Molecular hydrogen in agriculture

Faisal Zulfiqar*[1], Grace Russell[2] and John T Hancock[2]

[1]Department of Horticultural Sciences, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Pakistan.
[2]Department of Applied Sciences, University of the West of England, Bristol, UK.

*Correspondence:

Faisal Zulfiqar
Department of Horticultural Sciences,
Faculty of Agriculture and Environment,
The Islamia University of Bahawalpur, Pakistan
ch.faisal.zulfiqar@gmail.com
https://orcid.org/0000-0001-5428-5695

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Main conclusion

H_2 gas, usually in the form of H_2-saturated water, could play a useful role in improving many aspects of plant growth and productivity, including resistance to stress tolerance and improved post-harvest durability. Therefore molecular hydrogen delivery systems should be considered as a valuable addition within agricultural practice.

Abstract

Agriculture and food security are both impacted by plant stresses, whether that is directly from human impact or through climate change. A continuously increasing human population and rising food consumption means that there is need to search for agricultural useful and environmentally friendly strategies to ensure future food security. Molecular hydrogen (H_2) research has gained momentum in plant and agricultural science owing to its multifaceted and diverse roles in plants. H_2 application can mitigate against a range of stresses, including salinity, heavy metals and drought. Therefore, knowing how endogenous, or exogenously applied, H_2 enhances the growth and tolerance against numerous plant stresses will enhance our understanding of how H_2 may be useful for future to agriculture and horticulture. In this review, recent progress and future implication of H_2 in agriculture is highlighted, focusing on how H_2 impacts on plant cell function and how it can be applied for better plant performance. Although the exact molecular action of H_2 in plants remains elusive, this safe and easy to apply treatment should have a future in agricultural practice.

Keywords: abiotic stresses; heavy metals, hydrogen rich water; oxidative stress, salinity

Introduction

Plants are often grown in sub-optimal conditions, and plant stress has a significant adverse effect on global agriculture. Therefore, future food security will be dependent on better plant growth and higher productivity, especially as the human population expands and the demand for food increases (Molotoks et al. 2021). It has been shown that climate change-driven abiotic stresses causes a loss of food productivity, compromising the future of food security, with the cost of lost produce estimated to be more than US$170 billion per annum on a global scale (Razzaq et al. 2021). A challenge is to develop easy and cheap solutions that mitigate against plant stress and lead to better agricultural outputs, enhancing plant growth and productivity.

Hydrogen is the most abundant and lightest chemical element, constituting ~75% of the mass of the universe. At standard pressure and temperature, hydrogen gas (H_2) is highly flammable, colourless, tasteless and odourless. Atomic hydrogen (H) is rare in the Earth's atmosphere and hydrogen is more typically found as a diatomic molecule (H_2) that is electrochemically neutral and non-polar, which has previously been thought of as a relatively physiologically inert molecule (Zeng et al., 2014). H_2 emission from plant tissues was reported many years ago. Recently, H_2 has been found to play vital roles in biomedical fields, where it has been described as having anti-apoptotic, and anti-oxidant effects (Russell et al. 2020). Furthermore, the metabolism of H_2 in different organisms including bacteria, green algae, and higher plants, has also been widely reported (Renwick et al. 1964; Melis and Happe 2001; Bothe et al. 2010; Russell et al. 2020). Although the production of H_2 in higher plants remains somewhat unclear (Russell et al. 2020), a growing understanding of its physical and bio-regulatory roles hydrogen gas has recently gained attention in both plant and animal research. H_2 is becoming recognised as an integral signaling molecule that is likely to have influential roles in a broad array of adaptive and developmental responses. In plants, H_2 is known to combine a regulatory role in the control of gene expression and in signal transduction, mediating the management of numerous stress responses (Cui et al. 2020; Wang et al. 2020a,b), including cold stress (Xu et al. 2017), metal stress (Zhao et al. 2017; Wu et al. 2019; Wu et al. 2020a; Cui et al. 2020; Fan et al. 2020), UV stress (Xie et al. 2015; Zhang et al. 2018), high light stress

(Zhang et al. 2015), and salinity stress (Xie et al. 2012; Wu et al. 2020b). Therefore, a greater understanding of how H_2 acts in cells, and how it leads to better stress tolerance will be important for its future use.

Biological generation of H_2 in plants

Plant cells may be exposed to H_2 in different ways, either via endogenously production or by being applied exogenously. Endogenous production of H_2 occurs via the activity of hydrogenase enzymes (Vignais et al. 2001; Russell et al. 2020) ((FeFe)-hydrogenases) or nitrogenase enzymes (**Fig 1**). The metallo-protein complexes possess the potential of generating or removing H_2 via catalysing both forward and backwards reactions. The presence of phytohormones such as auxin, abscisic acid, jasmonate and ethylene can enhance endogenous production of H_2 in plants (Lin et al. 2014; Liu et al. 2016; Cao et al., 2017). To illustrate, Legume-rhizobia contributes to H_2 generation inside root nodules during biological nitrogen fixation process as an obligate by-product of the nitrogen fixing enzyme nitrogenase (Golding and Dong 2010). In this process of H_2 production, H_2 comes out of the root nodules leading to elevation of H_2 around the root surface (Porte et al. 2020). Increases in H_2 in the rhizosphere leads to beneficial impacts for subsequent plant growth resulting in 15–48% biomass increase in plants, a phenomenon termed as the hydrogen fertilization effect (Dong et al. 2003). Furthermore, H_2 production is also increased when plants are exposed to abiotic stress conditions such as drought and salt stress, suggesting this particular gasotransmitter may be important in stress signaling (Zeng et al. 2013).

H_2 delivery to plants

Although H_2 treatments to economically important crops are reported to have beneficial impacts in both yield and quality (Hu et al. 2021), H_2 is a gas and so is not easy to administer. As a gas, H_2 is is lighter than air, therefore under field conditions it is not pragmatic to apply in gaseous form. Furthermore, H_2 is highly explosive and is therefore not suitable to use at any significant concentrations due to safety and storage issues.

A more practical approach is the use of saturated forms of H_2 such as hydrogen rich water (HRW) (**Fig 2**). This can be used to apply H_2 directly onto plants, as used by Wu et al. (2020a). Briefly, HRW is prepared by pumping/bubbling H_2 into distilled water (or watering media) following H_2 gas production by hydrogen or oxy-hydrogen generators. Subsequently, this HRW can be diluted to make desired concentration for treatments. Other methods of HRW production include the mixing of magnesium-based tablets in the water, although these will leave behind by-products in the media. HRW can then be applied in spray form or as a soil drench to agricultural crop plants. If plants or plant cells are being grown in hydroponics or culture media, H_2 can be bubbled directly into the media. However, it needs to be borne in mind that H_2 is not very soluble (Wilhelm et al., 1977) and will readily diffuse into the atmosphere where, H_2 will be lost from the environment of the plant and can potentially build up to flammable levels if ventilation is limited, in laboratory conditions for example.

H_2 can be useful as a treatment for post-harvest storage. Here, fresh produce can be easily dipped, sprayed or misted with HRW. Moreover, under storage conditions the use of H_2 in gas form is also possible. Safety measures, again, are necessary on the use of large scale HRW or H_2 in gaseous form. HRW application has been shown to improve the vase life and quality of cut rose and lily flowers by decreasing leaf stomatal size and reducing oxidative damage by elevating ROS scavenging antioxidants such as ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) while reducing malondialdehyde (MDA) and electrolyte leakage (Ren et al. 2017).

The use of H_2 gas and HRW are relatively easy but not without problems, as discussed above. The future of H_2 in agriculture will need to look to more advanced solutions if the gas is to be widely used. Advances in development of solid hydrogen-storage materials may offer ways to improve the production and storage of H_2. When considering safety, sustainable hydrogen supply of solid-state storage, portable, and large hydrogen contents (Hirscher et al. 2020). Magnesium hydride (MgH_2) is also a promising low cost, abundantly available donor (Grochala and Edwards 2004), showing potential to be used as a H_2 source material in agriculture (Li et al. 2020a). Another potential method of H_2 delivery to agriculture is the use of nanotechnology that provides the opportunity for sustained delivery of H_2 via using hydrogen-releasing nanomaterials such as nanocapsule (AB@hMSN) by encapsulating ammonia borane (AB) into hollow mesoporous silica nanoparticles (hMSN) (Wang et al. 2021). In addition, the use of loaded hydrogen nanomaterials has the advantage of increased residence time in liquids such as water (Wang et al. 2021).

However, the release of by-products into the environment has to be considered before adopting any new technologies. In this regard, future studies should focus on the use of nanomaterial-based hydrogen release and their impacts on crops.

Physiological effects

Effect of H_2 on leaf stomata

Water uptake and transpiration are vital for plant growth and survival, but at the same time, climate change is leading to increased uncertainty of water availability for plants, with expanding areas of drought leading to declined agricultural output in some regions (Cook et al. 2018). On the other hand, other regions will be at more risk from flooding, a major climate-related calamity (Hirabayashi et al. 2013). Hydrogen treatment can mitigate drought stress (Zeng et al. 2013; Hancock and Russell 2021), whilst hydrogen is also known to be released from flooded soils (Piché-Choquette and Constant 2019), and therefore the role of H_2 in controlling the plants transpiration stream will be important to understand.

Stomata are tiny holes present on the aerial plant surfaces, especially the leaves that play vital role in regulating gas exchange rate and transpiration rates and are crucial for plant growth and survival. Studies have demonstrated that H_2 may have a crucial role in regulation of stomatal aperture via interaction with phytohormones (Liu et al. 2016). For example, in *A. thaliana* grown under drought stress conditions HRW application enhanced endogenous H_2 production, along with reduction in stomatal aperture, leading to drought tolerance (Xie et al. 2014). Zhang et al. (2020) tested the introgression of a *Chlamydomonas reinhardtii* hydrogenase gene (*CrHYD1*) for H_2 biosynthesis in *Arabidopsis thaliana* and demonstrated that increased H_2 resulted in closure of stomata under osmotic stress leading to osmotic stress tolerance. From these examples, it is clear that H_2 has a critical role in maintaining the stomatal closure particularly under drought/osmotic stress conditions, although the mechanism and pathways involved have yet to be fully delineated.

Drought is a major abiotic stress/challenge affecting agriculture productivity worldwide. Drought stress negatively affects physiological and biochemical mechanisms, and ultimately leads to plant growth reduction and diminishing agricultural productivity (Abideen et al. 2020; Zulfiqar et al. 2021). Chen et al. (2017b) reported that carbon monoxide and hydrogen gas (HRW as a source) co-ordinately improved growth, particularly during root formation, as well as other important traits such as chlorophyll content, relative water content and chlorophyll fluorescence characteristics, under drought-stress conditions. Furthermore, these compounds jointly increased the activities of SOD, POD, CAT, APX, proteins, water soluble carbohydrate, and proline content (also an antioxidant). These factors alleviated the drought induced oxidative stress, evidenced by decreases in hydrogen peroxide (H_2O_2), thiobarbituric acid reactive substances (TBARS), and superoxide radical ($O_2^{\cdot-}$) levels (Chen et al. 2017b). Moreover, exogenous H_2 (HRW) efficiently can lead to regulation of the stomatal aperture resulting in improved drought stress tolerance (Xie et al. 2014), as mentioned above. Jin et al. (2016) also demonstrated that under drought stress condition, H_2 promptly increased H_2O_2 signaling and altered the apoplastic pH of alfalfa leaves by influencing an abscisic acid (ABA)-based mechanism. The evidence, therefore, points to H_2-based treatment as being a useful adjunct for enhanced of drought tolerance in the future (Table 1).

Effect of H_2 on root development

The development of a good root system for the uptake of water and nutrients, as well as to ground the plants at its location, is vital for optimal plant growth and productivity. H_2 has been found to be influential in root growth (Lin et al. 2014; Zhu et al. 2016; Wu et al. 2020b).

Studies have demonstrated that H_2-induced root development is related to its influence on the endogenous level of plant hormones. In a recent study, Wu et al. (2020b) reported that HRW-treated mung bean seedlings enhanced the endogenous level of indole acetic acid (IAA) and gibberellic acid (GA_3) resulting in enhanced hypocotyl and root length. Augmentation of the endogenous levels of phytohormones is evidenced by up-regulation of related genes (Lin et al. 2014). Reports show that HRW seed treatment could induce phytohormone signalling pathways in response to environmental stresses (Zeng et al., 2013; Liu et al., 2016). In cucumber explants, Zhu et al. (2016) also demonstrated that the adventitious rooting-related target genes were upregulated in response to hydrogen-rich water (HRW). Additionally, it was found that HRW treatment upregulated the expression of cell cycle-related genes including A type cyclin (*CycA*), B type cyclin (*CycB*), cyclin-dependent kinase A (*CDKA*) and cyclin-dependent kinase B (*CDKB*) during adventitious rooting (Zhu et al. 2016). Similar to other accepted gasotransmitters such as NO, the endogenous regulatory functions of H_2 can be imitated through the exogenous treatment with hydrogen-based products/compounds.

The interaction of H_2 with other gaseous signals, such as nitric oxide (NO) and hydrogen sulfide (H_2S), is important to understand, as they may be working together or antagonistically. It was recently reported that NO is involved in the H_2-induced root formation (Li et al. 2020a). The combination of these gaseous molecules effectively regulates gene expression of plasma membrane H^+-ATPase and 14-3-3 proteins, which are integral for the proper growth, development and response to stresses. H_2 has also been reported to play a role in root formation via interacting with the NO and haemoxygenase-1/carbon monoxide pathways in plants (Lin et al. 2014; Zhu et al. 2016).

An alternative study of adventitious rooting describes H_2 as regulating target genes related to auxin signaling and root development such as *CsDNAJ-1*, *CsCPDK1/5*, *CsCDC6*, *CsAUX228-like*, and *CsAUX22D-like* through CO pathways. Such results confirm that H_2 promotes rooting by increasing the content of NO and the activities of NO synthase-like enzymes and nitrate reductase (Zhu et al. 2016). H_2 was also shown to activate the cell cycle and up-regulate cell cycle-related and rooting-related genes via the NO pathway (Zhu et al. 2016). Therefore, it is likely H_2 has a vital role in root formation through the interaction with endogenous plant signaling molecules, and their related downstream target genes.

Effect of H_2 on pre- and post-harvest senescence

The distance between food production sites and subsequent consumption sites is going to significantly increase due to continuous shift of population towards urban areas, a factor that becomes of increasing importance when considering the international distribution of food. Post-harvest losses in the supply chain of fresh horticulture commodities ahead of arrival to the consumer are estimated to be between 13–38% (Duan et al. 2020). The high market value of cut flowers in international markets compels major increases in the production of floricultural crops globally, particularly in developing countries, generating billions of US-dollars economically through worldwide trade and industry (Chandler and Brugliera 2011). Thus, minimizing post-harvest losses during storage and transportation, whilst also retaining the quality standard, is a great challenge for industries in pursuit of financial success. Therefore, further research in this area is needed if enhancing the post-harvest life of horticultural and floricultural produce and consequently reducing post-harvest losses is to be realised.

Of particular importance to the horticulture industries are the studies which describe the application of H_2 as enhancing the post-harvest longevity of variable crops (Su et al. 2019a; Li et al. 2020b). H_2 application has been reported to enhance the post-harvest life of many commodities including fruits, vegetables and floriculture crops (**Fig 3**). For instance, Hu et al. (2021) demonstrated that pre-harvest HRW treatment to daylily flowers not only enhanced the daily yield of budding flowers, but also reduce the chilling injury induced by elevated ROS levels and membrane oxidation. Whilst, HRW treated daylily buds generate less browning in storage conditions (Hu et al. 2021).

Treatment with HRW also improved the ornamental and aesthetics traits and enhanced the vase life rose (*Rosa hybrid*) and cut lily (*Lilium spp.)* flowers (Ren et al. 2017). Here, H_2 maintained membrane stability and water balance, whilst enhancing antioxidant activities and reducing oxidative damage and stomata size (Ren et al. 2017). It is generally observed that H_2 content decreases during senescence and therefore improving endogenous H_2 level during the senescence process might decrease the deterioration of produce (Hu et al., 2018). To illustrate, Su et al. (2019a) demonstrated that altering endogenous $H_{2,}$ by applying exogenous HRW, can extend vase life of lisianthus cut flowers by retaining redox homeostasis through enhancing endogenous antioxidant potential. Moreover, H_2 is reported to improve vase quality and extend the life of cut roses by inhibiting endogenous ethylene biosynthesis and mitigating ethylene signal transduction during senescence (Wang et al. 2020a). Recently, Li et al. (2020b) used magnesium hydride (MgH_2) as a source of H_2 in the vase solution, evaluating its role in prolonging the vase life of cut carnation flowers. An increase in H_2-induced H_2S was observed along with enhancement in the longevity of cut flowers via H_2S signaling, re-establishing redox homeostasis and decreasing the transcripts of representative senescence-associated genes, including *DcbGal* and *DcGST1* (Li et al. (2020b). A proteomic study revealed that HRW and NO (from sodium nitroprusside) application improved the post-harvest freshness of cut lilies, possibly via AtpA protein and activity of ATPase, as well as through regulating photosynthesis and describes the positive role of NO signaling in the H_2-induced enhancement in post-harvest of cut flowers (Huo et al. 2018).

As well as flowers, H_2 treatment of fruit can also be beneficial. It has been reported that H_2 can make a significant impact in the post-harvest preservation of kiwifruit (Hu et al. 2014). Here, H_2 treatment delayed ripening and senescence via constraining the respiration intensity, lowering the incidence of rot, decreasing lipid peroxidation levels and enhancing SOD activity, post-harvest. Furthermore, H_2 treatment was shown to prolong the post-harvest life of kiwifruit via restricting endogenous ethylene (Hu et al., 2018), a gaseous plant hormone known to be instrumental in the ripening process (Wei et al. 2021).

In tomato, during post-harvest treatment, H_2 was shown to not only reduce senescence and extend post-harvest life, but also to reduce the nitrite content, a substance that is harmful to human health (Zhang et al. 2019). In the edible mushroom *Hypsizygus marmoreus*, H_2 application in the form of HRW during post-harvest, improved quality through decreasing oxidative stress, depicted by lowered relative electrolyte leakage rate and MDA content and anti-superoxide-radical ($O_2^{\cdot -}$) activity (Chen et al. 2017a). The application of H_2 concertedly enhanced the activities of antioxidants including SOD, CAT, APX and glutathione reductase (GR) through inducing their gene expression levels (Chen et al., 2017b). Together, these reports (Chen et al., 2017a; Hu et al., 2018) demonstrate that H_2 is able to reduce endogenous ethylene production and prolong the longevity and shelf life of floriculture and horticulture crops by augmenting antioxidant activities and suppressing ethylene biosynthesis genes. However, further research is needed to ascertain the specific treatment regimens of H_2 that will be effective for individual species and genotypes of plants, particularly as phytotoxic reactions are highly variable between individual horticultural commodities. The authors suggest for floral longevity treatment of HRW as a vase solution, while for fruits and vegetables, fumigation at different intervals may be beneficial. As well as molecular hydrogen, other important signalling molecules such as hydrogen sulphide (H_2S) have been proposed as the post-harvest treatment in horticulture (Zulfiqar and Hancock 2020), although toxicity needs to be considered for their use, which does not seem to be an issue for H_2.

Seed germination

Seed priming is a process used to enhance seed germination, a process which inevitably leads to various desirable traits such as enhanced photosynthesis and tolerance to abiotic stresses (Zulfiqar 2021).

There are numerous seed priming methods available and many researchers have described the use of both chemical and non-chemical compounds effective for boosting seed germination, with varied responses being reported (Zulfiqar 2021). Importantly H_2 has also shown its potential as a seed priming agent. For example, Xu et al. (2013) demonstrated that HRW treatment to rice improved seed germination under salt stress condition, with the authors reporting activation of α/β-amylase activity resulting in the accelerated formation of reducing sugar and total soluble sugar content. Here, HRW treatment was also noted to trigger elevated antioxidant enzyme activity (SOD, CAT, APX) and decreases in oxidative stress markers (thiobarbituric acid reactive substances) (Xu et al. 2013).

H_2 and abiotic stress tolerance in plants

Metal stress can be alleviated with H_2

Plants depend on soil to acquire nutrients for their proper growth and development, although soils are often contaminated through anthropogenic activity (Okereafore et al. 2020). Increasing contamination of productive land has become a great concern for agriculture productivity (Okereafore et al. 2020). Productive soils can gain metal pollutants via solid or liquid fuel burning, industrial effluents, mining activities, sewage waste disposal, urban runoff, agrochemicals runoff and domestic garbage disposal in rivers and canals, for example (Hou et al. 2020). Few (potentially toxic) heavy metals including Cu, Co, Fe, Ni, Se, and Zn are vital elements for plants, but these are known to become toxic as soon as their excess accumulation occurs in soil solution. Accordingly, non-essential elements including arsenate (As), cadmium (Cd), and caesium (Cs) can threaten crop productivity should they accumulate in the soil, even in minute amounts (Okereafore et al. 2020).

Soil contamination with toxic heavy metals increases subsequent uptake in plants and their accumulation in plant tissues not only causes lowered crop productivity, but there is a concomitant risk to animal and human health (Couto et al. 2018). At the cellular level, elevated quantities of heavy metals can impose damage through numerous mechanisms. The most common of these is the production of reactive oxygen species (ROS) that can induce oxidative stress, although other consequences include inactivation of biomolecules through displacement of essential metal ions, or blocking essential functional groups, have also been described (Stohs and Bagchi 1995). ROS at normal physiological levels play an essential role in plant physiology (Hasanuzzaman et al. 2020), however enhanced generation disrupts homeostatic cellular functions (Jalmi et al. 2018), through oxidation of vital biomolecules including DNA, RNA, lipids, proteins and enzymes etc. (Dumanović et al. 2020). Fan et al. (2020) described that H_2 application alleviates the Cu toxicity in *Daphnia magna* by depressing Cu bioaccumulation and decreasing oxidative stress.

Redox active transition metals such as Fe and Cu, can generate ROS directly through redox reactions, as with the Fenton Reaction, for example; in contrast, other metals like Pb, Cd, Ni, Al, Mn, and Zn generate ROS by indirect mechanisms. The indirect mechanisms of ROS production include heightened ROS production within the mitochondria, stimulation of ROS-producing enzymes such as NADPH oxidases, or by displacing essential cations from the binding sites of functional enzymes and inhibiting their activities (Shahid et al. 2014; Stork and Li 2016).

Existing literature reveals positive influences of H_2 on alleviating metal accrued stress (**Fig 4**). In cucumber, under cadmium stress, HRW has been demonstrated to promote adventitious rooting and reduce the content of deleterious compounds such as hydrogen peroxide (H_2O_2), MDA, superoxide radical ($O_2^{\cdot -}$) and thiobarbituric acid reactive substances (TBARS), all of which are indicators of oxidative stress (Wang et al 2019). Furthermore, decreases in ascorbic acid (AsA), glutathione (GSH), lipoxygenase (LOX) activity, relative electrical conductivity (REC), AsA/docosahexaenoic acid (DHA) ratio, and GSH/oxidized glutathione (GSSG) ratio were observed as well, indicating a reduction in stress-associated biomarker activity. Concomitant increases in beneficial biomolecules GSSG and DHA, content under cadmium stress were also noted (Wang et al. 2019), evidencing that H_2 possesses the ability to induce adventitious rooting under Cd stress by decreasing oxidative damage. In alfalfa seedlings, application of HRW alleviated the mercury (Hg) toxicity reducing adverse effects of stunted growth as a result of Hg accumulation, through avoiding oxidative stress and maintaining redox homeostasis (Cui et al. 2014). HRW has also been demonstrated to regulate genetic expression related to glutathione and sulfur metabolism. Here, increased GSH (a key antioxidant that regulates the intracellular redox state of the cell) (Schafer and Buettner, 2001) metabolism resulted in enhanced Cd tolerance via Cd chelation and activating antioxidation pathways (Cui et al. 2020). An earlier proteomics study revealed that H_2 (HRW) eliminated cadmium toxicity through various mechanisms including altering genetic expression related to the reduction of oxidative damage, maintaining nutrient balance and by enhancing sulfur compound metabolism (Dai et al. 2017).

H_2 application is also reported to lower uptake of heavy metals and hence reduce toxicity in multiple plant species. For example, a study of the cadmium accumulation in Pak choi (*Brassica chinensis*) (Wu et al. 2019) observed that HRW applications repress the expression of cadmium absorption transporters (*BcIRT1* and *BcZIP2*) on exposure to increased Cd concentrations. It has also been reported that H_2 application mitigates the toxic effects of cadmium in *Brassica campestris* by upregulating the expression and ultimately the activity of nitrate reductase (NR) (Su et al. 2019b). Here, pre-treatment with HRW induced lower ROS, enhanced AsA content, increased activity of POD and SOD in seedling roots. Proteomic analysis revealed altered proteins related to antioxidants and oxidation-reduction processes in response to HRW treatment. Furthermore, mitigation of cadmium stress in *Brassica campestris,* through H_2 application was dependent on endogenous NO (Su et al. 2019b). A supporting study (Wu et al. 2015) reported alleviation of cadmium (Cd) toxicity in Chinese cabbage (*Brassica campestris* spp. chinensis L.). Molecular evidence showed that Cd-induced up-regulation of iron-regulated transporter1 (*IRT1)* and natural resistance associated macrophage protein genes, responsible for Cd absorption, was blocked, while expression of the *HMA3* gene, responsible for Cd sequestration into the root vacuoles, was substantially improved by HRW. Furthermore, it was demonstrated that the Cd-protective effect related to H_2 may be associated with its control of plasma membrane-based NADPH oxidase encoded by respiratory burst oxidase homolog D (*RbohD*), which activates upstream of *IRT1* and adjusts root Cd uptake at both the functional and transcriptional levels (Wu et al. 2020a). Chen et al. (2014) reported improvement in the aluminium induced inhibition of root growth through decreasing the endogenous NO level in alfalfa (Chen et al., 2014).

Work such as this shows that there is potential for H_2-based treatments to be used to mitigate against metal stresses, which may be seen in the future as increased anthropogenic activities and associated climate changes become more significant (Table 1).

Salinity stress

Salinity stress is a major environmental constraint globally. About 45 million hectares of productive, irrigated land have been estimated to be affected by salinity stress worldwide. This figure is increasing day-by-day (Munns and Tester 2008) largely due to climate change, especially in coastal areas (Hadley 2009). Salinity negatively affects plant productivity by causing imbalance in cellular osmotic and ionic equilibria. Major hostile effects of salinity include increased osmotic stress, specific ion toxicity, nutrient-acquisition and homeostasis/deficiencies, increased cell-turgor loss, and stress induced increased ROS causing oxidative stress (Flowers, 2004; Munns and Tester 2008). The role of H_2 in improving salinity stress tolerance has been reported in many crops including *Hordeum vulgar* (Wu et al. 2020c), *Oryza sativa* (Xu et al. 2013) and *Medicago sativa* (Wang et al. 2012).

Exogenous HRW application on *Hordeum vulgar* under salinity stress showed that H_2 increased the rate of Na^+ extrusion from roots, a mechanism mediated by salt-overly-sensitive SOS1-like Na^+/H^+ exchangers in the root epidermis. Furthermore, H_2 application enhanced root K^+ retention by preventing NaCl-induced membrane depolarization and reducing sensitivity of K^+ efflux channels to ROS, as detected by electrophysiological studies using non-invasive ion flux measuring MIFE techniques (Wu et al. 2020c). It has also been reported that endogenous H_2 levels are enhanced in response to salt stress where H_2 pre-treatment of *A. thaliana* modulated genes/proteins of the zinc-finger transcription factor ZAT10/12 and related antioxidant defence enzymes, resulting in reduction of oxidative stress (Xie et al., 2012). Here, H_2 pre-treatment was noted to regulate ion homeostasis by regulating the H^+ pump and antiporters responsible for Na^+ exclusion and compartmentalization. The same study suggested that APX genes such as *cAPX1*, and Salt Overly Sensitive1 (SOS1) protein gene, *SOS1*, might be the target genes of H_2 signaling (Xie et al. 2012). H_2 may also enhance total, isozymatic activities or corresponding transcripts of antioxidant enzymes, and reduced oxidative damage under salt stress during rice seed germination (Xu et al. 2013). More recently, a study on the role of *CrHYD1* transgenic *A. thaliana* under salinity stress reported that enhanced endogenous H_2 regulated the redox and ion homeostasis via interaction with melatonin (Su et al. 2021). It is clear, therefore, that the generation and accumulation of H_2 has an influence on stress-induced signaling pathways that can help mitigate the effects of high salt (Table 1), a factor which may become more significant as salinity continues to have an impact in the future (Munns and Tester, 2008).

UV and high light stress

Ultraviolet (UV) radiation of 280–400 nm is a minute portion of the solar energy that is able to modulate plant physiology upon reaching the terrestrial ecosystems (Paul and Gwynn-Jones 2003). Under open field conditions, plants are exposed to direct excess UV radiation, which can affect plant vitality and defence responses. Exposure to UV triggers alterations in fundamental cellular processes including production of ROS, DNA repair mechanisms and by causing damage to cellular structures (Jenkins 2009; Hideg et al. 2013; Li et al. 2013). Plants use their natural defence systems comprising of various antioxidant proteins and peptides, and osmoprotectants, to counteract the negative impact of ROS as a result of abiotic stresses (Zulfiqar and Ashraf 2021; Zulfiqar et al. 2020). Studies have demonstrated that H_2 application can induce tolerance to UV stress through modulation in the antioxidant defence system in plants (Xie et al. 2015; Zhang et al. 2015). HRW has been shown to confer tolerance to UVB-induced oxidative damage partially through the manipulation of (iso) flavonoid metabolism in *Medicago sativa* L. (Xie et al. 2015). Whilst studying the impact of light stress on maize seedlings, it was observed that HRW treated plants showed great tolerance to photo-oxidation by maintaining high levels of antioxidant activities, including SOD, CAT, APX, and GR (Zhang et al. 2015).

HRW significantly blocked UVA-induced accumulation of H_2O_2 and $O_2^{\bullet-}$ and increased anthocyanin production (Su et al. 2014). Interestingly, it has been demonstrated that incremental inositol 1,4,5-trisphosphate/calcium ($InsP_3$-dependent cytosolic Ca^{2+}) contributes to H_2-promoted anthocyanin biosynthesis under UVA irradiation in radish sprouts (Zhang et al. 2018).

Genetic approach

Modern breeding tools can enhance the development of new and refined cultivars which demonstrate higher tolerance toward environmental stresses, thereby achieving productivity goals within the agricultural and horticultural food industries. Classic breeding programs are time consuming and laborious, with very little success in developing desired traits in the intended crop, however with the advancement in molecular biology technology and improved techniques, promising opportunities are on the way to develop new cultivars inserted with the specific genes including osmolyte genes for abiotic stress resistance (Zulfiqar et al. 2020). Regarding H_2, genetic studies have revealed introgression of H_2-related genes into plants can enhance abiotic stress tolerance. For instance, the physiological roles of H_2 biosynthesis via expressing hydrogenase1 gene (*CrHYD1)* in *A. thaliana* demonstrates a contributing link among osmotic tolerance, endogenous H_2 level, and *CrHYD1* expression in transgenic lines (Zhang et al. 2020). However, introgression of *CrHYD1* from *Chlamydomonas reinhardtii* enhanced the salinity tolerance through enhancing endogenous production of H_2 and melatonin, depicting the crucial role of H_2 in response to salinity (Su et al. 2020). Results from such studies strongly suggest that future research should also focus on H_2-based genetic improvements of crops to further understand the role of H_2 in enhancing the stress tolerance in plants.

Possible mechanisms behind H_2 responses

The evidence from numerous studies, as discussed above, suggests that H_2 has a profound, and positive effect on the growth of plants, and their tolerance to stress; be that heavy metals (Cui et al. 2014; Dai et al. 2017; Wang et al. 2019), or salinity (Wang et al. 2012; Wu et al. 2020c; Xu et al. 2013). However, the molecular basis of these effects is far from certain. It is unlikely that H_2 is perceived by a classical receptor-type mechanism, as it is so small. It is also relatively inert, so reactions with thiol groups, as seen with other reactive biological gases including H_2O_2 (Ulrich et al. 2019), H_2S (Kumar et al. 2021) and NO (Gupta et al. 2020), are unlikely.

Many empirical investigations (Xu et al. 2013; Ren et al. 2017; Chen et al. 2017b) report beneficial effects of H_2 on antioxidant levels in cells, but how this is brought about is also unclear. It has been reported that H_2 can scavenge both hydroxyl radicals ($^{\bullet}OH$) and peroxynitrite ($ONOO^-$) (Ohsawa et al. 2007), although this has been brought into doubt when the reaction kinetics of H_2 and these distinct molecules were considered (Penders et al. 2014). It appears, therefore, that the direct scavenging of such reactive signaling molecules by H_2 can be ruled out. Hancock et al. (2021) have suggested that as the mid-point potential of the H_2 couple is -414 mV (relative to a Standard Hydrogen Electrode (SHE)), that iron ions, specifically Fe^{3+}, may be the target. If this is the case, many significant and relevant heme prosthetic groups could be targets, including those in mitochondria, chloroplasts and the respiratory burst oxidase homologues (RBOHs) (Hancock et al. 2021). This would lead to the reduction of Fe^{3+} to Fe^{2+}, altering the activity of such heme-based enzymes, potentially account for some of the effects seen. Furthermore, Fe^{3+}

has a role in hydroxyl radical formation (Fong et al. 1976) whilst Fe^{2+} has been shown to have pro-oxidant activity (Mozuraityte et al. 2006). The molecular basis of H_2 action in cells has recently been reviewed (Hancock and Russell 2021; Hancock et al. 2021).

Due to the physical characteristics of molecular hydrogen, an alternative mechanism of action may be through the spin states of H_2. The H_2 molecule can exist in two states, that is, para and ortho spin states (Shagam et al. 2015). It has been of the posited (Hancock and Hancock 2019) that this may allow an interaction with other signaling molecules in cells, such as NO. However, to date, there is no evidence that this is the case and is another aspect of hydrogen chemistry that is worthy of further investigation.

Finally, another mechanism of H_2 action may be through the alteration of the expression and/or activity of the enzyme haem oxygenase (HO-1). This enzyme aids in catabolism of haem (Wilks 2002). H_2 is reported to enhance the abiotic stress tolerance and adventitious root development via the modulation of HO-1 gene expression, a Nrf-2 regulated gene, that has key role in preventing both hypoxia and vascular inflammation through enhancing antioxidant, antiapoptotic, antiproliferative and immunomodulatory pathways in animals, and is likely to have a similar effect in plants (Kapitulnik et al. 2012). HO-1 may be part of vital signalling systems involved in the response to cellular stress in plants (Jin et al. 2013; Lin et al. 2014) and therefore its role in mediating H_2 effects would seem appropriate.

Although research findings are demonstrating the positive influence of H_2 usage in agriculture to manage the stress conditions, still much more work needs to be carried out as it is very unclear how H_2 actually works in the cell and what exactly the molecular targets are. This needs to be a focus for H_2-based research moving forward.

Conclusions and future perspectives

As a result of abiotic stresses, plants generally demonstrate retarded growth responses. Such stresses are only likely to be exacerbated by increased human activity and climate change. Therefore, mitigating treatments which are easy and safe to use would be of significance for future sustainable food security. H_2 application in the form of HRW has been suggested as such a beneficial remedy in agriculture. H_2 has been shown to mitigate against a range of abiotic stresses, including heavy metal tolerance (Dai et al. 2017; Wu et al. 2019; Wang et al. 2019; Dumanović et al. 2020; Fan et al. 2020; Cui et al. 2020), increased salinity (Wang et al. 2012; Xu et al. 2013; Wu et al. 2020c; Su et al. 2021) and UV exposure (Xie et al. 2015; Zhang et al. 2015; Su et al. 2014), as well as improving the post-harvest storage of flowers and fruits.

However, there are a few caveats which need to be the focus of future research. H_2 treatment is not always easy to use, with HRW being the most obvious and easiest, but the loss of H_2 from the liquid phase to the atmosphere will limit the longevity of such treatments and require regular administration. Using H_2 in the gaseous form is both dangerous (owing to flammability) and time limited as it would easily escape ground level atmospheres. Due to the modernity of molecular hydrogen research, most of the studies related to H_2 intervention for abiotic stress-induced negative impacts on crop plants are laboratory based and yield evaluations have not been completed, especially under field conditions where the severity of these stresses is more prevalent. Therefore the cost–benefit analysis of the viability of H_2 applications within industry, to both crop or produce, at pre-harvest and post-harvest stage respectively, should also be considered in future studies.

H_2 research for agriculture and horticulture is at its infancy stage and currently production costs are relatively high. For instance, green hydrogen production (by electrolysis) can range between $1.25 and $10.90 per Kg dependent on the generation method used (Calise et al. 2019). However, H_2 is likely to become cost effective as it is adopted by other industries, for example within the transport and energy sectors. Within agriculture and horticulture various possibilities could be evaluated to reduce labor cost for H_2 application. In this regard, H_2 application via irrigation systems such as drip irrigation could be an alternative to reduce labor costs and ensure uniform application. Therefore, the future progress in H_2-based mitigation of abiotic stresses and post-harvest benefits are totally reliant on the cost-effective technology of H_2 production and H_2 delivery on an agricultural scale, which is likely to be more attractive in the future.

In conclusion, H_2 treatments can be beneficial in many stages of agriculture, from seed germination (Xu et al. 2013), root growth (Lin et al. 2014; Zhu et al. 2016; Wu et al. 2020b), post-harvest storage (Jiang et al. 2021). Current and growing research literature supports the evidence that H_2 has positive effects on plants and appears to have no harmful biological effects. Future work on H_2 treatments and a comprehensive understanding of the molecular basis of how H_2 interacts in physiological systems are paramount and will require further research investment if this natural and effective compound is to be utilized to enhance food production on a commercial basis.

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Figure legends:

Figure 1. Abiotic Stresses - Highlights the potential causes of abiotic stress, with a focus on the particular vulnerability of commercial crops. Cellular effects of abiotic stress include elevation of ionic, osmotic and oxidative stress indicators that, if exposed for prolonged periods of time, can lead to reduced growth potential. H_2 Application and Stress Relief – Suggests possible application methods of molecular hydrogen and illustrates the positive cellular effects attributed to enhanced levels of cellular H_2, and how these can relate to increased growth potential.

Figure 2. Illustrates the known cellular effects of H_2 enrichment inferred from evidence delineated across both plants and animal species. From top left, running clockwise - The increase in activity of

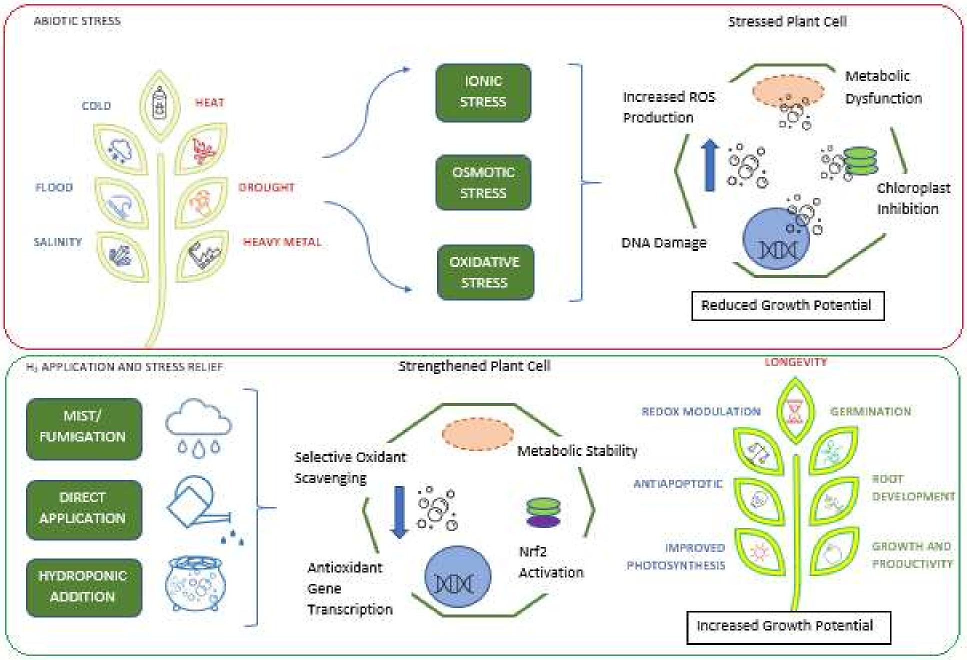
transcription factor (Nrf2) and downstream enzymes with anti-inflammatory properties (e.g. HO-1). Inhibition of multiple signalling pathways that activate the stress response (e.g. p38/MAPK) are detailed. The altered gene-expression profile described in the nucleus (Increased anti-oxidant profile, CAT, SOD e.g./Reduced pro-inflammatory profile NFkB e.g.). The reduction in oxidative degeneration to DNA and lipid membranes is also described. Furthermore, the reduced activity of caspase c, and resulting decline in apoptosis. Additionally, increased adenosine triphosphate (ATP) production and improved membrane potential of the mitochondria have been detailed. Finally, enhanced photosynthesis and energy metabolism is also shown. Thiobarbituric acid reactive substances (TBARS), MDA, lipoxygenases (LOX), ascorbic acid (AsA), gibberellic acid (GA_3), indole acetic acid (IAA), glutathione (GSH), Heme Oxygenase 1 (HO-1), hydroxyl group ($^{\cdot}OH$), peroxynitrite (ONOO-), (created in BioRender.com)

Figure 3. Demonstrates the possible methods of treating horticultural produce for enhancing post-harvest longevity.

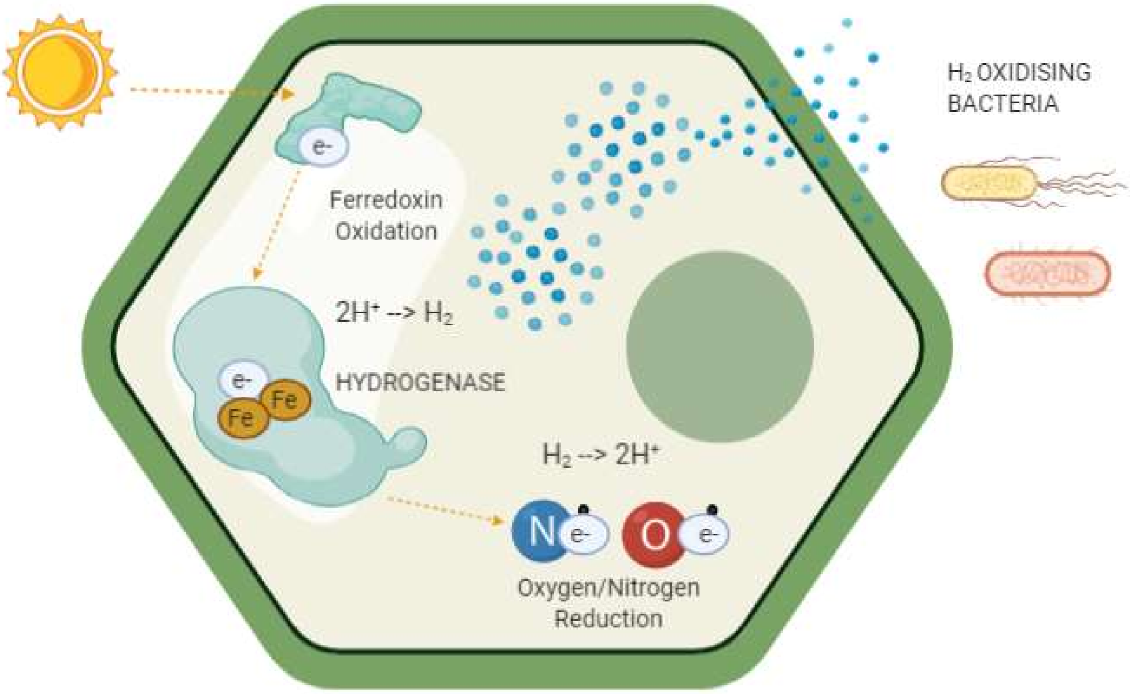
Figure 4. Depicts the possible mechanisms of H_2 during photosynthesis, describing the reduction of electrophilic ROS/RNS and the reversible activity of Fe-Fe hydrogenases. The cellular excess hydrogen produced in this manner is also hypothesised to enhance salubrious H_2-oxidising bacteria. (created in BioRender.com)

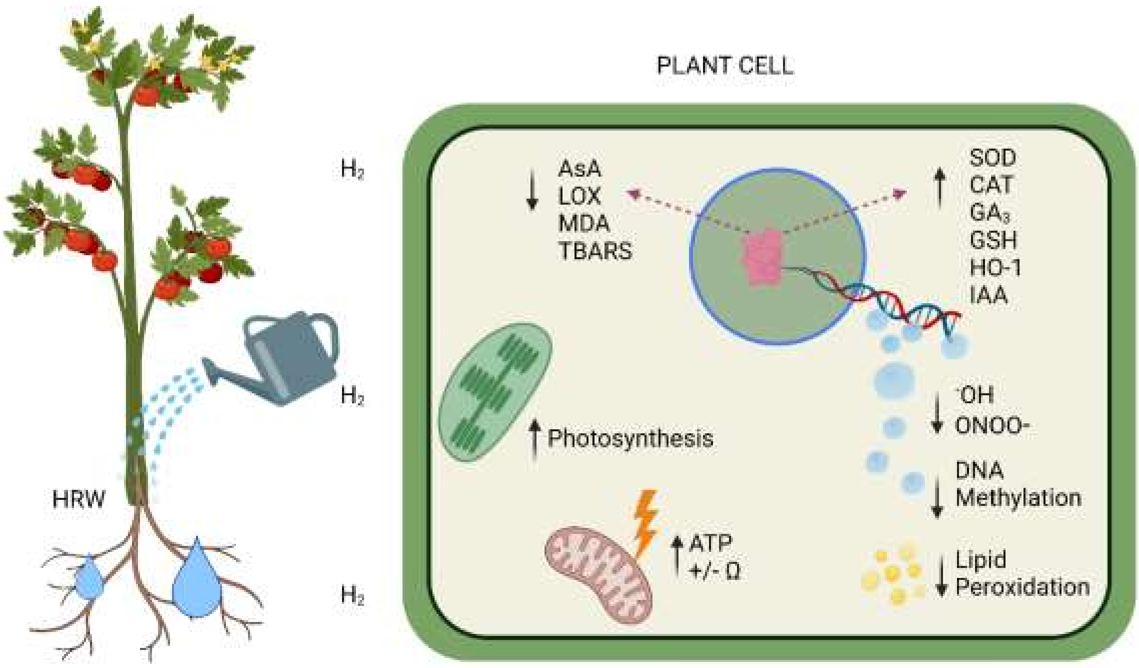
Table 1. Role of H_2 applied as HRW against different climate change induced abiotic stresse plants

Scientific name (plant)	Stress	Concentration used of H_2	Function	Refere
Alfalfa (*Medicago sativa*)	Mercury	1, 10, and 50% [v/v]	Decreased TBARS and oxidative stress; improved growth and biomass; modulated total and isozymatic antioxidant enzyme activities, re-established GSH and AsA homeostasis as well as genes relating to their expression	Cui et al.
Cucumber (*Cucumis sativus*)	Cadmium	50%	Recovered metal stress-induced decrease in adventitious roots growth; reduced oxidative stress; regulated ascorbate-glutathione cycle	Wang e (201
Alfalfa (*M. sativa*)	Cadmium	10%	Enhanced the level of genes related to sulfur metabolism; improved glutathione metabolism; alleviation of Cd toxicity, achieved through heightened ABC transporter-mediated secretion.	Cui et al.
Pak choi (*Brassica chinensis*)	Cadmium	50%	Decreased Cd uptake via inhibiting the expression of *BcIRT1* and *BcZIP2* genes related to metal transporters	Wu et al.
Barley (*Hordeum vulgare*)	Salt	830 μM	Alleviated detrimental impacts of salinity; maintained a higher rate of Na^+ extrusion mediated by SOS1-like Na^+/H^+ exchanger in the root epidermis; prevented NaCl-induced membrane depolarization and reduced sensitivity of K^+ efflux channels to ROS	Wu et al.
Arabidopsis (*Arabidopsis thaliana*)	Salt	10, 25, 50, and 75% [v/v]	Increased endogenous H_2 concentration; improved growth; modulated antioxidant defence enzymes; maintained ion homeostasis by regulating the antiporters and H^+ pump responsible for Na^+ exclusion	Xie et al.
Rice (*Oryza sativa*)	Salt	1, 5, 25, and 50 % [v/v]	Improved germination and seedling growth; activated α/β-amylase activity accelerating formation of reducing sugar and total soluble sugar. Enhanced total, isozymatic activities or corresponding transcripts of antioxidant enzymes, including superoxide dismutase, catalase, and ascorbate peroxidase.	Xu et al.
Alfalfa (*M. sativa*)	Drought	Irrigated with or without 50 % HRW	Exhibited more tolerance to drought stress via increased ABA activity.	Jin et al.
Arabidopsis (*A. thaliana*)	Drought	25%, 50%, and 75% [v/v]	Increased intracellular H_2 production and reduced stomatal aperture	Xie et al.
Cucumber (*C. Sativus*)	Drought	10, 30, and 50% [v/v]	Promoted development of adventitious roots; improved drought tolerance via enhancing endogenous antioxidant activities	Chen e (201


ABIOTIC STRESS
COLD
HEAT
FLOOD
DROUGHT
SALINITY
HEAVY METAL
IONIC STRESS
OSMOTIC STRESS
OXIDATIVE STRESS
Stressed Plant Cell
Increased ROS Production
Metabolic Dysfunction
Chloroplast Inhibition
DNA Damage
Reduced Growth Potential
H_2 APPLICATION AND STRESS RELIEF
MIST/ FUMIGATION
DIRECT APPLICATION
HYDROPONIC ADDITION
Strengthened Plant Cell
Selective Oxidant Scavenging
Metabolic Stability
Antioxidant Gene Transcription
Nrf2 Activation
LONGEVITY
REDOX MODULATION
GERMINATION
ANTIAPOPTOTIC
ROOT DEVELOPMENT
IMPROVED PHOTOSYNTHESIS
GROWTH AND PRODUCTIVITY
Increased Growth Potential

//1


Ferredoxin
Oxidation
$2H^+ \rightarrow H_2$
HYDROGENASE
e-
Fe
Fe
H_2 OXIDISING
BACTERIA
$H_2 \rightarrow 2H^+$
N
O
Oxygen/Nitrogen
Reduction


PLANT CELL
H_2
H_2
H_2
HRW
↓ AsA
LOX
MDA
TBARS
↑ SOD
CAT
GA_3
GSH
HO-1
IAA
↑ Photosynthesis
↓ ·OH
ONOO-
↓ DNA
Methylation
↑ ATP
+/- Ω
↓ Lipid
Peroxidation