



Review

Roles of Hydrogen Gas in Plants under Abiotic Stress: Current Knowledge and Perspectives

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Abstract: Hydrogen gas (H_2) is a unique molecular messenger, which is known to be involved in diverse physiological processes in plants, from seed germination to seedling growth to regulation of environmental stresses. In this review, we focus on the role of H_2 in plant responses to abiotic stresses, such as temperature, osmotic stress, light, paraquat (PQ)-induced oxidative stresses, and metal stresses. In general, H_2 can alleviate environmental stresses by improving the antioxidant defense system, photosynthetic capacity, re-establishing ion homeostasis and glutathione homeostasis, maintaining nutrient element homeostasis, mediating glucose metabolism and flavonoid pathways, regulating heme oxygenase-1 (HO-1) signaling, and interaction between H_2 and nitric oxide (NO), carbonic oxide (CO), or plant hormones. In addition, some genes modulated by H_2 under abiotic stresses are also discussed. Detailed evidence of molecular mechanisms for H_2 -mediated particular pathways under abiotic stress, however, is scarce. Further studies regarding the regulatory roles of H_2 in modulating abiotic stresses research should focus on the molecular details of the particular pathways that are activated in plants. More research work will improve knowledge concerning possible applications of hydrogen-rich water (HRW) to respond to abiotic stresses with the aim of enhancing crop quality and economic value.

Keywords: hydrogen gas; environmental stresses; regulatory pathways; metabolisms; genes



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1. Introduction

Hydrogen is the most abundant element on earth, comprising ~75% of the mass of the earth. Its molecular form, hydrogen gas (H_2), is a colorless, odorless and tasteless gas. Since H_2 was first purified by Robert Boyle in 1671, it has been known as a reducing gas [1]. The production of H_2 was first observed in bacteria [2] and then in green algae [3]. Sanadze [4] first found that the production of endogenous H_2 existed in higher plants. The release of endogenous H_2 has been widely found in higher plants since then [5,6]. It has been established that there are two pathways to produce H_2 in plant cells, via hydrogenases and nitrogenases [7–9]. Briefly, electrons are formed and transmitted in photosynthetic systems. Then, the electrons are activated, which reduces ferredoxin (Fd). Fd (red) can be reoxidized by hydrogenases to form H_2 . Moreover, rhizobia can release H_2 during the nitrogen fixation process (Figure 1; [7–9]). Additionally, environmental stress also promotes the production of endogenous H_2 in plants Figure 1; [10–12].

H_2 was reported as a therapeutic antioxidant and has become a hotspot in medical research since 2007. Researchers published more and more related papers in areas such as diabetes, organ ischemia-reperfusion injury, atherosclerosis, cancer, hypertension, and other major human diseases [13–16]. A review by Qian et al. [17] noted that H_2 was involved in treating some important diseases due to its antiapoptotic and anti-inflammatory effects, including nervous system disorders, liver diseases and metabolic diseases. In plants, H_2 acts as an important bio-regulator that modulates various physiological processes, including seed germination, seedling growth, adventitious rooting, root elongation, and post-harvesting. For example, Xu et al. [18] demonstrated that H_2 promoted remarkably

seed germination of rice (*Oryza sativa* L.) under salt stress. H₂ could increase the growth of seedlings of alfalfa (*Medicago sativa* L.), maize (*Zea mays* L.), and Chinese cabbage (*Brassica campestris* spp. *chinensis* L.) [19–21]. Adventitious root development in cucumber was improved significantly by H₂ [22,23]. H₂ significantly promoted the root length in cucumbers [18]. H₂ also significantly delayed the ripening process of kiwifruit (*Actinidia chinensis* cv. Huayo) [24].

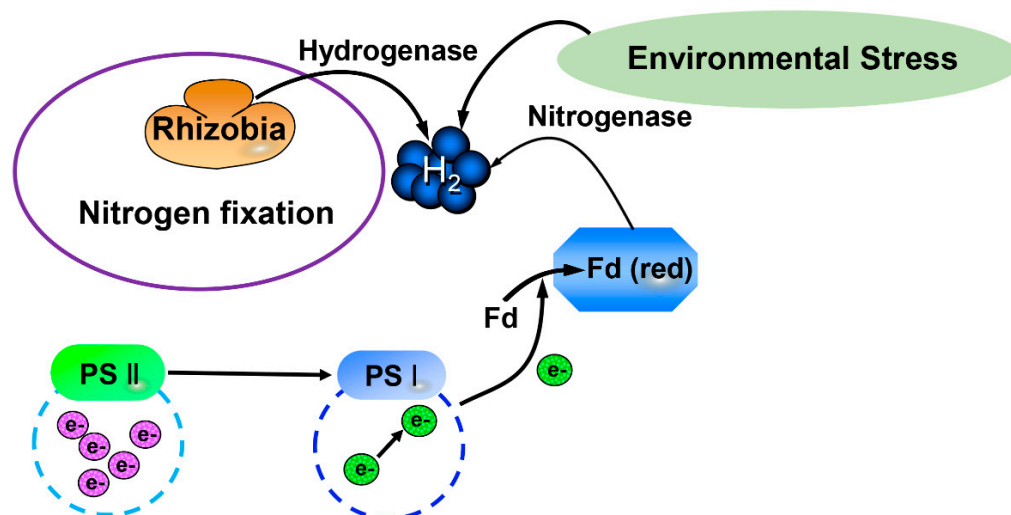


Figure 1. H₂ production pathway in organisms. H₂, Hydrogen gas; Fd, Ferredoxin; PS II, Photosynthetic system II; PS I, Photosynthetic system I.

In nature, plants are constantly challenged by a variety of environmental stresses [25,26]. In plants, numerous growth and development processes, such as root elongation, seedling growth and seed germination, are altered by environmental stresses. For instance, cadmium (Cd) stress significantly inhibited the root elongation and fresh weight of Pak Choi (*Brassica campestris* ssp. *Chinensis*) seedlings [10]. Zhao et al. [27] implied that the growth of maize seedlings was inhibited by aluminum (Al) stress. Oxidative stress usually appears when plants are subjected to various environmental stresses, indicated by an increase of reactive oxygen species (ROS) and responses by antioxidant systems. Chen et al. [28] reported that cucumber (*Cucumis sativus* L.) seedlings suffering from heat stress showed increased malondialdehyde (MDA) and hydrogen peroxide (H₂O₂) content and decreased activity of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POD) enzymes. Under cold stress, the levels of H₂O₂ and MDA in rice seedlings were remarkably elevated [29]. Al stress led to reductions in calcium (Ca), iron (Fe), and magnesium (Mg) uptake in both maize roots and shoots [27]. High light stress effectively blocked the photosynthetic capacity of maize seedlings [21]. Anthocyanin content of radish (*Raphanus sativus* L.) sprouts was decreased significantly by ultraviolet-A (UV-A) irritation [30]. Therefore, it is particularly important to explore the factors that improve the environmental stress-tolerance of plants. An increasing body of evidence suggests that phytohormones contribute to plant response to various environmental stresses. Nakashima et al. [31] summarized that abscisic acid (ABA) and ABA signaling factors may improve tolerance to environmental stresses. Li et al. [32] found that brassinolide enhanced cold stress tolerance of mango (*Mangifera indica* L.) through regulating plasma membrane proteins and lipids. The ethylene signaling pathway was also found to regulate salt stress response [33]. In addition, gas signaling molecules are involved in the regulation of abiotic stress. A review by Siddiqui et al. [34] showed that nitric oxide (NO) plays a vital role in resistance to salt, drought, temperature, ultraviolet-B (UV-B), and heavy metal stress. Hydrogen sulfide (H₂S) may improve plant tolerance to stresses [35]. Carbon monoxide (CO) was also reported to be involved in the regulation of various environmental stresses [36,37]. H₂ has emerged as an important antioxidant in plants that can respond to all kinds of environmental stresses

such as temperature, light, metals, osmotic, and paraquat (PQ) stresses [6,20,21,27–30]. For instance, H₂ could enhance cold tolerance in cucumber seedlings [29]. H₂ had a positive effect against high light-induced stress in maize seedlings [21]. Toxicity symptoms of Cd-induced in Chinese cabbage were alleviated by H₂ [20]. H₂ could enhance tolerance to osmotic stress in alfalfa [11]. The tolerance of PQ-induced oxidative stress was increased by H₂ in alfalfa seedlings [6]. Moreover, H₂ can crosstalk with other molecules to respond to various abiotic stresses. Interaction of H₂ and NO can enhance Al stress via alleviating Al toxicity symptoms in alfalfa [38]. Cross-talk between CO and H₂ could be involved in drought stress responses [39].

Considering the rapid increase of interest in the role of H₂ in plants' response to abiotic stress, this paper summarizes the information about H₂-mediated abiotic stress and regulatory pathways and mechanisms based on the most recent works in the literature. Additionally, we also briefly consider H₂-mediated related gene expression.

2. H₂ Confers Plant Tolerance to Environmental Stresses

Plants are invariably exposed to different environmental stresses in the process of growth and development, which has profound effects on crop production [40]. When plants are stressed, a variety of significant physiological changes will appear, such as reduced photosynthesis, ROS production, disturbed ion homeostasis of plant cells, and so on. Previous studies indicated that phytohormones and signaling molecules played an important role in plant resistance to environmental stress [31,33]. Nowadays, it has become increasingly evident that H₂ enhances plant resistance to environmental stimuli [19–21] (Table 1).

2.1. Temperature Stress

In general, temperature stress includes cold stress and heat stress [28,29] (Table 1). Purified H₂ (99.99%, *v/v*) was bubbled into 1000 mL Hoagland's solution (pH 5.87, 25 °C), which was used as 100% hydrogen-rich water (HRW). 50% HRW is diluted from 100% HRW. After the 3-week-old cucumber seedlings were pretreated with 50 and 100% HRW for 7 days, HRW pretreatment remarkably enhanced cucumber seedlings' heat-tolerance in comparison with heat stress treatment alone when cucumber seedlings were subjected to heat stress for 3 days (42/38 °C), and 50% HRW most effectively mediated heat-tolerance [28] (Table 1). The authors further proved that HRW pretreatment could enhance heat stress tolerance by improving photosynthetic capacity, antioxidant response, and the accumulation of HSP70 and osmolytes in cucumber seedlings [28]. Xu et al. [29] found that with 0.39 mM H₂ pretreatment, rice seedlings exhibited the maximum prevention of cold-induced growth inhibition and improved cold tolerance (0 °C). They also suggested that endogenous H₂ might contribute to the enhancement of cold tolerance by increasing photosynthetic capacity, improving antioxidant response, and reestablishing redox homeostasis mediated by miR398 and miR319 expression [29] (Table 1). Along with these studies, we suggest that H₂ might contribute to the enhancement of temperature tolerance in plants through improving photosynthetic capacity, increasing antioxidant response, promoting the accumulation of HSP70 and osmolytes, and regulating RNA expression that is required for redox homeostasis. As there are few studies about the roles of H₂ in developing temperature stress tolerance, more work is required on this topic.

2.2. Light Stress

The quality and amount of light that plants receive strongly influence their growth and development, but when more light is absorbed than is required for photosynthesis, the excess light can cause stress that can be harmful due to the production of ROS [41]. High light stress may result in stunted growth of maize seedlings, while exogenous H₂ supplementation could alleviate the phenomenon [21] (Table 1). These results confirmed that H₂ could alleviate high light stress in maize by decreasing the susceptibility of the PSII (Φ PSII) to photoinhibition and increasing antioxidant response [21]. As one of the main compo-

nents of light, UV radiation has been recognized as causing light stress on plant growth and development. In general, UV radiation may increase DNA damage, stimulate antioxidant response, induce flavonoid production, and alter the expression of defense-related genes. Su et al. [30] reported that H₂ significantly blocked the UV-A-induced antioxidant response of radish. Furthermore, H₂ markedly enhanced UV-A-induced increase of anthocyanin and total phenols contents in radish (high level of anthocyanin), implying that H₂ could alleviate UV-A radiation by reestablishing reactive oxygen species homeostasis and anthocyanin accumulation [30] (Table 1). The exposure of alfalfa seedlings to UV-B irradiation significantly increased endogenous H₂ content, suggesting that H₂ may play an important role in the response to UV-B irradiation [42] (Table 1). The authors suggest that H₂ conferred tolerance to UV-B irradiation via the manipulation of antioxidant defense and (iso)flavonoids metabolism in alfalfa [42]. Combined with the above results, we suggest that H₂ will have practical applications for enhancing the temperature tolerance of plants by improving photosynthesis, antioxidant response, and anthocyanin accumulation. These results will open a new window for increasing plant tolerance to light stress. However, whether there are other pathways that help alleviate light stress in response to H₂ still needs further investigation.

Table 1. Overview of the abiotic stresses alleviated by H₂ and regulatory pathways involved in the process in plants.

Stresses	Plants	Regulatory Pathways	References
Cold	Rice	ROS and antioxidant defense system	[29]
High light	Maize	Photosynthetic capacity	[21]
UV-A	Radish	ROS and antioxidant defense system	[30]
UV-B	Alfalfa	Flavonoid pathway	[42]
Hg	Alfalfa	Flavonoid pathway	[42]
		ROS and antioxidant defense system	
		Re-establishing ion homeostasis	[43]
		Re-establishing redox homeostasis	
Cd/Salt	<i>Hypsizygus marmoreus</i>	ROS and antioxidant defense system	[44]
		Glucose metabolism	
Cd	Chinese cabbage	ROS and antioxidant defense system	[20]
Cd	Alfalfa	ROS and antioxidant defense system	[45]
		Re-establishing redox homeostasis	
		ROS and antioxidant defense system	
Al	Maize	Photosynthetic capacity	[27]
		Maintaining nutrient element homeostasis	
	Alfalfa	NO production	[38]
		ROS and antioxidant defense system	
Cd	Alfalfa	Re-establishing ion homeostasis	[19]
		Maintaining nutrient element homeostasis	
	Pak Choi	Re-establishing ion homeostasis	[10]
Osmotic	Alfalfa	NO Signaling	[46]
		ROS and antioxidant defense system	
	Alfalfa	HO-1 signaling	[11]
Drought	Alfalfa	ABA	[47]
	Cucumber	CO signaling	[39]
		ROS and antioxidant defense system	
Salt	Arabidopsis	Re-establishing ion homeostasis	[5]
		ROS and antioxidant defense system	
	Rice	Glucose metabolism	[18]
		Re-establishing ion homeostasis	
Cd/Salt	Alfalfa	ABA, ethylene, or jasmonate acid	[12]
PQ	Alfalfa	ROS and antioxidant defense system	[6]
		HO-1 Signaling	

Notes: ROS, Reactive oxygen species; UV-A, Ultraviolet-A; UV-B, Ultraviolet-B; Hg, Mercury; Cd, Cadmium; NO, Nitric oxide; HO-1, Heme oxygenase-1; ABA, Abscisic acid; CO, Carbon monoxide; PQ, Paraquat.

2.3. Metals Stress

Metals such as mercury (Hg), Cd, and Al have become major environmental contaminants that restrict plant growth and development. Cui et al. [43] indicated that the growth of alfalfa seedlings was markedly inhibited when exposed to mercury chloride (HgCl₂), but the effect could be blocked by HRW. They also found that H₂ attenuated Hg toxicity via reducing Hg accumulation, improving the antioxidant defense system, and reestablishing redox homeostasis [43] (Table 1). In *Hypsizygus marmoreus*, 0.8 mM H₂ could remarkably reduce Cd toxicity, leading to a significant improvement of mycelial growth and biomass [44] (Table 1). These findings also suggested that H₂ could be an effective approach

for Cd detoxification via enhancing antioxidant response and glucose metabolism [44]. Cd toxicity caused root elongation and seedling growth inhibition in Chinese cabbage, but the addition of 50% saturation HRW significantly alleviated the symptoms that were reported by Wu et al. [20]. Their results also suggested that the improvement of Cd tolerance by HRW was closely associated with reduced Cd uptake and increased antioxidant defense capacities [20] (Table 1). Likewise, root elongation and seedling growth of alfalfa were inhibited by Cd stress, but the appearance of Cd toxicity symptoms was significantly alleviated when 10% HRW was added [45] (Table 1). These findings indicated that H₂ could improve Cd tolerance, which was consistent with reestablished glutathione homeostasis and a lesser quantity of accumulated Cd [45]. Al stress inhibited the growth of maize seedlings [27] (Table 1). However, 75% HRW markedly promoted root elongation of maize, thereby alleviating Al toxicity [27]. Zhao et al. [27] also concluded that H₂ could enhance Al tolerance by improving photosynthesis, reestablishing redox homeostasis, and maintaining nutrient homeostasis. Similarly, Chen et al. [38] indicated that H₂ can alleviate Al-induced alfalfa seedling growth inhibition. However, the difference was that H₂ improved Al tolerance by reducing NO production and decreasing Al uptake [38] (Table 1). Application of H₂ could alleviate Cd-induced growth inhibition of alfalfa seedlings [19] (Table 1). Wu et al. [10] showed that Pak Choi exposure to Cd resulted in a rapid increase in endogenous H₂ production, and exogenous application of HRW reduced a Cd stress-induced phenotype. Their results further indicated that H₂ operated upstream of the IRT1 transporter and regulated root Cd uptake by controlling plasma membrane-based nicotinamide adenine dinucleotide phosphate (NADPH) oxidase encoded by the *RbohD* gene [10] (Table 1). As mentioned above, H₂ alleviates metal toxicity mainly by reducing metal accumulation, improving the antioxidant defense system and glucose metabolism, reestablishing redox homeostasis, increasing photosynthesis, maintaining nutrient homeostasis, and reducing NO production. There is a real need for continued research in this area in order to reveal the underlying regulatory mechanisms of H₂ on metal stresses because these stresses impose considerable constraints on plant growth and development and crop production.

2.4. Osmotic Stress

Osmotic stress is one of the most significant abiotic stresses including the effects of drought and salinity, and affects virtually every aspect of plant physiology and metabolism. Su et al. [46] found that osmotic tolerance of alfalfa seedlings was closely linked with H₂ (Table 1). Under polyethylene glycol (PEG)-induced osmotic stress, H₂ alleviated root inhibition by decreasing lipid peroxidation, and H₂O₂ and heme oxygenase-1 (HO-1) were involved in H₂-induced osmotic stress [11] (Table 1). H₂-mediated enhancement of alfalfa seedlings' tolerance to drought stress was also suggested by Jin et al. [47] (Table 1). These responses were supported by Chen et al. [39] (Table 1). H₂ also could alleviate salt-induced growth inhibition in *Arabidopsis* (*Arabidopsis thaliana* L.) [5] (Table 1). These findings further indicated that H₂ improved the salt tolerance of *Arabidopsis* by modulating genes/proteins of zinc-finger transcription factor ZAT10/12, antioxidant defense, and maintenance of ion homeostasis [5]. Similarly, salt stress caused endogenous H₂ production in germinating rice seeds, and H₂ pretreatment more or less attenuated salt-induced inhibition of seed germination and seedling growth [18] (Table 1). The authors also suggested that H₂ alleviated salt stress in rice, which was consistent with sugar metabolism, the ratio of potassium (K) to sodium (Na), and antioxidant capability [18]. Under salt stress, H₂ improved salt tolerance in *Hypsizygus marmoratus* by increasing glucose metabolism and antioxidant response [44] (Table 1). Zeng et al. [12] also found that endogenous H₂ production in rice was induced under salt and drought stresses. They suggested that H₂ alleviated salt and drought stress in rice by modulating the output of hormone signaling pathways [12] (Table 1). In conclusion, H₂ could enhance the tolerance against osmotic stress by maintaining ion homeostasis, increasing antioxidant capacity, and regulating sugar metabolism.

2.5. PQ-Induced Oxidative Stress

Like many environmental stresses, PQ (a methyl viologen family of herbicide widely used to mimic oxidative stress) subjects plants to oxidative damage, leading to excessive ROS production [48]. When alfalfa seedlings were exposed to PQ stress, endogenous H₂O₂ production and lipid peroxidation were increased [6] (Table 1). The activities of antioxidant enzymes, the level of MsHO-1 transcript and HO-1 activity were increased after exposure to H₂ [6]. These results indicated that H₂ might alleviate PQ stress through the antioxidant system and HO-1 signaling [6]. However, only this report has been published to support the suggestion that H₂ could alleviate PQ stress in plants. Whether there are more pathways by which H₂ might alleviate the PQ stress still needs further investigation.

3. The Regulatory Pathways Abiotic Stress Alleviated by H₂ in Plants

3.1. ROS and Antioxidant Defense System

Environmental stress usually triggers ROS production in the chloroplasts, mitochondria, peroxisomes, plasma membranes, endoplasmic reticulum (ER), and the cell wall, and the accumulation of ROS can lead to lipid peroxidation, disruption of the cell membrane, and inhibition of plant growth [48,49]. The ROS mainly comprise oxygen (O₂), H₂O₂, superoxide anion (O₂^{•−}), and hydroxyl radical (OH^{•−}). H₂O₂ and O₂^{•−} are produced in plant cells under abiotic stresses, caused by factors like salt, heat, light, metal stresses and PQ-induced oxidative stress [25,50]. H₂O₂ and O₂^{•−} behave like double-edged swords in plant cells; they are beneficial at low concentrations, but damaging at higher concentrations. For example, PQ-induced oxidative stress in alfalfa seedlings resulted in an increase in H₂O₂ and O₂^{•−} [6]. UV-A induced significantly increased H₂O₂ and O₂^{•−} levels in radish [30]. To avoid ROS-induced cellular injury, plants utilize their antioxidant systems, notably SOD, CAT, APX, and monodehydroascorbate reductase (MDHAR), to scavenge the ROS [48,49]. SOD, CAT, and APX activities were increased by H₂ in response to salt stress in rice ([18], Table 1, Figure 2A). Lipids form a major portion of the plasma membrane which envelops the cell and helps it to adapt to the changing environment [51]. Sodium chloride (NaCl) induced ROS overproduction in rice, thereby leading to lipid peroxidation [18]. Salt-induced oxidative stress was alleviated by H₂ by increasing APX activity and decreasing thiobarbituric acid reactive substances (TBARS) content ([5], Table 1, Figure 1A). H₂ could enhance the heat-tolerance of cucumber seedlings via increasing SOD, POD, CAT, and APX activities, which reduced the increased MDA and H₂O₂ content [28] (Table 1, Figure 2A). The elevated H₂O₂ and MDA contents under cold stress were also decreased by H₂ via significant increases in SOD, POD, and CAT activities [29] (Table 1, Figure 2A). Zhang et al. [28] also found that heat stress resulted in the increase of O₂^{•−} and H₂O₂ contents. When H₂ was added, the enhancement of SOD, CAT, APX and glutathione reductase (GR) activities decreased O₂^{•−} and H₂O₂ contents, thereby improving the high light-tolerance of maize seedlings (Table 1, Figure 2A). HgCl₂ triggered the increase of ROS, which lead to lipid peroxidation in alfalfa, but the adverse effect was reversed by H₂ by enhancing the activities of SOD, POD and APX enzymes [43] (Table 1, Figure 2A). H₂ enhanced tolerance of PQ-induced oxidative stress in alfalfa seedlings through increasing the activities of SOD, CAT, POD and APX [6]. Under Al stress, TBARS, O₂^{•−} and H₂O₂ levels increased significantly in maize leaves and roots, whereas the increase was suppressed by H₂ [27] (Table 1, Figure 2A). Simultaneously, H₂ also played a promoting role in the Al stress-inhibited the activities of POD, SOD, APX and CAT [27]. H₂ increased significantly the activities of SOD, CAT and GR enzymes, thus decreasing remarkably the levels of O₂^{•−} and H₂O₂ in *Hypsizygus marmoreus*' response to Gd and salt stresses [44] (Table 1, Figure 2A). Su et al. [30] reported that H₂ remarkably blocked UV-A-induced increase of O₂^{•−} and H₂O₂ levels though enhancing UV-A-induced decrease of SOD and APX activities (Table 1, Figure 2A). Wu et al. [20] found that H₂ could improve the tolerance of Cd via enhancing the activities of SOD, POD, CAT and APX and decreasing TBARS content and ROS production in Chinese cabbage (Table 1, Figure 2A). H₂ prevented ROS accumulation and lipid peroxidation against Cd stress, followed by the increase of SOD, POD, APX and

GPX activities [19] (Table 1, Figure 2A). H₂ could alleviate Cd stress, as indicated by the decrease of TBARS and ROS production [20] (Table 1, Figure 2A). Under PEG-induced osmotic stress, the lipid peroxidation was decreased when H₂ was administrated [11] (Table 1, Figure 2A).

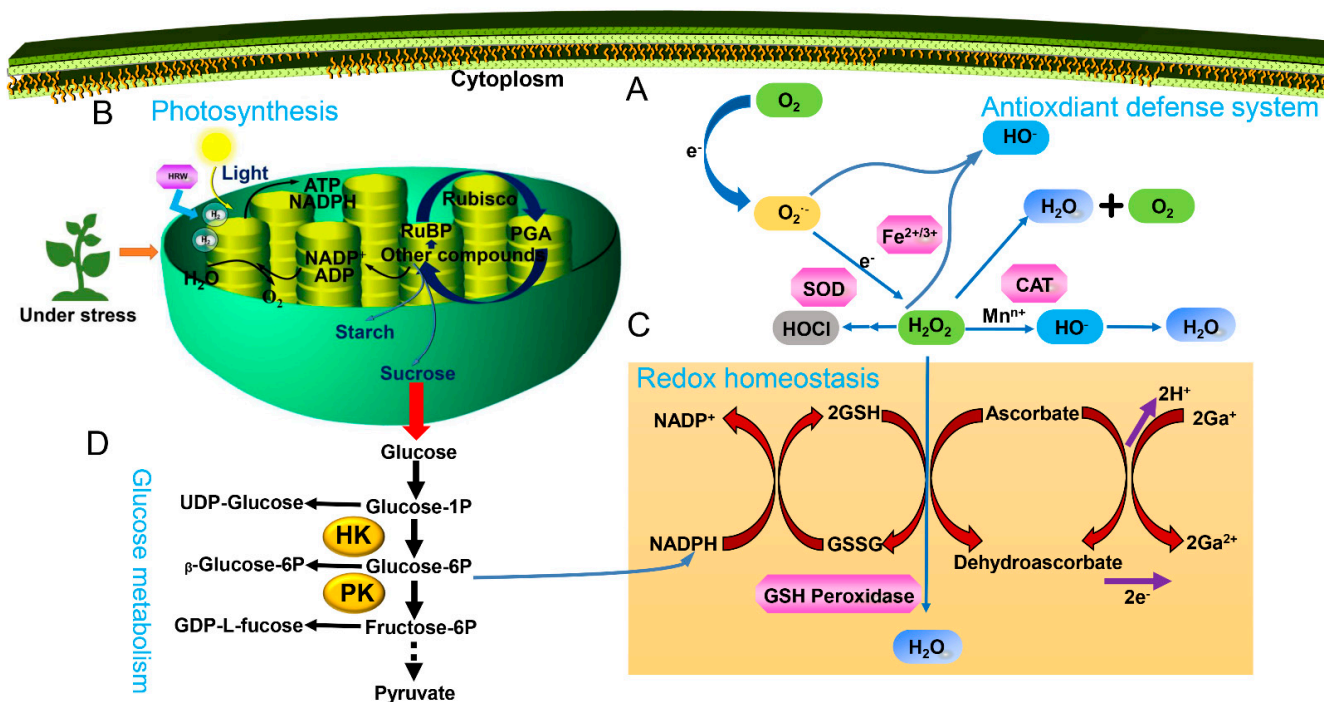


Figure 2. The regulatory mechanism of H₂-mediated abiotic stress. (A), Antioxidant defense system. H₂ could regulate the antioxidant system by increasing the activities of antioxidant-related enzymes and decreasing ROS accumulation and lipid peroxidation to relieve environmental stresses in plants. (B), Photosynthesis, H₂ could relieve abiotic stresses due to photosynthesis. (C), Redox homeostasis. H₂ could also regulate abiotic stresses via re-establishing redox homeostasis. (D), Glucose metabolism. Glucose metabolism is also involved in the regulation of abiotic stresses. H₂O₂, Hydrogen peroxide; O₂⁻, Superoxide anion; O₂, Oxygen; Water, H₂O; SOD, Superoxide dismutase; CAT, Catalase; NADPH, Nicotinamide adenine dinucleotide phosphate; ATP, Adenosine triphosphate; RuBP, Ribulose 1,5-bisphosphate; PGA, 3-phosphoglyceric acid; ADP, Adenosine diphosphate; NADP⁺, Nicotinamide-adenine dinucleotide phosphate; Glucose-1P, Glucose-1-Phosphate; Glucose-6P, Glucose-6-Phosphate; Fructose-6P, Glucose-6-Phosphate; HK, Hexokinase; PK, Pyruvate kinase; GSH, Glutathione; GSSG, Oxidized glutathione.

The plasma membrane which surrounds the entire plant cell plays an important role in interacting with the ever-changing environmental conditions and provides information necessary for the continual survival of the cell [51]. The root activity and plasma membrane integrity were maintained by H₂ under Al stress [27]. Plasma membrane integrity was maintained by H₂ via the increase of lipoxygenase (LOC) activity against Cd stress in alfalfa [19]. These results implied that H₂ could alleviate various environmental stresses via ROS and antioxidant defense systems. However, the molecular mechanisms regulated by H₂ during this process are still unknown. ROS functions, individual signaling pathways, and critical ROS concentrations regulated by H₂ against abiotic stress are unclear. Further studies are needed regarding the molecular mechanism of H₂ in regulating the ROS and antioxidant defense system and ROS functions, individual signaling pathways, and critical ROS concentration regulated by H₂ under abiotic stresses.

3.2. Photosynthetic Capacity

H₂ could improve the heat-tolerance of cucumber seedlings by mitigating the reduction of heat-induced photosynthetic rate (Pn), stomatal conductance (gs), mesophyll conductance (Gm) and water use efficiency (WUE) and the increase of heat-induced intercellular CO₂ concentration (Ci) and transpiration rate (E) [28]. Simultaneously, H₂ also significantly increased PSII maximal photochemistry efficiency (Fv/Fm), effective quantum yield of PSII, electron transport rate (ETR), the excitation capture efficiency of open centers (Fv'/Fm') and photochemical quenching (qP) during this process [28]. The light absorbed in PSII antennae can be divided into three parts: (1) energy utilized in photosynthesis (P), (2) energy dissipated by photo-protective mechanisms (D) and (3) the excess light energy that is neither utilized nor dissipated (E) [52]. Among them, P reflects the photosynthetic capacity of the entire PSII. Chen et al. [28] also reported that heat induced decreased P and increased D and E, but that H₂ reversed the effects of heat, suggesting that H₂ could improve the heat-tolerance of cucumber seedlings by enhancing photosynthetic capacity (Table 1, Figure 2B). Under cold stress, chlorophyll a, b, and total chlorophyll contents of rice seedlings were significantly reduced, which were reversed by H₂ [29] (Table 1, Figure 2B). Similarly, H₂ also blocked cold stress-induced reduction of Pn and gs and elevation of intercellular CO₂ concentration (Ci) [29]. H₂ could increase significantly and bring rapid recovery of the PSII maximal Fv/Fm and PSII performance index on absorption base (PI-ABS) response to high light stress which indicated that H₂ could alleviate effectively high light stress-induced damage to PSII [21]. The authors also found that H₂ could remarkably reduce the high light-induced damage to the acceptor side, reaction centre, as well as donor side of the photosystem II [21]. H₂ ameliorated Al stress-inhibited total chlorophyll content, net photosynthesis rate, stomatal conductance and phosphoenolpyruvate carboxylase (PEPC) of maize leaves [27] (Table 1, Figure 2B). Electron transport flux per excited cross section (ETo/CSO) and the number of active PSII reaction centers per excited cross section (RC/CSO) are important here. The decrease of Fv/Fm, PIABS, ETo/CSO, RC/CSO and (trapped energy flux per excited cross section) Tro/Cso caused by Al stress was suppressed when HRW was added to the Al treatment [27]. However, the molecular mechanism of the H₂-mediated photosynthetic system response to abiotic stress is unclear.

3.3. Re-Establishing Redox Homeostasis

Under Hg stress, H₂ not only differentially strengthened the increased tendencies in the contents of GSH (in particular), GSSG, hGSSGh, and AsA, but also blocked the hGSH and DHA levels [43] (Table 1, Figure 2C). High ratios of GSH/GSSG, hGSH/hGSSGh, and AsA/DHA were exhibited in HRW pretreatment compared with Hg treatment [43]. These data imply that H₂ was able to alleviate Hg toxicity in alfalfa seedlings via reestablishing glutathione homeostasis [43]. Similarly, H₂ significantly eliminated the Cd-induced decrease of GSH content and increase of GSSG content [45] (Table 1, Figure 2C). Under Cd stress, a higher ratio of GSH/GSSG responsive for intracellular redox status was exhibited in the HRW treatment [43]. Research showing that H₂ alleviated abiotic stress via reestablishing redox homeostasis is scarce.

3.4. Glucose Metabolism

H₂ could alleviate salt stress in rice seeds by activating α/β -amylase activity and accelerating the formation of reducing sugar and total soluble sugar [18]. Under Cd and salt stresses, H₂ enhanced PK activity, suggesting that H₂-mediated glucose metabolism regulated Cd and salt stresses [44] (Table 1, Figure 2D). Whether H₂ mediated glucose metabolism during other stresses is unclear.

3.5. Re-Establishing Ion Homeostasis

The ratio of potassium (K) to sodium (Na) of shoot and root parts in rice was increased by H₂ in response to salt stress [18] (Table 1). Under salt stress, H₂ regulated the antiporters and H⁺ pump responsible for Na⁺ exclusion (in particular) and compartmentation that

maintained ion homeostasis in *Arabidopsis* [5] (Table 1). Hg-induced increase in relative ion leakage was significantly reversed by H₂ [43] (Table 1). H₂ reduced Cd uptake of Pak Choi via Ca²⁺ transport across the plasma membrane and apoplastic H₂O₂ generation by BcRbohD under Cd stress [10] (Table 1). Metal ion homeostasis was associated with Cd resistance conferred by H₂ [19] (Table 1). Therefore, in the future, more detailed study is needed of the regulatory mechanisms by which H₂ alleviated stress through re-establishing ion homeostasis.

3.6. Maintaining Nutrient Element Homeostasis

Al stress led to reductions in Ca, Fe, and Mg uptake in both maize roots and shoots, while H₂ significantly improved the uptake of P and Fe in roots and Ca, P, Fe, and Mg in shoots, implying that H₂ could alleviate Al stress via maintaining nutrient element homeostasis [27] (Table 1). Under Cd stress, nutrient element homeostasis was closely associated with H₂-improved Cd tolerance of alfalfa [19] (Table 1). Studies on the abiotic stresses alleviated by H₂ via maintaining nutrient element homeostasis are scarce, however. Further research is required into the abiotic stresses alleviated by H₂ and the regulatory mechanisms for maintaining nutrient element homeostasis.

3.7. Flavonoid Pathway

Flavonoids are recognized as compounds with potential health benefits due their valuable nutritional antioxidant activities and are typical substances that plant produce in response to environmental stresses. Anthocyanin is a flavonoid, and its biosynthesis pathway is well described in plants. It is derived from phenylalanine, catalyzed by phenylalanine ammonialyase (PAL). Then it is mediated by a common step with chalcone synthase (CHS), chalcone isomerase (CHI), and flavanone 3-hydroxylase (F3H) and fluxed into anthocyanin biosynthesis by dihydroflavonol 4-reductase (DFR) and anthocyanidin synthase (ANS). Su et al. [30] showed that H₂ could up-regulate UV-A-induced anthocyanin biosynthesis-related genes by molecular analyses, as cyanidin content was enhanced significantly. They also implied that H₂ could alleviate UV-A irradiation by the anthocyanin accumulation pathway [30] (Table 1, Figure 3). Xie et al. [42] identified 40 (iso)flavonoids under UV-B irradiation by using ultra performance liquid chromatography-mass spectrometric (UPLC-MS), and H₂ remarkably increased 22 of them including afromosin, afromosin 7-O-β-D-glucoside-malonate, daidzein, formononetin 7-O-β-D-glucoside-6''-O-malonate, garbanzol, matteucin, naringenin and so on. H₂ significantly up-regulated UV-B-induced upregulation in the expression levels of (iso)flavonoids biosynthetic-related genes, suggesting that H₂ conferred tolerance to UVB irradiation by regulating the (iso)flavonoids metabolism pathway [42] (Table 1, Figure 3). Information is lacking on whether flavonoids are involved in the H₂-mediation of other stress in plants.

3.8. HO-1 Signaling

H₂ or the heme oxygenase-1 (HO-1) inducer hemin could reduce lipid peroxidation, O₂⁻ and H₂O₂ levels which enhanced tolerance of alfalfa seedlings to oxidative stress induced by PQ [6] (Table 1, Figure 4). Further results indicated that H₂ dramatically regulated the gene expression of *MshO-1*, corresponding protein expression and HO-1 activity, suggesting that H₂ improved the PQ-induced oxidative stress tolerance of alfalfa seedlings by regulating HO-1 signaling [6]. Under PEG-induced osmotic stress, exogenously applied H₂O₂ could mimic the protective effect of H₂ on alfalfa seedlings [11] (Table 1, Figure 4). H₂- and H₂O₂-induced activities of SOD, POD and APX enzymes could be inhibited by HO-1 inhibitor ZnPP, suggesting that H₂O₂ might be involved in H₂-mediated osmotic stress via HO-1 signaling [47] (Table 1, Figure 4). However, the exact mechanisms by which this process occurs are not yet known.

3.9. NO, CO, and Plant Hormones

Chen et al. [38] reported that Al-induced inhibition of alfalfa root growth was alleviated when NO was removed by a NO scavenger followed by decreasing NO production, suggesting that H₂ alleviated Al stress by NO signaling (Table 1, Figure 4). Proline synthesis and antioxidant defense were stimulated by H₂ and NO under osmotic stress [46] (Table 1, Figure 4). H₂-triggered S-nitrosylation was inhibited when endogenous NO was removed [46]. Therefore, they suggested the involvement of NO in H₂-triggered osmotic tolerance by S-nitrosylation, inducing proline synthesis and re-establishing redox balance [46]. Chen et al. [39] indicated the involvement of CO in H₂-induced adventitious rooting under drought stress via activating antioxidant enzymes, reducing TBARS, O₂⁻, and H₂O₂ levels, enhancing RWC and photosynthetic capacity (Table 1, Figure 4). H₂ may have an effect on Cd and salt stress in rice by modulating ABA, ethylene, or jasmonate acid hormone signaling pathways [12] (Table 1, Figure 4). Jin et al. [47] revealed that H₂ as a positive regulator alleviated drought stress through ABA and modulation of apoplastic pH. However, the interaction roles of H₂ and other signalling molecules under abiotic stress remain unclear (Table 1, Figure 4). Further research is required to investigate the interaction between H₂ and other signalling molecules in alleviating abiotic stress.

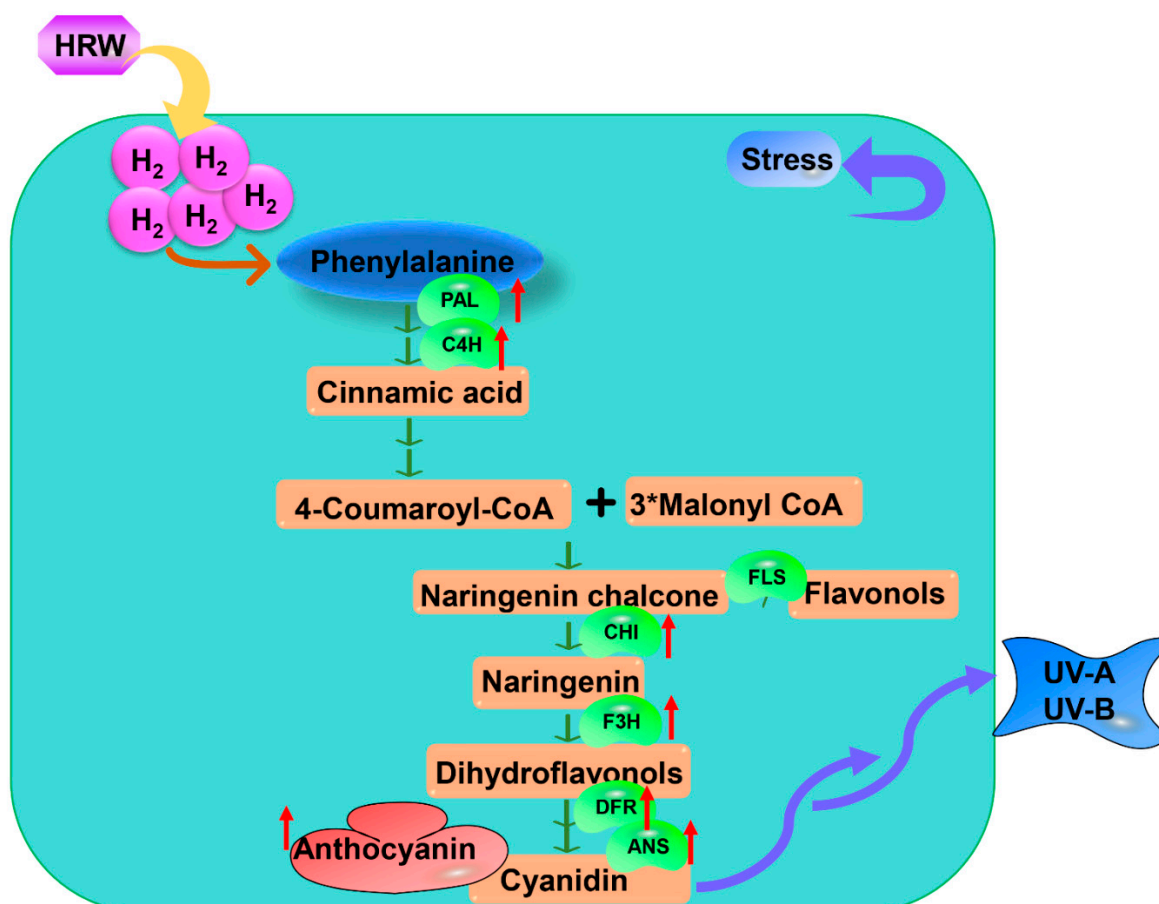


Figure 3. The mode of H₂-mediated flavonoid pathway under abiotic stress. H₂-mediated phenylpropanoid pathway promotes anthocyanin biosynthesis under abiotic stress. Some enzymes up-regulated by H₂ are involved in this process, including PAL, C4H, FLS, CHI, F3H, DFR, and ANS. HRW, hydrogen-rich water; H₂, Hydrogen gas; PAL, phenylalanine ammonia-lyase; C4H, cinnamic acid 4-hydroxylase; FLS, Flavonol synthase; CHI, chalcone synthase; F3H, flavanone-3-hydroxylase; DFR, Dihydroflavonol 4 reductase; ANS, anthocyanidin synthase; UV-A, Ultraviolet-A; UV-B, Ultraviolet-B.

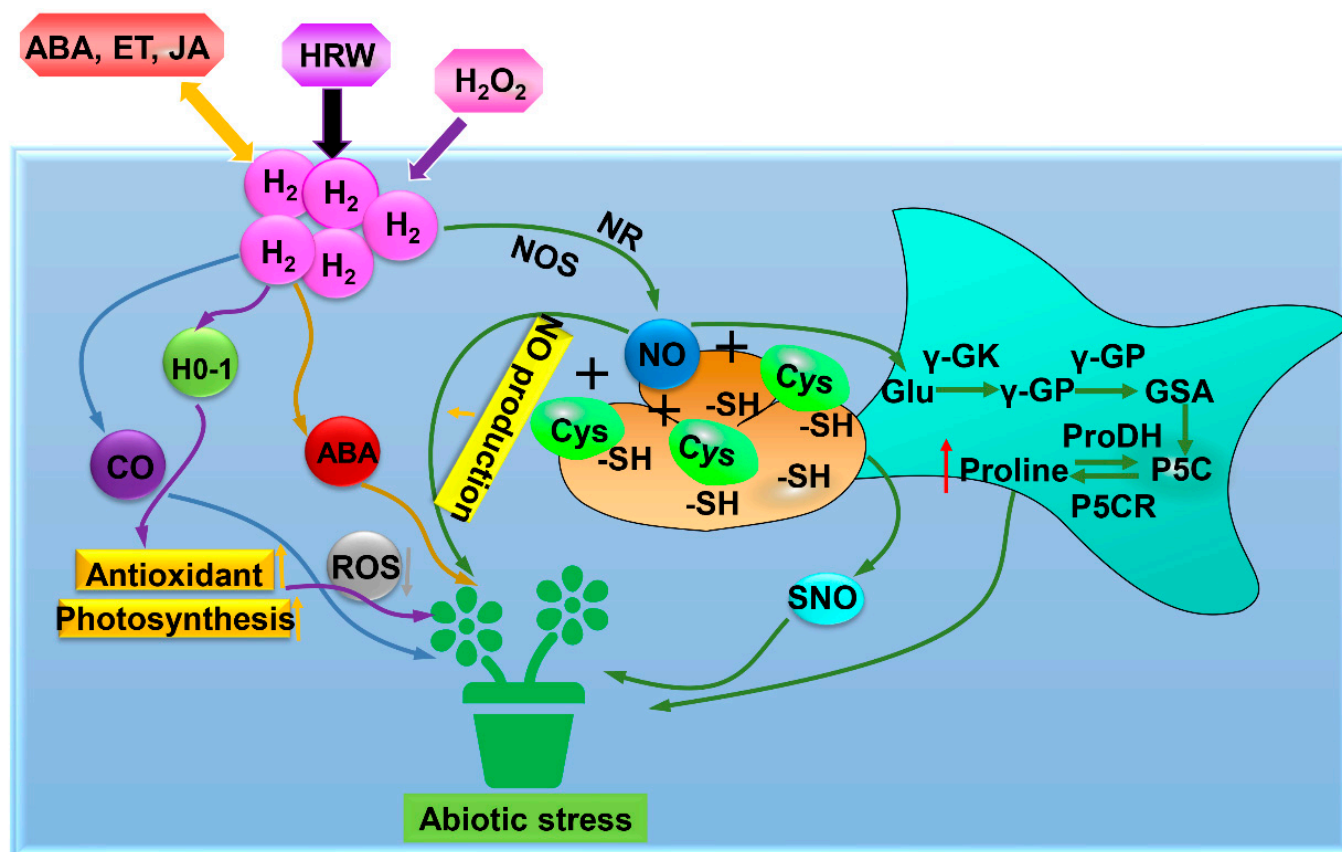


Figure 4. The mode of H_2 -mediated HO-1 signaling, NO, CO, and plant hormone action under abiotic stress. Under abiotic stress, H_2 could relieve abiotic stresses by S-nitrosylation, inducing proline synthesis and re-establishing redox balance. H_2 could also regulate the antioxidant system to relieve abiotic stresses in plants. Besides, H_2 responded to abiotic stresses by modulating ABA, ethylene, or jasmonate acid hormone signaling pathways. ABA, abscisic acid; ET, Ethylene; JA, Jasmonic acid; HRW, hydrogen-rich water; H_2O_2 , Hydrogen peroxide; H_2 , Hydrogen gas; CO, carbon monoxide; HO-1, Heme oxygenase-1; ROS, Reactive oxygen species; NOS, nitric oxide synthase; NR, nitrate reductase; NO, nitric oxide; Cys, Cysteine; -SH, sulfhydryl group; SNO, S-nitrosylation; Glu, Glutamate; γ -GK, Glutamyl kinase; γ -GP, Glutamyl phosphate; GSA, Glutamyl semialdehyde; P5C, Pyrroline-5-carboxylic acid; P5CR, Pyrroline 5-carboxylate reductase; Proline, Proline dehydrogenase.

4. Modulation of Gene Expression by H_2 under Abiotic Stress

Some gene activity must inevitably be changed when H_2 alleviates environmental stresses. At the transcript level, H_2 -upregulated the relative expression of antioxidant defense system-related genes *SOD*, *CAT*, *GR* and *noxR* to improve tolerance of $CdCl_2$ and NaCl in *Hypsizygus marmoreus* [44] (Table 2). H_2 promoted anthocyanin accumulation via exerting different effects on the expression levels of flavonoid-related genes *PAL*, *CHS*, *CHI*, *F3H*, *DFR* and *ANS* under UV-A [30] (Table 2). Under PQ-induced oxidative stress, H_2 could dramatically increase levels of the antioxidant defense system-related genes *Cu/Zn-SOD*, *Mn-SOD*, *POD*, *APX2* and *HO-1* transcript and HO-1 protein [6] (Table 2). Wu et al. [20] reported that improvement of Cd tolerance by H_2 was closely associated with H_2 -upregulated expression levels of the antioxidant defense system-related genes *IRT1*, *IRT2*, *Nramp1*, *HAM2*, *HAM3* and *HAM4* in Chinese cabbage (Table 2). H_2 up-regulated significantly the expression levels of ROS and antioxidant defense system and ion homeostasis-related genes *Cu*, *Zn-SOD*, *Mn-SOD*, *POD*, *APX1/2*, *GPX*, *ECS*, *GS*, *hGS* and *GR1/2* under Cd stress [45] (Table 2). Direct exposure to H_2 via treatment with HRW induced the expression of ROS and antioxidant defense system-related genes *POD*, *APX1/2*, *MDHAR*, *ECS*, *GS* and *GR1*, suggesting that H_2 response to Hg stress entailed regulat-

ing the expression of ROS and antioxidant defense system-related genes [43] (Table 2). *miR398* transcripts were downregulated by H₂, and the expression levels of its targets *Cu/Zn-SOD1 (CSD1)* and *Cu/Zn-SOD2 (CSD2)* were increased [29] (Table 2). By contrast, *miR319* transcripts were differentially increased, showing a relatively negative correlation with its target genes *PROLIFERATING CELL FACTOR5 (PCF5)* and *PROLIFERATING CELL FACTOR8 (PCF8)* [29]. Recently, Dai et al. [19] indicated that H₂ alleviated the detrimental effects of Cd stress on the growth of alfalfa seedlings by upregulating the expression levels of antioxidant defense system and glutathione homeostasis-related genes *IDH*, *Cu*, *Zn-SOD*, *NAD-dependent aldehyde dehydrogenase*, *Amine oxidase*, *Cysteine desulfurase*, *Peroxidase* and *Cysteine synthase* and downregulating the expression levels of ion homeostasis and nutrient element homeostasis-related genes *Phosphoenolpyruvate carboxylase* and *Ferritin* (Table 2). H₂ enhanced heat resistance by upregulating the expression of heat response gene *HSP70* in cucumber leaves [28] (Table 2). In Arabidopsis, genetic evidence suggested that *SOS1* and *cAPX1* might be the target genes of H₂ signaling response to salt stress [5] (Table 2). H₂ also modulated genes/proteins of zinc-finger transcription factor ZAT10/12 in Arabidopsis under salt stress [5]. As described above, H₂ improved the stress tolerance of plants by regulating the transcription of some specific genes, including ROS and the antioxidant defense system, including flavonoid, ion homeostasis, glutathione homeostasis, and nutrient element homeostasis-related genes. Although these specific genes may function in H₂ metabolism, their regulation network remains unclear.

Table 2. Overview of H₂-mediated genes under abiotic stresses in plants.

Plants	Tissues	Genes	Stresses	References
<i>Hypsizygyus marmoreus</i>	/	<i>SOD, CAT, GR, noxR</i>	Cd/Salt	[44]
Radish	Hypocotyls	<i>PAL, CHS, CHI, F3H, DFR, ANS</i>	UV-A	[30]
Alfalfa	Leaves	<i>Cu/Zn-SOD, Mn-SOD, POD, APX2, HO-1</i>	PQ	[6]
Chinese cabbage		<i>IRT1, IRT2, Nramp1, HAM2, HAM3, HAM4</i>	Cd	[20]
Alfalfa	Roots	<i>Cu, Zn-SOD, Mn-SOD, POD, APX1/2, GPX, ECS, GS, hGS, GR1/2</i>	Cd	[45]
Rice	Seedling	<i>POD, APX1/2, MDHAR, ECS, GS, GR1</i>	Hg	[43]
Alfalfar		<i>CSD1, CSD2, PCF, PCF8</i>	Cold	[29]
Alfalfar		<i>IDH, Cu, Zn-SOD, NAD-dependent aldehyde dehydrogenase, Amine oxidase, Cysteine desulfurase, Peroxidase, Cysteine synthase, Phosphoenolpyruvate carboxylase, Ferritin</i>	Cd	[19]
Arabidopsis	Roots/Leaves	<i>SOS1, cAPX1, ZAT10/12</i>	Salt	[5]

Notes: PQ, paraquat; UV-A, Ultraviolet-A; Hg, Mercury; Cd, Cadmium.

5. Conclusions and Perspectives

Abiotic stresses have caused great threats to plant growth and development. The effects of H₂ on abiotic stresses have drawn more and more attention. Accumulating evidence presented in this review indicates that H₂ is one of the important signaling molecules alleviating various abiotic stresses, including temperature, osmotic stress, light, PQ-induced oxidative stress, and metal stresses. The regulation of the antioxidant defense system, flavonoid pathway, photosynthetic capacity, re-established ion homeostasis, re-established glutathione homeostasis, maintained nutrient element homeostasis, glucose metabolism, and HO-1, NO, CO and plant hormone signaling by H₂ are essential strategies to alleviate abiotic stresses. The expression levels of some related genes are also regulated by H₂ under abiotic stresses.

Although it is now well-known that H₂ confers tolerance to various abiotic stresses in plants, many pieces of the puzzle are still missing. The regulatory mechanisms associated with its responses to environmental stimuli are still a subject of great interest. Further research is required to explore the physiological and molecular mechanisms underlying the role of H₂ in modulating plant abiotic and biotic stresses. Very little is known about the relationship between H₂ and other signaling molecules or plant hormones in response to abiotic stresses. Future studies regarding stress resistance enhanced by H₂ should

focus on the interaction between H₂ with other signaling molecules or plant hormones. Simultaneously, it will be important to explore whether H₂ increases resistance to pests and diseases or improves the yield and quality of crop plants. Although H₂ is now the subject of increasing research effort, there are many challenges to address.

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