Molecular Hydrogen in Horticulture

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Improvements in the growth, yield, and quality of horticultural crops require the development of simply integrated, cost-efficient, and eco-friendly solutions. Hydrogen gas (H2) has been observed to have fertilization effects on soils by influencing rhizospheric microorganisms, resulting in improvements in crop yield and quality. Ample studies have shown that H2 has positive effects on horticultural crops, such as promoting root development, enhancing tolerance against abiotic and biotic stress, prolonging storage life, and improving postharvest quality of fruits, vegetables and cut flowers.

hydrogen gas | hydrogen-rich water | hydrogen nanobubbles | solid H-storage material | horticultural crops | metabolism

1. Introduction

Horticultural crops are grown for food, medical use, and aesthetic enjoyment. They form an important part of agricultural production and contribute to food security as well as nutritional quality. The improvement in the growth, yield, and quality of horticultural crops has attracted widespread attention, especially for developing easy, cheap, and eco-friendly solutions, which is a challenge for a low-carbon society.

Hydrogen is the lightest and most abundant chemical element in the universe. Researchers have proposed that hydrogen gas (H_2) played a critical role in the origin of eukaryotes [1]. Meanwhile, the production and release of H_2 has been observed in algae, animals, and plants [2][3][4]. Thus, it is not surprising that H_2 has increasingly been attached to various biological functions in animals and plants, which have been observed during the last two decades of studies [5][6][7].

Despite its low mixing ratio (~530 parts per billion by volume) in current Earth's atmosphere, H_2 contributes to the homeostasis of the oxidation state in the atmosphere [8]. In the context of H_2 biogeochemical cycles, the most important source of H_2 for the atmosphere is methane, while other sources are non-methane hydrocarbons and photochemical oxidation. Conversely, microbial-mediated soil uptake is responsible for ~80% of the tropospheric H_2 losses. H_2 has been shown to maintain microbial viability and activity and, in turn, driven carbon cycling [9]. Since H_2 exposed soil improved plant growth, it has been proposed that H_2 fertilization of soil can be attributed to H_2-oxidizing bacteria in the rhizosphere [10]. Accordingly, the deliberate application of H_2 might have substantial potential in agricultural benefits.

In 2003, Dong et al. [10] observed that H_2-treated soil improved growth in canola (*Brassica napus*) and first proposed the “H_2 fertilization” hypothesis. Since then, a growing number of studies on the application of H_2 in horticulture have been carried out due to its unique properties in stimulating or sustaining plant growth and development, as well as postharvest preservation in particular (**Figure 1**). So far, there are a total of 62 publications on horticultural H_2 application from China (59), Australia (2), and Canada (1). In 2013, H_2 supplied by hydrogen-rich water (HRW) was observed to enhance plant tolerance with respect to herbicide (paraquat), drought, salinity, and cold stress in alfalfa seedlings [11]. Subsequently, many additional functions of H_2 have been discovered, such as promoting root development in cucumber (*Cucumis sativus*) [12] and tomato (*Lycopersicon esculentum*) [13] and alleviating heavy metal toxicity in pak choi (*Brassica rapa* var. *chinensis*) [14] and alfalfa (*Medicago sativa*) [15]. In addition, H_2 has been shown to improve the yield and quality of daylily (*Hemerocallis fulva* L.) [16], as well as prolonging the shelf life and vase life of fruits and flowers including kiwifruit (*Actinidia chinensis* var. *deliciosa*) [17], lychee (*Litchi chinensis*) [18], rose (*Rosa chinensis*) [19], and carnation (*Dianthus caryophyllus*) [20] (**Figure 2**). As the mechanism underlying the positive effects of H_2 on horticultural crops is progressively being revealed, the values of the application of H_2 in horticulture are being increasingly realized.

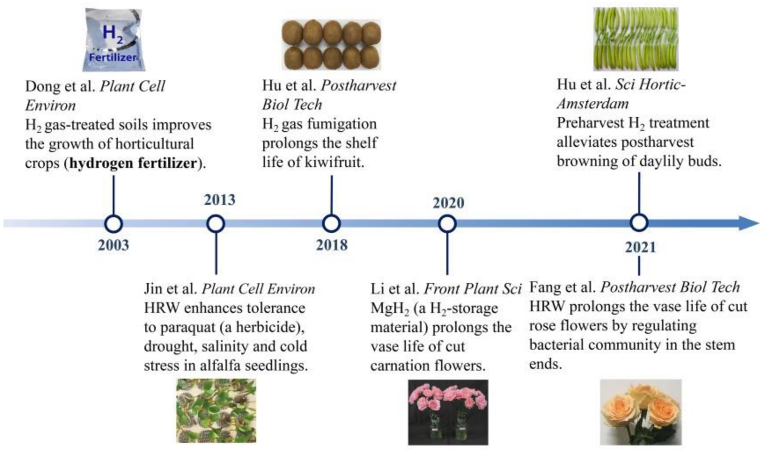

Figure 1. The developing profiles of the application of H_2 in horticulture.

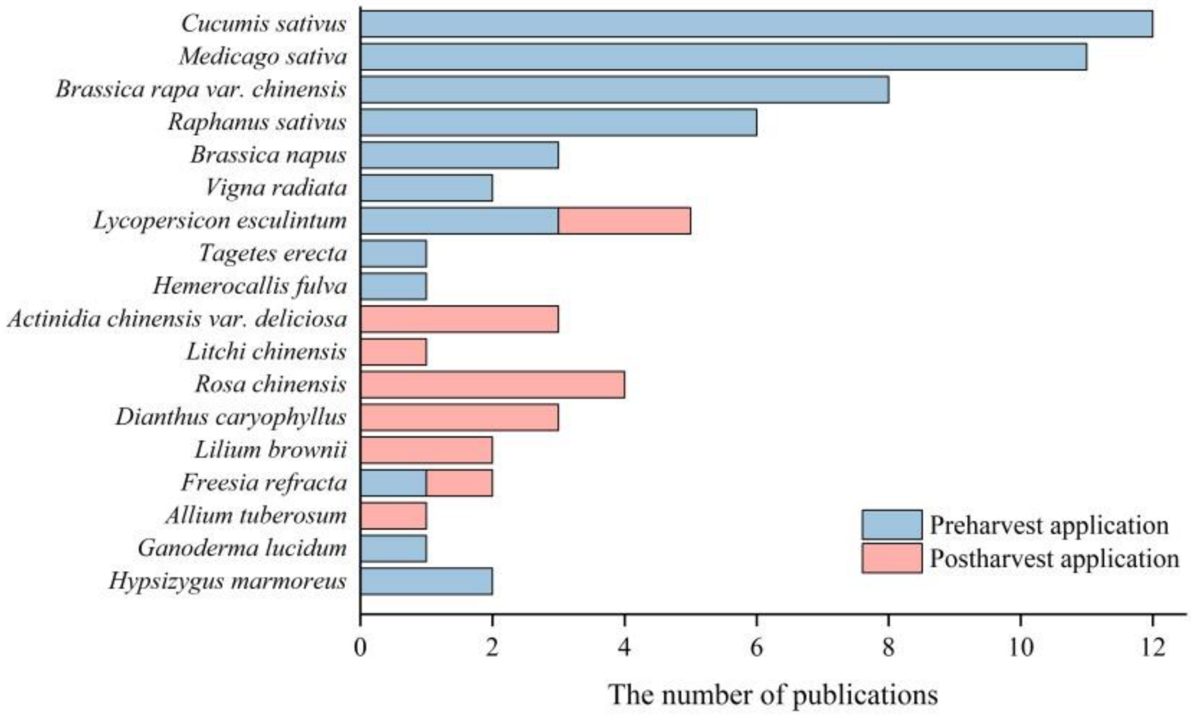

Figure 2. The species of the publications studied on the application of H_2 in horticulture.

2. Possible Mechanisms Underlying H_2 Responses in Horticultural Crops

2.1. Involved in Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) Metabolism

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are commonly involved in plants responses to various stresses [21]. For example, chilling [22], osmatic [23][24], paraquat stresses [11], and metal exposure [25][26][27] can induce ROS (including superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (·OH), etc.) and RNS (nitric oxide (NO), peroxynitrite ($ONOO^-$), etc.), disturbing the delicate redox homeostasis and causing cellular damage inside the plant cells. In postharvest fruits, vegetables, and cut flowers, ROS overproduction accelerated senescence process [16][17][18][28][29]. Additionally, ROS and RNS are vital signaling transducers in plant signaling networks for stress and development [30]. Therefore, the metabolic regulation of ROS and RNS is crucial for stress responses, growth, and development in plants.

Endogenous H_2 could be produced under abiotic stresses and senescence conditions in plants [11][31][32][29]. H_2 can increase the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POD), and ascorbate peroxidase (APX) and the transcript levels of corresponding genes, thus resulting in scavenging overproduced ROS and reestablishing redox homeostasis in alfalfa seedlings subjected to osmotic stress [11][24] (**Table 1**).

Table 1. Role of H_2 involved in reactive oxygen species (ROS) and reactive nitrogen species (RNS) metabolism in horticultural crops.

Materials	Treatment Stage	H_2 Delivery Methods and Treatment	Effective Concentration of H_2	Functions of H_2	Mechanism	Ref. No.
Brassica rapa var. chinensis 'Dongfang 2′	Preharvest	1/4 Hoagland's nutrient solution with H_2 (830 µM); the seedlings were pretreated for 48 h	~415 µM	Alleviates cadmium toxicity	Regulates NR-dependent NO signaling and enhances antioxidant capacity	[26]
		1/4 Hoagland solution with H_2 (865 µM); the seedlings were pretreated for 2/3 d (replaced every 12 h)	865 µM	Reduces cadmium uptake in plant roots	Control of NADPH oxidase encoded by *RbohD*, which operates upstream of IRT1, and regulates root Cd uptake at both the transcriptional and functional levels	[33]
Medicago sativa 'Biaogan'	Preharvest	HRW (220 µM); the seedlings were pretreated for 12 h	~110 µM	Enhances tolerance to paraquat	Modulates HO-1 signaling	[11]
				Alleviates aluminum toxicity	Decreases NO production	[25]
		HRW (780 µM); the seedlings were pretreated for 12 h	~390 µM	Induces osmotic stress tolerance	Regulates H_2O_2 and HO-1 signaling	[23]
					NO-mediated proline accumulation and reestablishment of redox balance	[24]
Cucumis sativus 'Xinchun 4′	Preharvest	HRW (450 µM); the	~225 µM	Promotes adventitious rooting	Regulates CO signaling and	[32]

Materials	Treatment Stage	H_2 Delivery Methods and Treatment	Effective Concentration of H_2	Functions of H_2	Mechanism	Ref. No.
		seedlings were incubated for 2/5 d (changed daily)			activates antioxidant system	
					Regulates NO signaling	[34] [35]
				Induces adventitious rooting under cadmium stress	Decreases oxidative damage, increases osmotic adjustment substance content, and regulates rooting-related enzyme activity	[27]
Cucumis sativus 'Jinyou 35'	Preharvest	HRW (450 µM); the seeds were soaked for 8 h	450 µM	Enhances cold tolerance	Enhances antioxidant capacity and slows dehydration rate by improving osmotic adjustment ability	[22]
Solanum lycopersicum 'Baiguoqiangfeng'	Preharvest	AB@hMSN (10 mg/L); the seedlings were incubated for 2/5 d	~400 µM	Induces lateral root formation	Modulates NR-dependent NO synthesis, cell cycle regulatory genes, and miRNAs expression	[36]
Hypsizygus marmoreus	Preharvest	HRW (1000 µM); the mycelia were cultivated until harvesting	~250 µM	Increases postharvest quality	Enhances antioxidant defense	[37]
Hemerocallis fulva 'Dawuzui'	Preharvest	HRW (1.6 µM); irrigation at the stages of bolting, growing and the day prior to the period of harvest	~0.8 µM	Promotes daylily bud yield and alleviation of bud browning	Decreases ROS level, increases the unsaturated:saturated fatty acid ratio, endogenous H_2 and total phenol content, and reduces PAL and PPO activity	[16]

Materials	Treatment Stage	H_2 Delivery Methods and Treatment	Effective Concentration of H_2	Functions of H_2	Mechanism	Ref. No.
Actinidia chinensis 'Huayou'	Postharvest	HRW (660 µM); the fruits were soaked for 5 min	~528 µM	Delays postharvest ripening and senescence	Enhances antioxidant defense	[17]
Litchi chinensis 'Huaizhi'	Postharvest	HRW (500 µM); the fruits were soaked for 3 min	~350 µM	Delays the pericarp browning	Induces antioxidant system-related characters	[18]
Rosa chinensis 'Kardinal'; *Lilium brownii* 'Manissa'	Postharvest	HRW (450 µM); cut flowers were incubated for vase period (changed daily)	~225 µM (Rose); ~45 µM (Lily)	Improves the vase life and quality	Maintains water balance and membrane stability by reducing stomatal size and oxidative damage	[19]
Allium tuberosum	Postharvest	Gas; the leaves were fumigated for storage period (renewed daily)	~1.2×10^3 µM	Prolongs the shelf life and maintain storage quality	Increases antioxidant capacity	[28]
Dianthus caryophyllus 'Pink Diamond'	Postharvest	HNW (~500 µM); cut flowers were incubated for 3 d (changed daily)	~50 µM	Prolongs the vase life	Reduces ROS accumulation and senescence-associated enzyme activities	[38]
Rosa chinensis 'Carola'	Postharvest	MgH_2 (0.001 g/L); cut flowers were incubated for vase periods	Not shown	Prolongs the vase life	Maintains ROS balance by modulating NO synthesis	[39]

Materials	Treatment Stage	H_2 Delivery Methods and Treatment	Effective Concentration of H_2	Functions of H_2	Mechanism	Ref. No.
		(changed daily)				
Lilium brownii 'Manissa'	Postharvest	HRW; cut flowers were incubated for vase period (changed daily)	Not shown (1% saturation HRW)	Prolongs the vase life	Regulates NO signaling and regulates the expression of the photosynthesis-related AtpA	[40]
Freesia refracta 'Red passion'	Postharvest	HRW (75 μM); cut flowers were pretreated for 12 h	~0.75 μM	Prolongs the vase life	Improves antioxidant capacity	[41]
		HRW (780 μM); cut				

[42] [43][44] [45][46] [43] 2 γ [47] 2

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Materials	Treatment Stage	H_2 Delivery Methods and Treatment	Effective Concentration of H_2	Functions of H_2	Mechanism	Ref. No.
Brassica rapa var. *chinensis* 'Dongfang 2′	Preharvest	1/4 Hoagland's nutrient solution with H_2; the seedlings were incubated for 48 h (replaced every 12 h) after removing cadmium stress	Not shown (50% saturation HRW)	Enhances cadmium tolerance	Reestablishes reduced GSH homeostasis	[45]
Medicago sativa 'Victoria'	Preharvest	HRW (220 μM); the seedlings were pretreated for 12 h	~22 μM	Alleviates cadmium toxicity	Reduces cadmium accumulation and reestablishes GSH homeostasis	[15]
					Expression regulation of genes relevant to sulfur and	[43]

Materials	Treatment Stage	H_2 Delivery Methods and Treatment	Effective Concentration of H_2	Functions of H_2	Mechanism	Ref. No.
					glutathione metabolism, resulting in enhanced glutathione metabolism and activating antioxidant defense and cadmium chelation	
					Decreases oxidative damage, enhances sulfur compound metabolic process, and reestablishes nutrient element homeostasis	[44]
				Alleviates mercury toxicity	Reduces mercury accumulation and reestablishes redox homeostasis (GSH, AsA, and antioxidant enzymes)	[46]
Solanum lycopersicum ‘Baiguoqiangfeng’	Preharvest	HRW (780 µM); the seedlings were incubated for 4 d (changed daily)	~390 µM	Influences lateral root branching	Promotes γ-ECS-dependent GSH production	[47]
Ganoderma lucidum strain HG	Preharvest	HRW (220 µM); added to the medium after 4 days of mycelium culture.	~11 µM	Regulates morphology, growth, and secondary metabolism	Increases glutathione peroxidase activity under HAc stress	[48]

2.3. Involvement in Flavonoids Metabolism

Materials	Treatment Stage	H_2 Delivery Methods and Treatment [50]	Effective Concentration of H_2 [51]	Functions of H_2	Mechanism	Ref. No.
Dianthus caryophyllus 'Pink Diamond'	Postharvest	MgH_2 (0.1 g/L MgH_2 and 0.1 M PBS (pH 3.4); cut flowers were incubated for vase period	~400 µM	Prolongs the vase life	H_2S-mediated reestablishment of redox homeostasis and increased transcript levels of *DcbGal* and *DcGST1*	[49]
Materials	**Treatment Stage**	**H_2 Delivery Methods and Treatment**	**Effective Concentration of H_2**	**Functions of H_2**	**Mechanism**	**Ref. No.**
Raphanus sativus 'Qingtou'; *R. sativus* 'Yanghua'	Preharvest	HRW (220 µM); 1/4 Hoagland's nutrient solution with H_2 (220 µM H_2); the seeds were soaked in HRW for 12 h; sprouts were incubated in nutrient solution with H_2 for 3 d (replaced every 12 h) under UV-A	~220 µM	Regulates anthocyanin synthesis under UV-A	Reestablishes ROS homeostasis and regulates anthocyanin biosynthesis-related gene expression	[52]
Raphanus. sativus 'Yanghua'	Preharvest	HRW (781 µM); the seedlings were incubated for 48/60 h (replaced every 12 h) under UV-A	~781 µM	Promotes the biosynthesis of anthocyanin under UV-A	Regulates $InsP_3$-dependent calcium signaling	[53]
					Involved in phytohormones, MAPKs and Ca^{2+} signaling	[54]
		HRW (220 µM); the seedlings were incubated for 72 h (replaced every 12 h) under short wavelength light	~220 µM	Promotes anthocyanin accumulation under short wavelength light	Promotes activities and transcription of anthocyanin biosynthesis-related enzyme (including CHS and UFGT)	[55]

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Materials	Treatment Stage	H_2 Delivery Methods and Treatment	Effective Concentration of H_2	Functions of H_2	Mechanism	Ref. No.
Medicagosativa 'Victoria'	Preharvest	HRW (781 μM); the seedlings were pretreated for 12 h	~390 μM	Alleviates UV-B-triggered oxidative damage	Regulates (iso)flavonoids metabolism and antioxidant defense	[51]

Table 4. Roles of H_2 involved in carbon and nitrogen metabolism in horticultural crops.

Materials	Treatment Stage	H_2 Delivery Methods and Treatment	Effective Concentration of H_2	Functions of H_2	Mechanism	Ref. No.
Cucumis sativus 'XinJinchun No. 4′	Preharvest	Hoagland's nutrient solution with H_2 (220 μM H_2); the seedlings were pretreated for 7 d (replaced daily)	~110 μM	Improves heat tolerance	Improves photosynthetic and antioxidant and increases HSP70 content	[31]
Brassica rapa var. *chinensis* 'Dongfang 2′	Preharvest	HRW; 1/4 Hoagland's nutrient solution with H2 (835.1 μM H_2); regarding soil cultivation, sprays with HRW (50 mL) at every 12 h for 17 d; for hydroponic solutions, the seedlings were incubated in 1/4 Hoagland solution with H_2 for 4 d (replaced every 12 h) with $Ca(NO_3)_2$	~417 μM	Reduces $Ca(NO_3)_2$ toxicity and improves the growth of seedlings	Enhances antioxidant capacities and reestablishes nitrate homeostasis	[56]
Cucumis sativus ′Jinyou 35′	Preharvest	HRW (450 μM); the seeds were soaked for 8 h	~450 μM	Enhances lower temperature tolerance	Increases the activities of key photosynthetic enzymes and maintains a high level of carbon and	[57]

Materials	Treatment Stage	H_2 Delivery Methods and Treatment	Effective Concentration of H_2	Functions of H_2	Mechanism	Ref. No.
					nitrogen metabolism	
Hypsizygus marmoreus	Preharvest	HRW (800 µM); mycelia were incubated for 5 d (replaced every 12 h) after removal of cadmium stress	~800 µM	Alleviates salinity and heavy metal toxicity	Activates pyruvate kinase, along with its induced gene expression	[58]
Solanum lycopersicum 'Jiafen No. 2′	Postharvest	HRW (780 µM); the fruits were soaked for 20 min	[61] ~585 µM	Reduces nitrite accumulation during storage	Inhibits/increases the activity and transcript level of NR/NiR	[59]

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Na/K ratio

Materials	Treatment Stage	H_2 Delivery Methods and Treatment	Effective Concentration of H_2	Functions of H_2	Mechanism	Ref. No.
Brassica rapa var. *chinensis* 'Dongfang 2'	Preharvest	1/4 Hoagland's nutrient solution with H_2; the seedlings were pretreated for 1 d (replaced every 12 h)	Not shown (50% saturation HRW)	Reduces cadmium accumulation	Inhibits the expression of *BcIRT1* and *BcZIP2*, and reduces cadmium absorption	[62] [63]
Brassica napus 'Zhongshuang 11′	Preharvest	Ammonia borane (NH_3·BH_3; 2 mg/L); the seedlings were incubated for 3 d (changed daily) under NaCl, PEG, or Cd stress	~300 µM	Enhances the tolerance against salinity, drought, or cadmium	Decreases cell death rebuilds redox and ion homeostasis, increases proline content, thus reducing cadmium absorption and accumulation	[61]
Cucumis sativus 'Xinchun 4′	Preharvest	HRW (450 µM); the seedlings incubated for 2/5 d (changed daily)	~450 µM	Induces adventitious rooting	Regulates the protein and gene expressions of PM H^+-ATPase and 14-3-3 mediated by NO.	[64]

2.6. H_2 Is Involved in Phytohormones Signaling

Abscisic acid (ABA), ethylene (ETH), and jasmonate acid (JA) can induce H_2, but the specific biosynthesis pathway has yet to be elucidated [65][66]. For alfalfa drought response, H_2 acted as a positive regulator in the ABA signaling cascade to regulate stomatal movement [66] (**Table 6**). H_2-modified apoplastic pH by H^+-ATPase might be involved in this signaling process. Moreover, H_2 differentially increased the transcriptional factor genes involved in ABA signaling, including *MYB102*, *MYC2*, and *ABF*/*AREB2* [67].

Table 6. Roles of H_2 involved in phytohormones signaling in horticultural crops.

Materials	Treatment Stage	H_2 Delivery Methods and Treatment	Effective Concentration of H_2	Functions of H_2	Mechanism	Ref. No.
Medicagosativa ‘Victoria’	Preharvest	HRW; the seedlings were irrigated for 7 d before 15-d drought treatment	Not shown (50% saturation HRW)	Induces drought tolerance	Modulates stomatal sensitivity to ABA and Apoplastic pH	[66]
Medicagosativa ‘Victoria’	Preharvest	1/4 Hoagland’s nutrient solution with H_2 (780 µM H_2); the seedlings were pretreated for 12 h	~390 µM	Induces tolerance against osmotic stress	Involved in phytohormone signaling	[67]
Cucumis sativus ‘Xinchun 4′	Preharvest	HRW (680 µM); the seedlings were incubated for 7 d (changed daily)	~350 µM	Induces adventitious rooting	Ethylene may be the downstream signaling molecule during H_2-induced adventitious rooting, and proteins RuBisCo, SBPase, OEE1, TDH, CAPX, and PDI may play important roles	[68]
Cucumis sativus ‘Lufeng’	Preharvest	HRW (220 µM); incubated for 4 d	~110 µM	Regulates adventitious root development	Regulates HO-1 signaling	[12]

Materials	Treatment Stage	H_2 Delivery Methods and Treatment	Effective Concentration of H_2	Functions of H_2	Mechanism	Ref. No.
Vigna radiata; *Cucumis sativus* 'Jinchun 4′'; *Raphanus sativus* 'Yanghua'	Preharvest	1/8 strength Hoagland nutrition solution with H_2 (800 µM); the seedlings were incubated for 5 d (replaced every 12 h)	~480 µM	Promotes elongation of hypocotyls and roots	Increases GA and IAA contents in the hypocotyl and the root	[69]
Vigna radiata	Preharvest	HRW; seeds were soaked for 3 d	100/250 µM	Promotes the growth of shoots and roots	Involved in phytohormone signaling	[65]
Freesia refracta	Preharvest	HRW (75 µM); the bulbs were soaked for 6 h; irrigated HRW at every 7–10 d and total 3 times after scape sticking out	~37.5 µM	Promotes early flowering; increases the number and diameters of florets	Regulates phytohormone and soluble sugar content	[70]
Actinidia deliciosa 'Xuxiang'	Postharvest	Gas; the fruits were fumigated for 24 h/12 h + 12 h	~0.2 µM	Prolongs the shelf life	Decreases ethylene biosynthesis	[71]
Rosa chinensis 'Movie star'	Postharvest	HRW (235 µM); cut flowers were incubated for vase periods (changed daily)	~2.35 µM	Alleviates postharvest senescence	Inhibits ethylene production and alleviates ethylene signal transduction	[72]

, or other ... arrier that ... made by ... hich may ... [73] ... lysis, are estimated to produce H_2 for ~USD 5.50 per kilogram of H_2. Although renewable H_2 is relatively expensive, its production costs are reducing. According to the BloombergNEF's report of "Hydrogen Economy Outlook" [74], between 2014 and 2019, the cost of alkaline electrolyzers fell 40% in North America and Europe, and systems made in China are already up to 80% cheaper than those made elsewhere. They forecast that renewable H_2 could be produced for USD 0.7 to USD 1.6/kg H_2 in most parts of the world before 2050. Thus, the cost for applying H_2 in horticulture is primarily dependent on the cost of labor, which is both feasible and affordable, at least under current economic conditions.

H_2 has been applied in the above-mentioned important horticultural crops, confirming its positive effects both on plant growth, development, stress tolerance, and postharvest storage (**Figure 3**). A recent field trial has observed that H_2 infusion increased H_2-oxidizing bacteria activities, accompanied with an alteration of composition and structure of the microbial community [75]. However, the above effects of H_2 on soil microbe were significantly influenced by environmental conditions, which would be taken into account in further H_2 field trials. The potential negative effect of H_2 on soil ecosystems should also be concerning. For example, H_2 exposure may stimulate methane oxidation and the activities of pathogens that use H_2 as an energy source [9]. Therefore, long-term and large-scale commercial field trials of H_2 require further investigation, especially in the evaluation of resistance to pests and diseases, yield, and quality, as well as environmental impact. In addition, enhanced understanding is required with respect to the causal mechanisms underlying plant H_2 production and action.

Overall, H_2 has a substantial potential in horticultural applications to reduce fertilizer and pesticide use, providing higher-value and nutrient-rich horticultural crops. Since making technology cheap requires technological advance, we urge the cooperation of the industrial community. The next step may focus on practical application of H_2 in horticulture.

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