_2O_2 can be sensed by the HPCA1 (*hydrogen-peroxide-induced Ca^{2+} increases*-1) protein, which is a leucine-rich-repeat receptor kinase (LRR-RK) [43]. Downstream of H_2O_2 can be alterations in the pattern of gene expression. For example, in Arabidopsis it was found that the presence of H_2O_2 increased the expression of some genes, while depressing the expression of others [44,45]. Similar data was obtained in tobacco [46]. Many of these encoded proteins were involved in metabolism and cell signalling processes, showing that the cells are adapting to survive and thrive in the future, perhaps being more resilient to future stress challenges. If H_2 is having an effect on ROS levels, mediated by a rise in the total intracellular antioxidant capacity, then this would include a lowering of H_2O_2 levels and therefore an alteration of H_2O_2-mediated gene expression. As discussed above, H_2 is likely to have a short-term effect on plants, but is also known to have a long-term influence too. Some studies treat with for H_2 for a relatively short period, and then responses are measured hours, or even days, into the future. It is unlikely that at the point of measurement of the response there is much, if any, H_2 present, as it is likely to have dispersed to the atmosphere by that point in time. Therefore, an alteration of gene expression would allow the altered production of proteins. Specific proteins may accumulate if gene expression is increased, or their cellular concentration may decrease if gene expression is depressed but they are still being degraded. The complement of active proteins can be considerably altered, allowing short-term H_2 treatment to have a long-term cellular effect.

Mechanisms have been mooted to account for how H_2 may alter gene expression. As mentioned above, one such mechanism involves Nrf-2, at least in animals [34]. Further to the earlier studies, it has been suggested that H_2 targets oxidized form of Fe-porphyrin [47]. Fe-porphyrin can conjugate with hydroxyl radicals to produce PrP-Fe(III)-OH, which can react with H_2 to produce PrP-Fe(III)-H, which can subsequently oxidise Kelch-like ECH-associated protein 1 (Keap1) which leads to the activation of Nrf-2, and hence altered gene expression. Nrf-2 will influence antioxidant response elements (AREs) in the nucleus [48], and hence the antioxidant capacity of the cell will be altered, but protecting against oxidative stress and altering cellular activity, such as gene expression, as discussed. The interaction of H_2 with haem (Fe-porphyrin) has also been suggested by others [49,50] where chemical mechanisms have been proposed. There is certainly growing evidence that this is a plausible manner in which H_2 brings about cellular effects, at least in animals. The evidence for related mechanisms in plants needs to be sought.

Another mechanism which has been suggested that connects H_2 to gene expression involves the alteration of the oxidation of phospholipids [51]. Low levels of H_2 (~1% *v*/*v*) lowered the autoxidation of linoleic acid. In a cell-free system, H_2 modified the oxidation of lipids, and when these were used to treat cells there was a subsequent lowering of Ca^{2+}-based signalling and altered gene expression. This cellular work was carried out using cultured

THP-1 cells (a monocyte cell line from an acute monocytic leukaemia), so again, evidence of a H_2-mediated lipid-based signalling system in plants needs to be sought.

As well as effects of gene expression, it is well known that ROS has a major control over the central metabolism of cells. For example, the cytosolic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is covalently modified in the presence of H_2O_2, and then migrates to the nucleus [52,53]. This has two consequences. Firstly, it removes the enzyme from its activity as part of the glycolytic pathway, and hence the support of energy metabolism. Secondly, it controls the levels of gene expression and hence the future protein complement of the cell. If H_2 has no effect on H_2O_2 levels in the cell, then there can be no direct action on this enzyme and the oxidative response will not be altered. On the other hand, as argued above, if H_2 increases the antioxidant capacity of the cell, then the levels of ROS in cells, including H_2O_2, will be altered and so H_2 can have an indirect, or downstream, effect on both metabolism and gene expression of cells.

As well as its influence on ROS, it would be amiss not to discuss the influence of H_2 on other reactive signalling molecules, such as RNS. The main molecule here is NO. Levels of NO in plant cells can be influenced by phytohormones, such as cytokinins [54]. It was established by Ohsawa et al. [3] that there was no direct interaction between H_2 and NO, and hence it would be assumed that there would be no alteration of NO accumulation in cells when H_2 is present. It was established that H_2 interacts with $ONOO^-$, which itself can act as a signalling molecule [55,56]. Therefore, H_2 will have some influence on RNS signalling, but not directly through the most influential molecule, i.e., NO. But is this a too simplistic view?

Certainly, if one looks at the animal literature there are many which invoke NO metabolism in the effects of H_2. In chondrocytes (cells in cartilage) H_2 was found to reduce $ONOO^-$ derived from NO, and this was suggested as a mechanism for altered gene expression [57]. In rat retina, H_2 lowered $ONOO^-$ levels, reduced oxidative stress and decreased levels of tyrosine nitration on proteins [58], leading to downstream alteration of protein activity. In macrophages (derived from monocytes, but in the tissues) lipopolysaccharide/interferon g (LPS/IFNg)-induced nitric oxide (NO) accumulation was reduced by H_2 [59]. This was by influencing the expression of inducible nitric oxide synthase (iNOS). In plants, NO was involved in the H_2 enhancement of adventitious root formation [60]. On treatment of cucumber with HRW, the levels of NO accumulation rose, and the activity of nitric oxide synthase (NOS) was increased, as was the activity of another enzyme which produced NO in plants, i.e., nitrate reductase (NR). It was therefore concluded that NO was downstream of H_2 in the signalling pathway. In alfalfa, H_2 reduced the effects of aluminium inhibition of root elongation by lowering the production of NO [61]. Also in alfalfa, NO was involved in the mediation of H_2 effects during osmotic tolerance [62]. This also involved an accumulation of proline which aided in the maintenance of the intracellular redox poise. Even postharvest, NO has been implicated in mediating H_2 effects. In cut lilies (*Lilium* "Manissa"), Huo et al. [63] used NO inhibitors (sodium azide (NaN_3) or tungstate) to show that H_2 effects were mediated by NO.

There seems to be little doubt, therefore, that NO and H_2 pathways are often linked to lead to the effect or response seen, both in animals and plants. Other small redox-active compounds may well be involved too. Hydrogen sulfide (H_2S) is known to be such a signalling molecule in plants [64,65], and indeed a link between H_2 effects and H_2S signalling has been noted, significantly involving the enzyme cysteine synthase (CS) [66]. Therefore, when looking at H_2 signalling a holistic look at redox active molecules, the molecules that produce them and remove them, ought to be sought. Taking NO as an example, the effects of H_2 on enzymes that produce NO such as NR need to be characterised, and along with enzymes which remove the downstream signalling products. For example, *S*-nitrosylated proteins are acted on by protein–SNO reductase [67], and it would be useful to know if H_2 has any effect on this activity in a range of species.

Much of the work on the molecular effects of H_2 have been carried out on animal species, including a lot in mammals. However, there needs to be a note of caution in translating this work across to plants. For example, there is evidence of H_2 effects on NOS [e.g., Zhu et al. [60]), but the presence of NOS in plants is still being disputed. In a genetic analysis study of higher plants there is little evidence of a NOS being encoded for in their genomes [68–70], suggesting that any effect on H_2 reported on a plant NOS needs to be treated with caution. No doubt, animal-based research will progress similar research in plants in the future, and *vice versa*, but some of the translational research needs to be looked at critically.

4. Effects of Molecular Hydrogen on Plant Physiology

Hydrogen gas is relatively insoluble in water, relatively unreactive and such a small molecule that it is hard to envisage it having a receptor for its perception. The direct, but rather unspecific, direct interaction of H_2 with proteins has been suggested [71], but there is no experimental evidence for this. There are mechanisms mooted to account for how H_2 may work in biological systems, but what seems to be of little doubt is that the treatment of plants with H_2 have revealed a wide range of effects, as listed in Table 1.

Table 1. Examples of the effects of H_2 on plants and plant materials.

Effect Seen	Plant Used	Treatment Used/Effect	Reference(s)
		Germination	
	Rice	HRW: alleviated salt stress	[72]
	Cucumber	HRW: regulation of sugar/starch metabolism	[73]
	Cucumber	HRW: increased trehalose biosynthesis	[74]
	Barley	HRW: enhanced under drought stress	[75]
	Barley	HRW: enhanced under drought, mediated by ASA/GSH cycle and sugar metabolism	[76]
	Wax gourd (*Benincasa hispida*)	HRW: involves GA and ABA signalling	[77]
	Lentil	HRW: enhanced germination	[78]
		Plant growth	
	Rice	HRW: enhanced during salinity stress	[79]
	Rice	HRW: under nitrogen deficiency	[80]
	Rice	HRW: during Cd and Pb stress	[81]
	Mung bean, cucumber & radish	HRW: better hypocotyl elongation mediated by GA and auxin	[82]
	Cucumber	HRW: under salinity stress	[83]
	Maize	HRW: enhanced root growth under salinity stress	[84]
	Wheat	HRW: better seedlings and drought tolerance	[85]
		Plant stress	
Drought	Barley	HRW: better seed germination under drought stress	[75,76]
	Wheat	HRW: better drought tolerance	[85]
	Tomato	HRW: drought resistance involved ABA	[86]
	Rapeseed (*Brassica napus* L.)	HRW (prepared by ammonia borane): better drought tolerance.	[87]
Salt	Cucumber	HRW: enhanced salt resistance	[83]
	Maize	HRW: enhanced salt tolerance	[84]
	Rapeseed (*Brassica napus* L.)	HRW: better salinity tolerance	[87]
	Barley	HRW: better salinity tolerance	[88]
	Rice	HRW: altered salt tolerance and antioxidant levels	[89]
Heavy metals	Rice	HRW: Cd and Pb stress	[81]
	Rapeseed (*Brassica napus* L.)	HRW, Cd stress	[87]
	Medicago sativa	HRW: alleviates Cd stress	[90]
	Chinese cabbage	HRW: increased Cd tolerance and better antioxidants.	[30]
	Alfalfa	HRW: enhanced Hg tolerance	[91]
UV light	Radish	HRW: improves ROS homeostasis	[92]
	Radish	HRW: involves anthocyanin biosynthesis	[93,94]
	Medicago sativa	HRW: alleviates stress and (iso) flavonoids	[32]
Herbicide	Rice	HRW: gives better tolerance	[95]
Temperature	*Tetrastigma hemsleyanum*	HRW: enhanced cold tolerance	[96]
	Cucumber	HRW: enhanced cold tolerance	[97]
	Cucumber	HRW: enhanced cold tolerance	[98]
	Cucumber	HRW: better tolerance to heat stress	[20]
		Better crops	
	Rice	HRW: better fitness of plants	[99]
	Rice	HNW: enhanced qualitative and quantitative traits in field trials	[100]
	Barley	HRW: Altered nutrient and antioxidant content	[101]
	Tomato	hydrogen nanobubble water: better yield and quality	[102]
		Post harvest	
	Kiwifruit	HRW: delays ripening and senescence	[28]

	Okras	HRW: delays fruit softening and prolongs shelf life	[103]
	Okras	HRW: increases shelf life and phytohormones	[104]
	Litchi	HRW: delays pulp breakdown	[105]
	Rosa sterilis fruit	HRW: maintains fruit quality and alters antioxidants	[106]
	Lanzhou lily	HRW: enhanced quality of scales	[107]
Flowers	Lily and Rose	HRW: better vase life and flower quality	[108]
	Carnation	Magnesium hydride: prolonged vase life	[109]
	Carnation	HRW: enhanced vase life	[110]
	Carnation	HNW: delays senescence and longer vase life	[111]
	Lisianthus	HRW: delays petal senescence	[112]
	Lily	HRW: improved quality, involved NO	[63]

ABA: abscisic acid; ASA: ascorbate; GA: gibberellic acid; HNW:hydrogen nanobubble water.

The presence of H_2 can have a range of effects on plants at different developmental stages. Seed germination is enhanced, particularly under stress conditions such as salinity [72] or drought, [75,76], for example. Some of the molecular effects here involves changes in sugar metabolism [73,74] and mediation by the ASA/GSH cycle [76], or by phytohormones such as abscisic acid (ABA) and gibberellic acid (GA) [77]. Enhanced plant growth has been seen on a variety of species, including rice, mung bean, cucumber, radish, maize and wheat as listed in Table 1.

Many of the effects seen are under stressful conditions, which seems to mirror that which is seen in animals, such as during disease, such as reports about H_2 and liver disease [113], or ophthalmic diseases [114]. As can be seen in Table 1, H_2 alleviates, or at least enhances tolerance, a range of stresses in plants, including drought, salinity, heavy metals such as Cd and Pb, temperature extremes, such as cold, excessive UV light, or the presence of herbicides.

One of the most important observations is that H_2 treatment can produce fitter plants and better crops. In field trials by Cheng et al. [100], this is particularly interesting to note. Here, they grew rice in the presence of hydrogen nanobubble water (HNW) or ditch water, and found that the inclusion of the hydrogen increased length, width and thickness of the rice grains. The authors then went on to look at the molecular changes induced, which included changes in amylose metabolism and heavy metal uptake. As rice is such a major world food, this seems to be a significant observation and shows the potential for the use of H_2 treatments in future food security.

As well as plant growth, H_2 has significant benefits post-harvest. As listed in Table 1, there are several fruits which have been investigated, including kiwifruits, okras, and lichi. When such postharvest material is treated with H_2 the ripening is delayed, as is the fruit senescence, and therefore the shelf life is increased, a positive characteristic for food transport or storage, and overall, for food security. Phytohormones and antioxidants are involved, as might be expected.

Flowering in plants can be influenced by a range of signalling molecules, including NO [115], and H_2 can have an effect too. Flowers have been studied with H_2 treatment postharvest, and as might be predicted, the vase life of the flowers is increased, and flower senescence is delayed. This use of H_2 is both inherently safe–the flowers would not normally be eaten–and easy to do, and as can be seen in Table 1, several treatments were used, including HRW, but also HNW and magnesium hydride, which would release H_2 in solution. Therefore, floriculture may well be a potential future use of H_2, with such treatments simply added to the feed water and then thrown away when no longer needed.

What is interesting with many of the data found in the literature is that H_2 treatment increases the antioxidants of the plant tissues, whether this is still growing or postharvest, for example with the Chinese cabbage work [30]. Furthermore, many of the treatments are carried out when the plant is under stress conditions, such as drought, salt, temperature, etc. However, plants cells under stress generally have an increased oxidative stress–more ROS production and accumulation–and this leads to an increase in the antioxidants of the cell [116,117], in the absence of H_2. But H_2 enhances the cells antioxidants. If, as normally seen, these studies are carried out during plant stress, why is the H_2 having an effect? Is the normal antioxidant response in the cell inadequate? And if not, as cells normally can adapt and survive numerous stress challenges, why does the H_2-induced antioxidant capacity show such dramatic effects? Or is the H_2 presence doing something else as well? On the work described in the section above [44], in animals the H_2 induces Nrf-2-mediated gene expression, often leading to enhanced antioxidants, so does the same issue apply? What is required is a holistic view of two major points:

- How does H_2 fit into the milieu of redox signalling, which involved ROS, RNS, sulfur compounds etc.?
- Is H_2 having other actions, perhaps at the same time as moderating hydroxyl radicals, and antioxidants?

There seems to be no doubt that from a physiological perspective H_2 has a range of effects in plants. However, a greater understanding of how these effects are brought about is needed.

5. Conclusions and Future Perspectives

There seems to be no doubt that H_2 has significant effects in plants, and H_2 aids plant cells and tissues to respond to a range of stresses, including heavy metal, drought, or conditions of storage and use postharvest. Some mechanisms of action of H_2 are being unravelled, but there is still much to learn. The studies of the molecular effects of H_2 need to take a holistic view of all the redox/reactive molecules involved in signalling. This should include those designated as ROS or RNS, but also the sulfur-based compounds such as glutathione and H_2S, as well as methane and carbon monoxide.

Some of the effects and mechanisms of action of H_2 have been elucidated in animal systems, and there needs to be research into how well these translate across to plants. Some caution is needed here, but the principles of molecular biology will remain the same across the plant and animal kingdoms, so there is a lot of scope for learning from different species.

The use of H_2 in agriculture potentially has a bright future. However, to date, there has not been an in-depth cost/benefit analysis, and unless the production, transport, storage and application of H_2 treatments is not offset by the increased value of the crops produced, either in yield or quality, or both, then H_2 treatments are not likely to be widely adopted. Floriculture may be the first to target, as it is relatively easy and cheap to use, and the benefits are visually seen. However, the use of hydrogen in general is becoming more common, with hydrogen-fuelled vehicles being developed and studied, including cars, trucks [118], and buses [119].

It is hoped that this overview will encourage researchers to look at H_2 in biological systems, and in particular in plant sciences, a little more closely, and perhaps an increase in research and understanding in this field will one day see the widespread use of H_2 in the agricultural sector.

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