Magnesium Hydride-Mediated Sustainable Hydrogen Supply Prolongs the Vase Life of Cut Carnation Flowers via Hydrogen Sulfide

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Magnesium hydride (MgH2) is a promising solid-state hydrogen source with high storage capacity (7.6 wt%). Although it is recently established that MgH2 has potential applications in medicine because it sustainably supplies hydrogen gas (H2), the biological functions of MgH2 in plants have not been observed yet. Also, the slow reaction kinetics restricts its practical applications. In this report, MgH2 (98% purity; 0.5–25 µm size) was firstly used as a hydrogen generation source for postharvest preservation of flowers. Compared with the direct hydrolysis of MgH2 in water, the efficiency of hydrogen production from MgH2 hydrolysis could be greatly improved when the citrate buffer solution is introduced. These results were further confirmed in the flower vase experiment by showing higher efficiency in increasing the production and the residence time of H2 in solution, compared with hydrogen-rich water. Mimicking the response of hydrogen-rich water and sodium hydrosulfide (a hydrogen sulfide donor), subsequent experiments discovered that MgH2-citrate buffer solution not only stimulated hydrogen sulfide (H2S) synthesis but also significantly prolonged the vase life of cut carnation flowers. Meanwhile, redox homeostasis was reestablished, and the increased transcripts of representative senescence-associated genes, including DcbGal and DcGST1, were partly abolished. By contrast, the discussed responses were obviously blocked by the inhibition of endogenous H2S with hypotaurine, an H2S scavenger. These results clearly revealed that MgH2-supplying H2 could prolong the vase life of cut carnation flowers via H2S signaling, and our results, therefore, open a new window for the possible application of hydrogen-releasing materials in agriculture.

Keywords: magnesium hydride, hydrogen gas, hydrogen sulfide, vase life, cut carnation flowers

INTRODUCTION

Hydrogen is an ideal energy carrier that is being increasingly used in both power generation applications and transportation. Besides, hydrogen gas (H2) has been documented having a range of biological effects and gradually utilized in medicine and agriculture (Ohsawa et al., 2007; Xie et al., 2012; Zeng et al., 2013; Wu et al., 2019). Clearly, the storage of hydrogen is one of the key challenges in developing a hydrogen economy. The storage methods include pressurized gas, a cryogenic
liquid, and solid fuel as chemically or physically combination with materials, such as metal hydrides (Sakintuna et al., 2007). At present, the supplementation of H\textsubscript{2} for biological research includes a gas cylinder and water electrolysis, and H\textsubscript{2} is normally dissolved in water and saline (Ohta, 2011; Xie et al., 2014; Li et al., 2018; Su et al., 2018). However, the extensive application of the hydrogen-rich liquid solution is limited due to the low solubility and short residence time of H\textsubscript{2} in water. Fortunately, the growing development of solid hydrogen-storage materials may provide ways to improve the issues about production and storage of H\textsubscript{2}, considering portable, safety, large hydrogen contents, and sustainable hydrogen supply of solid-state storage (Hirscher et al., 2020).

Magnesium hydride (MgH\textsubscript{2}) stands as a promising hydrogen source because of its high hydrogen-storage capacity (7.6 wt%), abundant resources, and low cost (Grochala and Edwards, 2004). The research on applications of MgH\textsubscript{2} and its related compounds has focused on thermal storage for solar power stations and hydrogen supply for vehicles (Bogdanović et al., 1995; Schlabbach and Zuttel, 2001; Baricco et al., 2017; Lototskyy et al., 2018; Hirscher et al., 2020). It is well documented that MgH\textsubscript{2} can produce a desired quantity of H\textsubscript{2} by the following hydrolysis reaction at room temperature: MgH\textsubscript{2} + 2H\textsubscript{2}O \rightarrow Mg(OH)\textsubscript{2} + 2H\textsubscript{2}, the by-product of which is environmentally friendly. This property of MgH\textsubscript{2} makes it a possible for biological application. Amazingly, Kamimura et al. (2016) discovered that orally given MgH\textsubscript{2} could increase the content of blood H\textsubscript{2} and decrease the level of plasma triglyceride in rats, thus extending their average lifespan. These results indicated that MgH\textsubscript{2} with biosafety might also have potential roles in medical applications.

In fact, there are two disadvantages of MgH\textsubscript{2} restricting its further practical application: (1) the reaction kinetics of MgH\textsubscript{2} hydrolysis is extremely slow in pure water; (2) the insoluble layer of magnesium hydroxide [Mg(OH)\textsubscript{2}] rapidly coated on the outer surface of the unreacted MgH\textsubscript{2} to further hide reaction as the pH increases (Hiraki et al., 2012). Subsequently, some organic acids (including citric acid, ethylenediamine-tetracetic acid, and tartaric acid) were found as good buffer agents to effectively accelerate the reaction, finally improving H\textsubscript{2} generation by decreasing the pH and suppressing Mg(OH)\textsubscript{2} formation (Hiraki et al., 2012; Chao, 2018). On the other hand, it is well-known that organic acid-induced decrease in pH of vase solutions inhibits bacterial growth and increases the water conduction in the xylem of cut flowers, thus prolonging the vase life (van Doorn, 2010).

The postharvest senescence of cut flowers results in significant commercial losses, which is closely associated with a series of signaling molecules, including ethylene (Kumar et al., 2008), reactive oxygen species (ROS; van Doorn and Woltering, 2008), nitric oxide (NO; Naing et al., 2017), and hydrogen sulfide (H\textsubscript{2}S; Zhang et al., 2011). Highly coordinated changes in gene expression are also involved (Shahri and Tahir, 2011). Many senescence-associated genes (SAGs) have been cloned from carnation petals, and their expression patterns were examined as well. For example, transcripts of representative genes encoding β-galactosidase (DcbGal) and glutathione-S-transferase (DcGST1), previously described as SR12 and SR8, are increased during flower senescence (Lawton et al., 1989; Meyer et al., 1991).

Recently, the usage of H\textsubscript{2} in the form of hydrogen-rich water (HRW) was observed to delay postharvest senescence and improve the quality of cut flowers (Ren et al., 2017; Su et al., 2019; Wang et al., 2020). Subsequent biochemical analysis showed that H\textsubscript{2} prolonged the vase life of cut rose and lily was mediated by maintaining water balance, increasing antioxidant defense, and prolonging cell membranes stability (Ren et al., 2017). Meanwhile, H\textsubscript{2} can inhibit ethylene synthesis and corresponding signal transduction via regulating the expressions of related genes (such as ethylene synthesis genes Rh-ACS3 and Rh-ACO1 and ethylene receptor genes Rh-ETR1), thus delaying rose senescence during the vase period (Wang et al., 2020). In addition, H\textsubscript{2}-stimulated NO, another gaseous molecule, can act as a downstream signal molecule involving keeping postharvest freshness in cut lily (Huo et al., 2018). However, the effects of sustained hydrogen supply on prolonging the vase life of cut flowers and related mechanisms are still elusive.

In this study, we firstly aim to find an optimized condition for using MgH\textsubscript{2} in the flower vase experiment. It was confirmed that the application of citrate buffer solution (CBS) could greatly accelerate the reaction rate of MgH\textsubscript{2} hydrolysis, confirmed by the rapid and sustainable increased H\textsubscript{2} generation, thus showing more efficiency in the residence time of H\textsubscript{2} in solution, compared with HRW. By using pharmacological and molecular approaches, we discovered that the combined treatment of MgH\textsubscript{2} and CBS could remarkably prolong the vase life of a cut carnation flower, compared with either treatment with MgH\textsubscript{2} or HRW, or CBS alone. It is a new finding. Further results suggested that the discussed MgH\textsubscript{2}-CBS response is mediated by influencing H\textsubscript{2}S signaling. Together, this work will not only extend the application of MgH\textsubscript{2} to agricultural practices but also provide a new idea for the development of new plant growth regulators.

**MATERIALS AND METHODS**

**Chemicals**

All chemicals used in our experiments were purchased from Sigma-Aldrich (St. Louis, MO, United States) unless stated otherwise. MgH\textsubscript{2} was obtained from the Center of Hydrogen Science, Shanghai Jiao Tong University (Ma et al., 2019). MgH\textsubscript{2} was further characterized by using scanning electron microscopy (SU-8010, Hitachi, Tokyo, Japan), X-ray diffraction (D/MAX-Ultima III, Rigaku, Tokyo, Japan) with Cu K radiation source, differential scanning calorimetry (STA449F3, Netzsch, Selb, Germany), and thermogravimetry (TG209F3, Netzsch, Selb, Germany). In addition, sodium hydrosulfide (NaH\textsubscript{2}S) and hypotaurine (HT) were used as an H\textsubscript{2}S releasing compound and a specific H\textsubscript{2}S-scavenger, respectively (Ortega et al., 2008). H\textsubscript{2}S fluorescent probe 3-oxo-3H-spiroisobenzofuran-1,9’-xanthene]-3,6’-diyl bis(2-(pyridin-2-ylsulfanyl)benzoate) (WSP-5; MKBio, Shanghai, China) was used to monitored endogenous H\textsubscript{2}S in cut flowers (Peng et al., 2014). The
concentrations of these chemicals were selected based on the results of pilot experiments.

**Plant Material and Treatments**

Cut carnation “Pink Diamond” flowers at the typical commercial stage (the petals form a right angle with the stem axis) were purchased from a flower market in Nanjing City, Jiangsu Province, China, from July to September of 2019. They were transported within 1 h to the laboratory. Subsequently, the cut flower stems were placed in distilled water and re-cut underwater to a length of 25 cm. The top two leaves were kept as well.

The cut flower stems were incubated in glass bottles with 150-ml distilled water (control) and 0.1-M CBS (pH 3.4) containing 0.01, 0.1, and 1 g L\(^{-1}\) MgH\(_2\). Because the treatment with 0.1-M CBS (pH 3.4) plus 0.1 g L\(^{-1}\) MgH\(_2\) showed the most obvious effects on prolonging the vase life of a cut flower in a pilot experiment (Supplementary Figures 1A–C), this combined treatment was applied subsequently. Meanwhile, 0.1 g L\(^{-1}\) MgH\(_2\), 0.1 M CBS (pH 3.4), or 10% HRW (obtained by water electrolysis) alone was, respectively, regarded as controls, and HRW was prepared according to the previous method (Su et al., 2019).

To confirm the possibility that the effect of MgH\(_2\) was only due to molecular hydrogen and not associated with magnesium ion, MgH\(_2\)-CBS solution was boiled for three times, 5 min each to remove the generated H\(_2\), followed by keeping under the normal temperature condition for 1 day until no H\(_2\) was detected. Because 600-µM NaHS and 10-mM HT showed the obviously promoting and repressing effects on prolonging the vase life of a cut flower in pilot experiments, respectively (Supplementary Figures 1D,E), these treatments were also chosen. For further tests, the cut flower stems were incubated in treatment solutions (150 ml) containing distilled water (control), 0.1 g L\(^{-1}\) MgH\(_2\)-CBS, 600-µM NaHS, or 10-mM HT, alone and in combination. For the entire tests, all stems were continuously kept in the treatment solutions throughout the vase period at 25 ± 2°C, 60–70% relative humidity, and 12 h per day of light (20 µmol m\(^{-2}\) s\(^{-1}\)). All treatment solutions were renewed daily as well.

**Determination of Hydrogen Gas Concentration**

The concentration of H\(_2\) in solutions was measured by a portable dissolved hydrogen meter (ENH-1000, TRUSTLEX, Osaka, Japan) that was calibrated by gas chromatography (Su et al., 2019).

**Vase Life, Relative Fresh Weight, and Flower Diameter**

The vase life of each flower was calculated as the number of days from the day that the stems were placed in the vase solutions (recorded as day 0) until the day that 50% of petals had wilted or the stems had bent (bent-neck angle greater than 45°). During the vase period, the fresh weight of each sample was measured daily using an analytical balance. The relative fresh weight (RFW) was calculated as following: RFW% = (FW\(_t\)/FW\(_0\)) × 100, where W\(_t\) is the fresh weight of the sample (g) at day t (t = 0, 1, 2, 3, etc.), and W\(_0\) is the fresh weight of the same sample (g) at day 0. Additionally, flower diameter was defined as the maximum width of each flower and measured daily using a digital caliper. In each experiment, 10 flowers were placed per treatment with three replications, and the means of the vase life, RFW, and flower diameter were determined.

**Measurement of Endogenous Hydrogen Sulfide**

With the aid of laser scanning confocal microscopy, H\(_2\)S level in vivo was determined as described previously with minor modification (Kou et al., 2018). The petals were incubated with 20-µM WSP5 (an H\(_2\)S fluorescent probe) in 20-mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid–sodium hydroxide buffer (pH 7.5) for 30 min in the dark (25°C). After three washes (10 min per time) with fresh 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid–sodium hydrogen buffer, the samples were observed using an LSM 710 microscope (Carl Zeiss, Germany) with excitation at 495 nm and emission at 525 nm. The bright-field images were shown at the lower right corners of their corresponding fluorescent images. The relative fluorescence was presented as relative units of pixel intensities calculated by the ZEN software to the control samples. At least five sections per sample were determined, and three samples in each treatment were used.

**Histochromic Staining and Corresponding Measurement of Hydrogen Peroxide Content**

The hydrogen peroxide (H\(_2\)O\(_2\)) in petal was visually detected according to the method of Thordal-Christensen et al. (1997). The petals were stained with 0.1% 3,3-diaminobenzidine for 12 h at room temperature in the dark. Afterward, the petals were detected under a light microscope (Stemi 2000-C; Carl Zeiss, Germany).

The H\(_2\)O\(_2\) content was measured by the spectrophotography (Mei et al., 2017). The samples were incubated with assay reagent (containing 50-mM H\(_2\)SO\(_4\), 200-µM xylenol orange, and 200-mM sorbitol) for 45 min in the dark at 25°C. Then, the absorbance values were determined at 560 nm. A standard curve was obtained by adding a variable amount of H\(_2\)O\(_2\).

**Analysis of Senescence-Associated Genes Transcription**

Quantitative real-time RT-PCR (qPCR) was used to analyze the expression of SAGs. Total RNA was extracted from petals using the SparkZol Reagent (SparkJade, Shandong, China). The concentration and quality of RNA were determined using a NanoDrop 2000 (Thermo Fisher Scientific, Wilmington, DE, United States), and RNA was treated with RNase-free DNase (TaKaRa Bio Inc., Dalian, China) to eliminate traces of DNA. Afterward, complementary DNAs were synthesized using HiScript III RT SuperMix (Vazyme, Nanjing, China). By using specific primers (Supplementary Table 1), qPCR was performed using a Mastercycler ep® realplex real-time PCR system (Eppendorf, Hamburg, Germany) with
Statistical Analysis
All values are means ± standard error (SE) of three independent experiments with three biological replicates for each. Data were analyzed by SPSS 22.0 software (IBM Corporation, Armonk, NY, United States). Differences among treatments were analyzed by one-way analysis of variance (ANOVA) followed by Duncan’s multiple range test or t-test, and P < 0.05 or 0.01 were considered as statistically significant.

RESULTS
Characterization of Magnesium Hydride
As shown in the scanning electron microscopy images (Figure 1A), the as-received MgH₂ particles are spherical with a diameter of 0.5–25 µm (mean diameter = 15 µm; Ma et al., 2019). The X-ray diffraction patterns (Figure 1B) confirmed that MgH₂ is the majority phase with a small amount of unhydried magnesium. The dehydriding properties of MgH₂ were investigated by using differential scanning calorimetry and thermogravimetry. It was observed that the peak temperature of decomposition is 405°C at a heating rate of 10°C min⁻¹ with a mass loss of about 7.2 wt% (Figure 1C).

The amount of H₂ generated from complete hydrolysis of MgH₂ was about 1,800 ml g⁻¹ (Figure 1D); namely, the concentration of H₂ in unit volume (1 m³) was 0.18% (v v⁻¹). It is not flammable and explosive when the H₂ concentration is less than 4% by volume (lower flammability limit of H₂). Thus, it is generally safe by using MgH₂ as a vase regent.

Magnesium Hydride–Citrate Buffer Solution Prolongs the Vase Life of Cut Carnation Flowers
In our experimental conditions, when 0.1 g L⁻¹ MgH₂ was dissolved in 0.1-M CBS (pH 3.4), this combined treatment (also abbreviated as MgH₂-CBS in the following experiments) was observed as the most obvious effect on prolonging the vase life of cut carnation flowers, compared with different doses of MgH₂, various CBS, or 10% HRW alone (Supplementary Figures 1A–C and Figures 2A,B). In the presence of 0.1 g L⁻¹ MgH₂-CBS (0.1 M, pH 3.4), for example, the vase life of the fresh cut flowers was the longest among all the treatment and was 11.4 days, which prolonged 3.9 days compared with the control, which was also significantly different from the treatments of 0.1 g L⁻¹ MgH₂ (prolonged about 2.0 days), 0.1-M CBS (pH 3.4; about 1.6 days), or 10% HRW (about 1.5 days) alone. This conclusion correlates with the data from other phenotypic parameters, including RFW and flower diameter in carnation (Figures 2C,D). By contrast, the removal of H₂ by heating solution impaired the positive effects of MgH₂-CBS. It was also confirmed that the boiling used in our experiment was sufficient to remove H₂ from solutions (Figure 2E), thus suggesting the function of MgH₂-CBS is H₂-dependent.

Consistently, the contents of dissolved H₂ in MgH₂-CBS and 0.1 g L⁻¹ MgH₂ solutions ranked the first and second (rapidly peaking at 0.80 and 0.48 ppm) and remained in higher levels until 6 and 12 h, respectively. Meanwhile, H₂ existing in 10% HRW progressively decreased, just from an initial 0.16 ppm to the basal level after 6 h (Figure 2E).

Hydrogen Sulfide Is Involved in Magnesium Hydride–Citrate Buffer Solution-Prolonged Vase Life of Cut Carnation Flowers
To investigate whether H₂S is involved in MgH₂-CBS-prolonged vase life of cut carnation flowers, both MgH₂-CBS and HT (a specific H₂S scavenger; Ortega et al., 2008) were applied alone and in combination. Meanwhile, NaHS (an H₂S releasing compound) was used as a positive control. The response of the endogenous H₂S level in the petal was monitored by labeling H₂S using an H₂S-specific fluorescent probe (WSP-5; Peng et al., 2014) and imaging by laser scanning confocal microscopy (Kou et al., 2018). As shown in Figure 3, the WSP-5-dependent fluorescent intensity was increased by NaHS but was greatly impaired by HT. In addition, HT alone decreased fluorescent intensity in comparison with the chemical-free control. It was confirmed that some, if not most, of the WSP-5-related fluorescence is caused by H₂S. Further results demonstrated that MgH₂-CBS significantly increased endogenous H₂S production. Consistently, the inducing effect achieved by MgH₂-CBS could be prevented by HT. Moreover, there was no additive response in fluorescence when MgH₂-CBS was added together with NaHS.

The subsequent experiment was to assess the contribution of H₂S in prolonging carnation vase-life achieved by MgH₂-CBS. Consistently, three parameters, in terms of vase life, RFW, and flower diameter, were used. As expected, compared with the responses of NaHS, the prolonged vase life of cut carnation flowers was intensified in the presence of MgH₂-CBS, which was abolished when HT was added (Figure 4). In contrast, compared with control, HT alone shortened the vase life. However, MgH₂-CBS co-treated with NaHS cannot result in an additive extension of carnation vase-life. Correlating with the changes in endogenous H₂S production (Figure 3), the results indicated that endogenous H₂S might participate in MgH₂-CBS-prolonged the vase life of cut carnation flowers.

Magnesium Hydride–Citrate Buffer Solution Maintains Redox Homeostasis via Hydrogen Sulfide
Histochemical staining of ROS (H₂O₂) accumulation was then adopted to reveal the detailed mechanism underlying MgH₂-CBS-prolonged carnation vase-life. As expected, it was observed that a gradual increase of 3,3-diaminobenzidine-dependent staining in the control during the vase period (Figure 5A). The change of endogenous H₂O₂ level determined
with spectrophotography displayed a similar tendency (Figure 5B), indicating that redox homeostasis was disrupted during senescence.

Compared with the control, the treatments with MgH₂-CBS and NaHS individually resulted in slight staining patterns (Figure 5A). By contrast, the mentioned responses elicited by MgH₂-CBS, and NaHS was reversed by the removal of endogenous H₂S when HT was applied. Alone, HT brought out extensive staining compared with the control (5 days). No additive responses were observed in MgH₂-CBS plus NaHS. Meanwhile, changes in endogenous H₂O₂ contents showed similar patterns (Figure 5B). These results suggested that MgH₂-CBS could reestablish redox homeostasis in carnation flowers, which might be mediated by H₂S.

**Role of Hydrogen Sulfide in Magnesium Hydride–Citrate Buffer Solution-Modulated Senescence-Associated Genes During Postharvest Senescence**

To further elucidate the molecular mechanism of how H₂S is involved in MgH₂-CBS-prolonged carnation vase-life, several molecular probes responsible for senescence, including DcbGal and DcGST1, were analyzed by qPCR. The time-course experiment showed that the expression levels of DcbGal and DcGST1 were increased during postharvest senescence, and those in petals of control were much higher than those in the presence of MgH₂-CBS (Figures 6A,B). Similar to the responses of H₂S, MgH₂-CBS could also downregulate the transcripts of DcbGal and DcGST1 (5 days; Figures 6C,D). In contrast, the inhibition mentioned earlier was attenuated by the depletion of H₂S with HT. Additionally, HT alone could greatly increase the expression levels of these two genes. No additive inhibition responses occurred in co-treatment of MgH₂-CBS and H₂S as well. Therefore, H₂S was involved in MgH₂-CBS-induced reduction of DcbGal and DcGST1 expression in carnation during the vase period.

**DISCUSSION**

At present, HRW is a major route of H₂ administration (Shen and Sun, 2019). Ample evidence showed that HRW has positive effects on postharvest physiology. For example, HRW can prolong the shelf life (Hu et al., 2014) and decrease nitrite accumulation of fruits during storage (Zhang et al., 2019), as...
FIGURE 2 | Changes in vase life, relative fresh weight (RFW), and flower diameter of cut carnations and dissolved H₂ in solution subjected to MgH₂, citrate buffer solution (CBS), MgH₂-CBS, heated MgH₂-CBS, and hydrogen-rich water (HRW). (A) Representative photographs of cut flowers (scale bar = 2 cm). Cut flower stems were incubated in untreated (control) and treatment solutions containing 0.1 g L⁻¹ MgH₂, 0.1-M CBS (pH 3.4) with or without 0.01, 0.1, and 1 g L⁻¹ MgH₂, 10% electrolytic HRW during vase period. Afterward, vase life (B), RFW (C), maximum flower diameter (D), and H₂ content in solutions (E) were expressed as mean ± standard error (SE). There were three replicates and 10 flowers per each for (A–D), and three replicates per each for (E). Experiments were conducted for three times. Bars with different letters are significantly different (P < 0.05), as determined by Duncan’s multiple range test.
FIGURE 3 | MgH₂-CBS triggers H₂S accumulation. (A) Cut flower stems were incubated in untreated (control) and treatment solutions containing 0.1 g L⁻¹ MgH₂-CBS, 600-µM NaHS, 10-mM HT (a scavenger of H₂S), alone or their combinations for 3 days. Afterward, epidermis of petals was loaded with 20-µM WSP5 (an H₂S fluorescent probe) and detected by laser scanning confocal microscopy (scale bar = 200 µm). Bright-field images corresponding to the fluorescent images were at the bottom right corner. (B) Relative fluorescence was also presented as values relative to control. Mean and SE values were calculated. At least five sections per sample were determined, and three samples in each treatment were used. Bars with different letters denoted significant differences in comparison with control at P < 0.05, according to Duncan’s multiple range test.

well as prolong the vase life of cut flowers (Ren et al., 2017; Su et al., 2019; Wang et al., 2020). Importantly, the HRW is presently mainly obtained by water electrolysis, which requires a hydrogen gas generator. Moreover, the solubility of H₂ in water is very low (approximately 1.84 ml in 100-g H₂O at 20°C, 1 atm; Safonov and Khitrin, 2013), and especially, the residence time of H₂ in HRW is shorter, as the half-time of dissolved H₂ in HRW is less than 1 h (Figure 2E), at least under our experimental conditions. The discussed disadvantages may restrict the practical applications of the electrolytic produced HRW.

In this study, H₂ was generated by MgH₂ hydrolysis, which was intensified when dissolved in CBS. Additionally, it can remain in higher amounts of dissolved H₂ over a relatively longer period than the electrolytic HRW (Figure 2E). It has been reported that hydrolysis of magnesium particles can produce hydrogen nanobubbles that can exist in the water solution of a dietary supplement for a sufficiently long time (Bunkin et al., 2009; Safonov and Khitrin, 2013). A balance between surface tension and repulsive forces between surface electric charges is responsible for the stabilization of nanobubbles (Bunkin et al., 2009). We also found that the dissolution of MgH₂ in water and CBS (in particular) was accompanied by a large number of small bubbles in the first 1–2 min. Thus, MgH₂ may also produce hydrogen nanobubbles that increase the solubility and the residence time of H₂. However, the dissolution of MgH₂ in water led to a strongly
alkaline environment (approximately pH 10; Supplementary Figure 1F). By contrast, the administration with CBS significantly accelerated the reaction of MgH$_2$ hydrolysis and increased H$_2$ generation (Figure 2E) by decreasing the pH, which is consistent with the previous studies (Hiraki et al., 2012; Chao, 2018).

It is worth noting the safety of MgH$_2$ use. In fact, the concentration of H$_2$ generated from MgH$_2$ hydrolysis is far less than the lower flammability limit of H$_2$ (4% in air). Therefore, it is safe by using MgH$_2$ as a vase regent. It has been reported that the citric acid buffered around pH 3 can effectively prolong the vase life of cut flowers by reducing bacterial growth and maintaining the water balance (van Doorn, 2010). A similar result was observed in this study (Supplementary Figures 1B,C and Figures 2A,B). Although the combination of MgH$_2$ and acid solutions is impractical for industry application because it causes equipment corrosion, it precisely favors postharvest preservation. We also observed that combining MgH$_2$ with CBS may produce additive or synergistic effects in prolonging the vase life of cut carnation flowers. Together, MgH$_2$ might be used as a promising chemical for producing a hydrogen-rich solution in horticulture.

**FIGURE 4** MgH$_2$-CBS-prolonged vase life of cut carnation flowers is sensitive to the scavenger of H$_2$S. (A) Cut flower stems were incubated in untreated (control) and treatment solutions containing 0.1 g L$^{-1}$ MgH$_2$-CBS, 600-µM NaHS, 10-mM HT (a scavenger of H$_2$S), alone or their combinations throughout the vase period. Representative photographs of cut flowers were taken (scale bar = 2 cm). Vase life (B), relative fresh weight (RFW; C), and maximum flower diameter (D) were expressed as mean and SE values. There were three replicates and 10 flowers per each. Experiments were conducted for three times. Bars with different letters are significantly different ($P < 0.05$), as determined by Duncan’s multiple range test.
**FIGURE 5** | MgH$_2$-CBS maintains redox homeostasis via H$_2$S. (A) Cut flower stems were incubated in solutions containing 0.1 g L$^{-1}$ MgH$_2$-CBS, 600-µM NaHS, 10-mM HT, alone or their combinations throughout the vase period. The petals were stained with 3,3-diaminobenzidine (DAB), then photographed under a light microscope (scale bar = 1 mm). (B) Spectrophotography also determined H$_2$O$_2$ contents. Values are mean ± SE of three independent experiments with three replicated for each.

H$_2$S is a well-known important gaseous signaling molecule involved in plant developmental and environmental responses, such as root organogenesis, response to abiotic stresses, and delayed senescence of vegetables, fruits, and flowers (Zhang et al., 2011; Li et al., 2012, 2013; Wang et al., 2012; Ali et al., 2019; Corpas, 2019; Mei et al., 2019). It has been confirmed that L-cysteine desulphhydrase-dependent H$_2$S acts as the downstream signal molecule involved in NO-induced heat tolerance of maize seedlings (Li et al., 2013) and methane-induced tomato and Arabidopsis lateral root formation (Mei et al., 2019). Interestingly,
a similar requirement of H$_2$S for MgH$_2$-prolonged vase life of cut carnation flowers was discovered in this work. The conclusion is supported by the following pharmacologic and molecular evidence.

HT, a scavenger of H$_2$S (Ortega et al., 2008; Fang et al., 2014; Mei et al., 2019), was used in our experiments, and its inhibitory role was confirmed. The increase in endogenous H$_2$S accumulation triggered by MgH$_2$-CBS was observed to be sensitive by HT (Figure 3). Correlating with the changes in the phenotypes of vase life, relative fresh weight, and flower diameter (Figure 4), the results presented here further revealed a requirement for endogenous H$_2$S in MgH$_2$-CBS-prolonged carnation vase-life.

Furthermore, ROS (especially H$_2$O$_2$) has been observed to increasingly produce during the senescence process in cut flower (Hossain et al., 2006; Kumar et al., 2007; Su et al., 2019). It has been demonstrated that H$_2$S could inhibit ROS overproduction by increasing activities of antioxidant enzymes (Zhang et al., 2011; Hu et al., 2012, 2015). In this study, the contents of H$_2$O$_2$ gradually increased during the normal senescence of cut carnation flowers, which indicated the disruption of redox homeostasis (Figure 5B). The lower H$_2$O$_2$ levels maintained by MgH$_2$-CBS might be, at least partially, responsible for delaying senescence. By contrast, the discussed responses of MgH$_2$-CBS were reversed by the removal of endogenous H$_2$S with HT (Figure 5B). Changes in histochemical staining showed a similar pattern (Figure 5A). The discussed results, therefore, confirmed that MgH$_2$-CBS-reestablished redox homeostasis was closely associated with the alteration in endogenous H$_2$S.

Recent evidence proved that H$_2$S decreased the expression levels of SAGs, resulting in delaying the postharvest senescence of broccoli (Li et al., 2014). Furthermore, sucrose and silver thiosulphate (an inhibitor of ethylene receptor) could repress the upregulation of SAGs (including DcbGal and DcGST) in petals of carnation (Hoeberichts et al., 2007). Similarly, our further

**FIGURE 6 |** Changes in the transcripts of senescence-associated genes. Cut flower stems were incubated in solutions containing 0.1 g L$^{-1}$ MgH$_2$-CBS, 600-µM NaHS, 10-mM HT, alone or their combinations throughout the vase period. After treatments for the indicated time points or 5 days, the transcript levels of DcbGal (A,C) and DcGST1 (B,D) in petals were analyzed by qPCR and presented as values relative to the control samples (0 days) after the normalization with the transcript levels of an internal control gene DcActin. Values are mean ± SE of three independent experiments with three replicated for each. Bars with asterisks were significantly different in comparison with control at *$P < 0.05$ and **$P < 0.01$ according to t-test. Bars with different letters are significantly different ($P < 0.05$), as determined by Duncan’s multiple range test.
molecular data revealed that MgH₂-CBS could downregulate the expression of DcbGal and DcGST (Figure 6). By contrast, such inhibition effects of MgH₂-CBS were alleviated by HT. Combined with the changes in phenotypes and endogenous H₂S level (Figures 3, 4), we also speculated that SAGs might be the target genes responsible for MgH₂-CBS-triggered H₂S-prolonged vase life of cut flowers.

Accordingly, a schematic model shown in Figure 7 summarizes the role of H₂S in the MgH₂-CBS-prolonged the vase life of cut carnation flowers.

CONCLUSION

This study revealed the effectiveness of MgH₂-mediated H₂ sustainable supply in postharvest preservation of cut flowers. Compared with hydrogen-rich water, the utilization efficiency of MgH₂ was improved by buffering with CBS. Thus, MgH₂ may have great potential for application in horticulture. In addition, it also demonstrated a vital role of H₂S in MgH₂-CBS-prolonged the vase life of cut flowers by modulating the expression of SAGs.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

WS and LL conceived and designed the research. LL, YL, and SW performed the experiments and analyzed the data. JZ and WD provided advice and materials for these experiments. LL, YL, SW, and WS wrote and revised the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2020.595376/full#supplementary-material
Li et al.

December 2020 | Volume 11 | Article 595376

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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