Editorial for Special Issue: “Production and Role of Molecular Hydrogen in Plants”

John T. Hancock

Department of Applied Sciences, University of the West of England, Bristol BS16 1QY, UK; john.hancock@uwe.ac.uk; Tel.: +44-(0)1173282475

1. Introduction

Molecular hydrogen (H\(_2\)) is an extremely small molecule, which is relatively insoluble in water and relatively inert. Regardless of this, there seems little doubt that H\(_2\) has profound effects in a range of organisms, from plants [1] to humans. In the biomedical arena, H\(_2\) has been suggested as a therapeutic agent [2] for a range of diseases, including neurodegenerative disease [3] and COVID-19 [4]. It has even been suggested to be useful as a sports supplement [5].

H\(_2\) can be administered to plants, or plant tissues, either as a gas or dissolved in a suitable medium and sprayed on. For the latter, H\(_2\) is usually bubbled through water to make a hydrogen-enriched solution, referred to as hydrogen-rich water (HRW). This can then be added to the soil (or feed solution) or sprayed on the foliage. As an example, Wu et al. [6] used this approach to look at effects of H\(_2\) on cadmium stress in cabbage.

Using such approaches there have been numerous reports of the effects of H\(_2\) in plants. H\(_2\) is involved in seed germination, for example, especially under salt stress [7]. Hydrogen-rich water (HRW) promotes root growth, again especially under stress conditions [8], such as when excess metal ions are present. It has been suggested that such stress relief by H\(_2\) may involve phytohormone signalling [9].

H\(_2\) is safe. Sun et al. [10] give three reasons why they consider H\(_2\) safe for humans, and, therefore, by extension it is safe for treatments of plants, which are used for food crops. Firstly, H\(_2\) has been used as a compressed gas for deep-sea diving for decades, with no ill effects reported. Secondly, H\(_2\) is an endogenous gas, being produced in the gut, for example [11]. Thirdly, experimental evidence has been reported that H\(_2\) is safe. On the other hand, H\(_2\) is highly inflammable, so some caution needs to be exercised if used in confined places, such as in a glasshouse.

How H\(_2\) is acting on plants is not well understood. If sprayed onto the foliage it is not known how much of the H\(_2\) enters the plant tissues but clearly it has to if there are effects seen. Even if used as HRW, H\(_2\) is very likely to enter the gas phase relatively quickly, so repeated treatments may be needed. The direct effects of H\(_2\) are also hard to understand, i.e., what the molecular targets of H\(_2\) are. H\(_2\) is probably too small to interact with a protein receptor in the classical manner. However, there are reports of H\(_2\) acting as an antioxidant [12], with the most likely target being the hydroxyl radical, or the peroxynitrite molecule (ONOO\(^-\)). The latter is formed through a reaction of nitric oxide (NO) and superoxide anions. It is thought that it is less likely that H\(_2\) is reacting with other reactive oxygen species (ROS), such as the superoxide anion or hydrogen peroxide (H\(_2\)O\(_2\)), or other reactive nitrogen species, such as NO. It is also unlikely that H\(_2\) is involved in the direct modification of amino acids in polypeptides, which would be the typical mechanism of H\(_2\)O\(_2\) (through oxidation [13]) or of NO (through S-nitrosylation [14]). Others have suggested that H\(_2\) acts on enzymes, such as heme oxygenase [15], but the direct interaction is not known.

Here, this Special Issue (SI) was an invitation to those in the field to give their up-to-date research and appraisal of the effects and uses of H\(_2\) in plant science.
2. Effects of H\textsubscript{2} on Plants

Here, in this SI, Nguyen and Lim ask if H\textsubscript{2} can be used for the extension of the vase life of flowers [16]. Post-harvest is a hugely important topic. In some countries, the postharvest waste has been estimated to be over 40% [17]. Crops need to be commercially viable, safe to consume and acceptable to the customers. This latter point is very pertinent for flowers where the expectation would be that they look good and last for as long as possible. Nguyen and Lim reviewed the various methods used to deliver H\textsubscript{2} to plants, including HRW, hydrogen nanobubble water (HNW) and magnesium hydride (MgH\textsubscript{2}). Plants used across studies included rose, carnation and lilies. Overall, the application of H\textsubscript{2} increased vase life of flowers, and the authors concluded that such work should be continued to release the potential for the use of H\textsubscript{2} in the floriculture industry. Interestingly, the authors also discuss the cost–benefit analysis of H\textsubscript{2} use and suggest that labour costs are an issue [18]. Certainly, as H\textsubscript{2} is developed more for other industries, such as a transport energy source [19], the cost and delivery of H\textsubscript{2} is likely to become cheaper.

Li et al. [20] continue the theme of using H\textsubscript{2} to extend the vase life of flowers. Here, the authors use HNW, which they say has a higher concentration of dissolved H\textsubscript{2} than conventional HRW, and the residual time that the H\textsubscript{2} remains dissolved for is longer, both properties, which would be of benefit if adopted for widespread use. Their data show that 5% HNW significantly lengthened the vase life of cut carnations. This concentration was better than other concentrations of HNW tried, and better than either water or HRW. Their measures of improvements included electrolyte leakage, oxidative damage and cell death in the petals. The authors concluded by suggesting that HNW may have future applications for postharvest preservation. Certainly, treatment with molecular hydrogen is likely to be much safer than the use of some of the alternatives mooted, such as hydrogen sulfide [21], which is known to have toxicity [22]. This might not be too much of an issue with flowers but may become a problem if the same treatments are used for postharvest preservation of food crops.

Cheng et al. [23] have taken H\textsubscript{2} applications out into the field, with trials of whether such treatments will improve rice. They too use HNW, comparing it to ditch water. Their data show that HNW increased the length, width and thickness of brown/rough rice and white rice. They then looked at gene expression in these plants and could correlate the physiological changes with the molecular alterations seen. In the white rice they saw no difference in total starch content, but the enzyme amylase was decreased. Cadmium accumulation was also decreased, which also correlated with gene expression patterns reported. Overall, such work shows that H\textsubscript{2} treatments can be taken to larger scales, not just in a laboratory setting. The authors conclude that H\textsubscript{2} application does increase the quality of the rice and should be considered as a future treatment. Although field trials with H\textsubscript{2} have been written about before [24], they are relatively rare and more large-scale work, such as this, certainly needs to be undertaken if H\textsubscript{2} treatments are to be used more widely in agriculture.

There seems little doubt that H\textsubscript{2} treatment of plants is beneficial, as exemplified by the papers in this SI and the further papers that these authors cite. There is a growing body of this evidence, and as more is reported, on different species, the use of H\textsubscript{2} will be seen to be advantageous, whether used in the field or postharvest. Despite the molecular mechanisms not being well understood, the next papers go someway to unravel what H\textsubscript{2} might be doing in plant tissues.

3. Mechanisms of H\textsubscript{2} Action

The mechanisms in the cells, which enable H\textsubscript{2} to have its effects, were also the focus of papers in this SI. Zhao et al. [25] show an interaction between H\textsubscript{2} and glucose in adventitious roots of cucumber. The effects of HRW were blocked by glucosamine, suggesting that glucose content may be mediating root development. HRW increased the cellular content of a range of sugar-based metabolites, including glucose, starch, sucrose, glucose-6-phosphate, fructose-6-phosphate and glucose-1-phosphate. HRW treatment resulted in the increase
in the activity of several relevant enzymes, including hexokinase, pyruvate kinase and sucrose synthase, and, furthermore, gene expression patterns matched these findings. Interestingly, the authors state that all the positive effects of HRW were inhibited by glucosamine, and they concluded that H₂ was regulating adventitious root growth by promoting glucose metabolism.

The literature on H₂ effects tends to support the notion that H₂ increases cellular antioxidant levels. For example, Wu et al. [6] reported increased antioxidants in cabbage under cadmium stress, whilst Chen et al. [26] showed that the antioxidant capacity of Hypsizygus marmoreus (mushroom) was increased by HRW use during postharvest. Here, in this SI, Jiang et al. [27] also look at antioxidant capacity and how this might be improved by H₂ treatment, using Chinese chive. In a similar manner to the vase-life work above, this is also being carried out postharvest. Chives were treated with a range of H₂ concentrations, in comparison to air. Shelf-life was improved most by 3% H₂, a conclusion supported by measurements of decay index, loss ratio of weight and protein content. Of pertinence to the discussion here, the content of total phenolics, flavonoids and vitamin C were maintained, whilst the activities of antioxidant enzymes were increased, including superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX). Clearly, H₂ treatment was protecting the plant material by an increase in antioxidant capacity, as suggested by others, for example [28].

Zhang et al. [29] looked at pesticide residues in plants. Using tomato and Arabidopsis, they found that the degradation of carbendazim (a benzimidazole pesticide) was increased by H₂. H₂ increased glutathione metabolism, which led to the increased degradation of carbendazim. Glutathione is an immensely important antioxidant in plants [30], so any effects seen may also alter cellular redox states [31]. Carbendazim is a fungicide [32]. Zhang et al. [29] comment that the antifungal action of carbendazim was not affected by the H₂ treatments, but they were convinced that glutathione was important for the H₂ detoxification of the fungicide. Therefore, as the authors point out, this is a previously unknown action of H₂ in plant cells.

Wuzhimaotao (Ficus hirta Vahl) was the plant of focus for the study by Zeng and Yu [33]. In China, this edible plant also has medicinal properties. Following treatment with H₂ (as HRW), the transcriptomic pattern of the roots was compared with controls. One hundred and seventy three genes were found to be down regulated, whilst 138 were up regulated. The authors also carried out a metabolomic analysis and found that nearly 200 metabolites had their levels altered by H₂ treatment. With further analysis, it was suggested that the biosynthesis and metabolism of phenylpropanoid were the main pathways being controlled by H₂ treatment. Although the authors suggest that H₂ application should be considered for future growth of this medicinal plant, the data also show the scale of the effects that can be reported when plants are treated with H₂. With so many genes being up- and down-regulated, H₂ must be having a profound effect on transcription factors in plant cells. With similar results emanating from work on mammalian cells [34], the future will no doubt allow the mechanisms behind these changes in gene expression to be unravelled.

Finally, a review of the direct actions of H₂ on plants was published as part of the SI [35]. There are reports that H₂ acts as a direct antioxidant [12], but the chemistry has been disputed [36]. It has been suggested that H₂ acts through its redox state [37], and there is some precedent for this in bacterial systems [38]. Alternatively, H₂ may be acting through its spin states [39], but there is no evidence of this. Clearly, there is a lot to explore here, and some of the focus of H₂ research needs to be pointed in this direction.

Whatever the mechanism, it would strengthen the argument for the use of H₂ in agriculture, and in the biomedical arena, if the mechanism(s) of the direct action of H₂ was resolved, but there is no doubt that such evidence, either ruling mechanisms in or out, will be forthcoming in the future.
4. Conclusions and Future Perspectives

As can be seen by the papers that were published in this Special Issue, H₂ has beneficial effects on plants. H₂ appears to increase the crop yields and can be used for improving postharvest storage of crops, as exemplified by the work on flowers here. Therefore, H₂ use should have a bright future in agricultural settings.

Plants may be exposed to H₂ naturally, either through the action of cellular enzymes [40], or through the metabolism of other organisms in the location, such as in the soil [41]. Alternatively, H₂ may be applied to the plant—either onto the soil or foliage—as a treatment. As can be seen in the papers in this SI, there are a variety of ways to achieve this. H₂ can be applied as a gas, or in an enriched solution, i.e., HRW. However, more recent advances in this area have seen the development of other solutions, which can be used, such as HNW. Alternatively, H₂ can be supplied from donor molecules, such as MgH₂, and no doubt the future will see better donor compounds being developed, which can deliver more H₂ for a longer period of time, rather than giving tissues a bolus effect.

One aspect of the reporting of the effects of H₂ that needs to be consistent is the quoting of the concentration of H₂ used. Often the percentage of HNW or HRW is quoted, but without knowing for sure exactly how concentrated the stock solution is it is hard to compare different studies and, therefore, the effects. H₂ does not last long in solution, so quoting the actual concentration of H₂ in the solutions used would be very beneficial to push this field forward.

Some evidence of the molecular aspects are presented here in this SI too, including the action through glucose metabolism, glutathione metabolism, as an antioxidant and in the control of gene expression. However, the direct targets of H₂ still remain elusive, not just in plant science but in all aspects of the action of H₂ in biological systems. Several ideas have been mooted but there is little evidence of them at present. Future work needs to be focussed on this aspect of H₂ biology, as this would really strengthen the argument for H₂ use. It would also give reassurance on the safety of H₂ treatments, especially if it is proposed to be used as a treatment for consumed crops, either in the field or postharvest.

Finally, more large field trials are needed on a range of crops. H₂ is being studied in some countries around the world, most notably China, but it needs to be looked at more widely, in different locations and with different plants. H₂ appears to be safe, albeit inflammable, but the cost–benefit needs to be well established before H₂ will be taken up widely in agriculture and floriculture. H₂ has benefits, especially if used when plants are stressed, and no doubt large scale trials will unlock the hesitation for the adoption of H₂ applications in the future.

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