

Article

Melatonin Promotes Post-Harvest Preservation of Cut Roses ‘Corolla’ by Facilitating the Production of Hydrogen Sulfide

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Abstract

In recent years, the applications of melatonin (MT) and hydrogen sulfide (H₂S) have both been proven to improve the post-harvest preservation of horticultural products. However, the specific regulatory mechanism between them in cut flower preservation remains unclear. Here, we conducted research on the combined effect of MT and H₂S during the senescence of cut roses ‘Corolla’, as well as the relationship between endogenous H₂S production and MT. We found that the cut roses treated with MT + H₂S exhibited the longest vase life, the largest flower diameter, and the highest increase of fresh weight compared with those under MT or H₂S treatment alone. We have found that both MT and H₂S can inhibit the contents of the ethylene precursor ACC and the key enzymes ACS and ACO involved in ethylene synthesis in cut roses. Further investigation revealed that the application of hypotaurine (HT), a specific chemical scavenger of hydrogen sulfide (H₂S), compromised the preservation of cut roses. In the combined treatment with HT and melatonin (MT), elevated levels of reactive oxygen species (ROS), along with reduced antioxidant enzyme activities and downregulated expression of associated genes, were observed. However, the combined treatment still demonstrated a superior preservative effect relative to the HT treatment alone. Conversely, MT treatment individually enhanced endogenous H₂S production and upregulated the transcript levels of the H₂S biosynthesis genes *RhLCD* and *RhDCD* in petal tissues. These results substantiate that MT extends the postharvest longevity of cut roses by stimulating H₂S synthesis, thus counteracting oxidative damage.

Keywords: cut flower; postharvest senescence; melatonin; antioxidant enzyme; hydrogen sulfide generation



Academic Editor: Rosario
Paolo Mauro

Received: 29 May 2026

Revised: 26 June 2026

Accepted: 1 July 2026

Published: 3 July 2026

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1. Introduction

Rose (*Rosa hybrida* L.) belongs to the Rosaceae family and the *Rosa* genus. They are thorny shrubs, serving as an important cultivated group for cut flower production and one of the most popular cut flower varieties in the global flower trading market [1]. After the cut roses are harvested from the industrial park, they are first subjected to pre-cooling treatment to reduce the respiratory rate and inhibit aging. Then, through grading and screening, as well as standardized packaging, they are transferred to low-temperature storage for transportation. After that, they are transported via cold chain to various markets and eventually reach consumers. During this process, the cut roses are prone to water loss and wilting due to water imbalance. At the same time, the emerging problems such as petal shedding, stem rot, and dull color may occur, which can seriously affect the

commercial quality and ornamental value of the cut roses. Therefore, the extension of the preservation period of cut roses is urgently needed to reduce post-harvest losses, enhance product value, and meet consumer demands. Additionally, the cut roses are one of the most favored cut flowers by consumers, but their petals are susceptible to being influenced by multiple factors, which then decreases the preservation time of the cut roses. Thus, developing efficient preservatives to prolong the post-harvest preservation time and ensure post-harvest quality of cut rose flowers, especially their petals, is significant for cut rose production. The preservation period of cut flowers depends on the genetic background of the variety and various external environmental factors, such as ambient temperature, humidity, light, and nutrients in the vase solution [2]. The internal physiological changes associated with the senescence of cut flowers include a decrease in the rate of fresh weight change, excessive release of ethylene, a decline in soluble sugar, soluble protein, and proline (Pro) contents, weakened antioxidant enzyme activities, and an increase in the production of reactive oxygen species (ROS) [3,4]. Although numerous studies have been conducted to extend the preservation period of cut roses, the development of highly effective preservatives remains a current research hot topic.

Hydrogen sulfide (H_2S) is an important gaseous signaling molecule that regulates fruit ripening and senescence, and it is primarily synthesized by two key enzymes: L-cysteine desulphydrase (LCD) and D-cysteine desulphydrase (DCD) [5,6]. For instance, H_2S donors delayed the decline in fruit firmness and the color change while increasing the contents of reducing sugars, soluble proteins, and free amino acids during the post-harvest storage of strawberry fruits [7]. However, the mechanism by which H_2S affects the ripening of fruits and the post-cutting storage of cut flowers has only been reported in recent years. For example, studies have shown that the use of exogenous H_2S had a significant impact on the antioxidant enzyme activity of carnation cut flowers, thereby extending the post-harvest preservation period of the flowers [8]. However, the interaction relationship between H_2S and other plant growth regulators is still unclear. For example, the relationship with melatonin (MT) remains unknown.

MT is a key antioxidant molecule involved in horticultural crop postharvest regulation [9]. MT also plays a key role in the ripening, aging regulation, and post-harvest preservation of horticultural crops. For example, during the normal temperature storage of grape fruits, the application of exogenous MT delayed the decrease in soluble solids content and inhibited the accumulation of ROS and hydrogen peroxide (H_2O_2), thereby improving the post-harvest storage quality [10]. When applied to the preservation of cut roses, the antibacterial effect of MT was better than 8-hydroxyquinoline (8-HQ), consistent with a better condition of water absorption and an increase in flower diameter [11]. Furthermore, research conducted on wheat (*Triticum aestivum* L.) demonstrated that both MT and H_2S conferred tolerance to salt stress by potentiating antioxidant enzyme activities and upregulating the expression of sodium transport genes. In addition, MT employed multiple potential signaling pathways to alleviate salt stress, with the H_2S -mediated pathway representing one of them [12]. Whereas the regulatory mechanisms governing the interactions between MT and other gas transmitters, including H_2S , during the preservation of cut flowers remain to be elucidated.

For now, most current studies have focused on the individual use of MT and H_2S for cut rose preservation. However, the aspects of their joint use and the role of MT in regulating H_2S in order to enhance the freshness of cut roses are unknown. In this study, the 'Corolla' rose (*Rosa hybrida* L.) was selected as the plant material, and the solutions of sodium hydrosulfide (NaHS) and hypotaurine (HT) were applied as H_2S donor and scavenger, respectively. The effects of combined MT and H_2S treatment on the vase life and senescence process of cut roses, as well as the relationship and specific regulatory

mechanisms between them, were analyzed. The core scientific hypothesis proposed in this study is that MT and H₂S function synergistically as vital signal substances to jointly mitigate postharvest senescence and sustain the storage quality of cut roses, with H₂S serving as a key downstream component participating in the MT-associated regulatory cascade governing cut flower senescence. Unlike prior investigations predominantly concentrating on the independent physiological effects of individual signaling molecules, the current research attempts to elucidate the interactive crosstalk between MT and H₂S during cut rose preservation. This research established a new framework of coordinated regulation by H₂S and MT, opening up a new direction for post-harvest physiology research of cut flowers.

2. Materials and Methods

2.1. Plant Materials and Experimental Conditions

Cut roses 'Corolla' were harvested from a commercial grower in Lanzhou (Gansu Province, China) early in the morning and transferred to the laboratory immediately. Buckets containing the flower stems were covered with a plastic film shroud to minimize moisture loss during transportation. In the laboratory, the cut rose stems were re-cut to a length of 35 cm under distilled water to avoid air embolism. The upper 3 leaves were retained on each stem. Then, the base of the stem was inserted into distilled water for a 2 h rehydration treatment. Among the properly selected roses, a certain number of flower buds with an open degree of four, a net weight of 23 ± 3 g, and without any mechanical damage or infection were initially selected to ensure the uniformity of the experimental materials. Three independent biological replicates were set up for each treatment, with six flowers used in each replicate. The experiment was conducted under controlled environmental conditions at 20 ± 1 °C, $60 \pm 5\%$ relative humidity, and a 12 h photoperiod (08:00–20:00 h) provided by cool fluorescent lamps at a photosynthetic photon flux density (PPFD) of $15 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

In the control group, the vase solution was a base solution consisting of $20 \text{ g}\cdot\text{L}^{-1}$ sucrose and $200 \text{ mg}\cdot\text{L}^{-1}$ 8-HQ. In the MT treatment group, the base solution was used as the solvent to prepare MT (Shanghai Yuanye Bio-Technology Co., Ltd., Shanghai, China) solutions at concentrations of 0, 1, 25, 50, and $100 \text{ mg}\cdot\text{L}^{-1}$. The concentration gradient of MT was screened and finalized according to our preliminary experimental results. We used NaHS (Tianjin Kemiou Chemical Reagent Co., Ltd., Tianjin, China) as H₂S scavenger in this study, whose reagent purity is $\geq 98\%$. It can effectively avoid the interference of impurities. The H₂S treatment group's vase solution contained $50 \text{ mg}\cdot\text{L}^{-1}$ sodium bisulfide (NaHS) in the base solution. This working concentration of NaHS was determined based on previous studies [7]. The HT treatment group's base solution was prepared by adding $60 \text{ mg}\cdot\text{L}^{-1}$ hypotaaurine (HT, Shanghai Macklin Biochemical Co., Ltd., Shanghai, China) in the base solution, and the applied HT concentration was also optimized via preliminary trials. The treated petals were used as tissue samples for subsequent analysis.

2.2. Measurement of Morphological Indexes

Following the methods of Ren et al. (2017) with slight modifications, we determined vase life duration, maximum flower diameter, and relative fresh weight [13]. The phenotypic changes of cut roses, including petal color, shape, and texture, were recorded daily throughout the experimental period. Vase life was considered terminated when any of the following symptoms occurred: substantial petal abscission; severe water loss accompanied by abscission of more than half of the outer petals; prominent yellowing and desiccation of petal apices; or obvious softening of the flower head. The maximum flower diameter was defined as the maximum width of each flower, which was the average value obtained

from two cross-sectional measurements. The measurement was conducted using a vernier caliper. On the 6th d of the bottle culture, a unified measurement and calculation were carried out. Starting from the first d of vase insertion, the fresh weight of the cut rose branches was measured daily at a fixed time (13:00 h) using a platform scale for 7 consecutive d. The daily measurements were then used to calculate the increase in fresh weight change rate of the cut roses. The average values of the lifespan, maximum petal diameter, and fresh weight of 6 flowers in each treatment group were calculated.

2.3. Determination of Soluble Sugar and Soluble Protein

The soluble sugar content in the experiment was determined using the anthrone colorimetric method. The cut rose petals that were treated with different solutions for 6 d were retained as the following experimental materials. 0.5 g of fresh-cut rose samples were accurately weighed, homogenized with distilled water, transferred to a centrifuge tube, and made up to 10 mL before being treated in a boiling water bath for 30 min. After the samples cooled to room temperature, filtration was performed, and the filtrate was further made up to 100 mL. 0.3 mL of the sample solution was mixed with 1.7 mL of distilled water, and the absorbance value was detected at a 630 nm wavelength using a spectrophotometer. The actual content was calculated using the established standard curve.

The determination of soluble protein content was accomplished using the coomassie brilliant blue staining method. The cut rose petals that had undergone 6 d of bottle insertion liquid treatment were retained as experimental materials for the following experiment. The rose samples were rapidly frozen with liquid nitrogen and ground, then fully lysed with phosphate buffer solution (PBS, pH 7.4). After centrifugation at 4 °C and 12,000 g, for 20 min, the supernatant was collected. An appropriate amount of protein extract was reacted with the coomassie brilliant blue reagent, and the absorbance value was measured at a wavelength of 595 nm. The soluble protein content was calculated based on a pre-prepared bovine serum albumin standard curve.

2.4. Determination of Pro Content

The content of Pro was determined by the sulfosalicylic acid method. The petals of the cut roses that had undergone 6 d of different treatments were retained as the experimental materials. Firstly, 0.5 g of fresh rose petals were weighed and placed in a capped test tube. Five mL of 3% sulfosalicylic acid was added, and the tube was covered. The mixture was heated for 10 min. When the temperature dropped to room temperature, it was centrifuged at 4 °C, 12,000 g, for 10 min. Two mL of the supernatant was withdrawn and mixed with 2 mL of ice-cold acetic acid and 3 mL of acidic ninhydrin. In the control group, 2 mL of distilled water was used instead of the supernatant. The mixture was heated for 30 min. After cooling to room temperature (25 °C), 5 mL of toluene was added for thorough shaking to extract the red substance. After the layers were separated, the toluene layer was taken and measured at an absorbance of 520 nm.

2.5. Determination of Malondialdehyde (MDA) Content

The experiment used the thiobarbituric acid (TBA) method to determine the MDA content. The cut rose petals that were collected on the 6th d from different treatments were used as the experimental samples. 0.8 g of fresh rose petals were cut, and a small amount of 10% trichloroacetic acid (TCA) and quartz sand were added. The mixture was ground into a homogenate, which was then centrifuged at 4 °C for 15 min at 4000 g. Next, 3 mL of the supernatant was mixed with 0.5% TBA in a 10% TCA solution and boiled for 15 min, then quickly cooled. The cooled solution was transferred to a centrifuge tube and centrifuged at 4 °C for 15 min at 4000 g. The absorbance values of the supernatant were measured at 450, 532, and 600 nm. MDA con-

centration was calculated using the following formula based on the method described: $C_{MDA} = [6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}] \times V_{total} / (V_{sample} \times FW)$. Where C_{MDA} is the MDA concentration ($\text{nmol} \cdot \text{g}^{-1}$); A_{450} , A_{532} , and A_{600} refer to absorbance values at corresponding wavelengths; V_{total} means the total extraction volume; V_{sample} stands for the volume of supernatant taken for reaction; FW indicates the fresh weight of petal samples [14].

2.6. Determination of Enzyme Activities of Ascorbic Acid Peroxidase (APX), Peroxidase (POD), and Catalase (CAT)

The cut rose petals that were collected on the 6th d from different treatments were used as the experimental samples. The enzyme activity of APX was determined using the ascorbic acid (AsA) oxidation method. Firstly, the crude enzyme solution was extracted. 0.5 g of frozen rose samples under different treatments were taken and placed in a pre-cooled mortar, with 1 mL of enzyme extraction solution added, which contained 2 mM AsA, 2 mM ethylene diamine tetraacetic acid (EDTA), and 1% polyvinyl pyrrolidone (PVP) in 200 mM PBS (pH 7.8). A small amount of quartz sand was then added, and the mixture was ground on ice until a homogeneous suspension was obtained. Subsequently, centrifugation ($4\text{ }^{\circ}\text{C}$, 4000 g, 20 min) was performed to obtain the supernatant, which was the crude APX enzyme solution. Based on this, the activity was measured. The reaction system was 3 mL containing 50 mM pH 7.0 PBS buffer, 0.1 mM EDTA, 0.5 mM AsA, 0.1 mM H_2O_2 , and an appropriate amount of the samples to be tested was added to initiate the reaction. The absorbance was measured at 290 nm immediately, and the amount of the enzyme with an A_{290} reduction of 0.1 within 1 min was defined as 1 APX activity unit ($\text{U} \cdot \text{g}^{-1} \cdot \text{min}$).

The enzyme activity of POD was determined by the guaiacol colorimetric method. Firstly, the crude enzyme solution was extracted. 0.5 g of different treated frozen rose samples were taken and placed in a pre-cooled mortar. Five mL of PBS (pH 7.0, 200 mM) and a small amount of quartz sand were added. The mixture was ground on ice until homogenized. The resulting supernatant was centrifuged at $4\text{ }^{\circ}\text{C}$ and 10,000 g for 20 min. The obtained supernatant was the crude POD enzyme solution. Then, the absorbance value was measured: Three mL of the reaction system included 2.6 mL of a 50 mM pH 6.0 PBS, 200 μL of 3% H_2O_2 and 200 μL of the crude enzyme solution. A blank tube without H_2O_2 was used as the reference solution. Finally, H_2O_2 was added to initiate the reaction, and the absorbance value at 470 nm of the reaction system was measured immediately on a spectrophotometer. The absorbance value was read every 10 s and recorded every 5 min.

The activity of the CAT enzyme was determined by the ultraviolet absorption method for H_2O_2 . The preparation method for the crude enzyme solution was the same as that used for determining POD enzyme activity. During the activity measurement, the reaction system contained 2.86 mL of 50 mM pH 7.0 PBS and 40 μL of 3% H_2O_2 in a 3 mL volume. Then, 100 μL of the crude enzyme solution was added to initiate the reaction. The absorbance value at 240 nm was recorded every 10 s after the reaction began. The amount of the enzyme that reduced A_{240} by 0.1 within 1 min was defined as 1 CAT activity unit ($\text{U} \cdot \text{g}^{-1} \cdot \text{min}$).

2.7. RNA Isolation and Real-Time Reverse Transcription Quantitative PCR (qRT-PCR)

On the 6th d of different treatments, the petals from 3 cut roses were collected in each treatment group and were ground into powder in liquid nitrogen. The Mini BEST PLANT RNA extraction kit was used to extract total RNA from the ground powder. Then, cDNA synthesis was carried out. Specifically, at a reaction temperature of $37\text{ }^{\circ}\text{C}$ for 15 min, $85\text{ }^{\circ}\text{C}$ for 5 s, and $4\text{ }^{\circ}\text{C}$ for an indefinite period, the bright and appropriately concentrated RNA was reverse-transcribed into cDNA. The resulting product was then stored at $-20\text{ }^{\circ}\text{C}$ for future use.

qRT-PCR was performed using the LightCycler 480 real-time fluorescence quantitative PCR system (Roche Applied Science, Penzberg, Germany) and the SYBR Green Premix Pro Taq HS Premix kit. The primers used in qRT-PCR were designed by Primer Premier 5.0 and were listed in Supplemental Table S1. The tissue samples in each treatment were analyzed with three replicates to monitor possible sampling and experimental errors. The CT values were within the linear amplification range to ensure the reliability of the data. The internal reference gene was *RhActin*. The amplification efficiency of each gene-specific primer pair was evaluated via serial 5-fold dilutions of pooled cDNA templates to generate standard curves before formal qRT-PCR detection. Only primers with amplification efficiency ranging from 90.0% to 110.0% and correlation coefficient $R^2 > 0.98$ were adopted for subsequent quantification. The specificity of all primers was verified by melting curve analysis following each qRT-PCR run, which produced a single sharp peak for each target amplicon. In addition, agarose gel electrophoresis was performed to confirm a single expected band size for each PCR product, eliminating non-specific amplification and primer-dimer interference (Supplement Figure S1). Relative gene expression levels were calculated using the classic $2^{-\Delta\Delta C_t}$ comparative Ct method.

2.8. Determination of ROS Content

The petals of the cut roses treated with different solutions for 6 d were collected as the plant samples. Firstly, 0.5 g of fresh rose petals was taken as the sample. Acetone was added to a pre-cooled mortar, and the petals were ground at an ice bath to form a homogenate, then diluted to 5 mL with pre-cooled acetone. Next, the mixture was centrifuged for 20 min (4 °C, 12,000 g) to obtain the supernatant. Then, 1 mL of the supernatant, + 0.1 mL of $TiCl_4$ + 0.2 mL of concentrated ammonia water were added successively to a 5 mL centrifuge tube and mixed well. Then, the tube was centrifuged at 5000 g for 10 min, the supernatant was removed, the pigments were removed, and the precipitate was washed with acetone 2–3 times. Three mL of 2 MH_2SO_4 was added to dissolve the precipitate. After complete dissolution, the absorbance was measured at a 412 nm wavelength; then the H_2O_2 content was calculated through the standard curve.

The content of superoxide anion $O_2^{\cdot-}$ was determined by the hydroxylamine reduction method. Firstly, 1 g of fresh rose petals was taken and added to a mortar with PBS (0.05 M, pH 7.8). The solution was then made up to 5 mL and homogenized at 4 °C under an ice bath. After centrifugation at 12,000 g for 20 min at 4 °C, the supernatant obtained was the crude enzyme extract. 0.5 mL of the extract was added to a centrifuge tube, and 0.5 mL of PBS (0.05 M, pH 7.8) and 1 mL of hydroxylamine (1 mM) were mixed. Then, it was placed at 25 °C for 1 h, followed by the addition of 1 mL of 1-naphthylamine (7 mM) and 1 mL of sulfanilic acid (17 mM). The mixture was shaken up and down and then incubated at 25 °C for 20 min. Finally, the absorbance value was measured at a wavelength of 530 nm.

2.9. Determination of 1-Aminocyclopropane-1-carboxylic Acid (ACC) Content and Enzyme Activities of ACC Synthase (ACS) and ACC Oxidase (ACO)

The determination of ACC content was performed following previously reported protocols [15]. Petals from three randomly selected cut roses per treatment were sampled and ground into fine powder with liquid nitrogen. Briefly, 3 g of the petal powder was homogenized in 10 mL of 95% alcohol, followed by centrifugation at $4000\times g$ for 20 min. The resulting supernatant was concentrated at 55 °C, resuspended with 0.5 mL trichloromethane and 5 mL distilled water, and centrifuged again at $4000\times g$ for 20 min. For the assay tube, 0.5 mL supernatant was mixed with 10 μM $HgCl_2$ and 0.5 mL of a 2:1 (v/v) solution of 5% $NaClO$ + saturated $NaOH$. The standard tube was prepared identically except for the absence of ACC. After a 30-min incubation on ice, both tubes were vortexed, and 1 mL of headspace gas was collected to quantify ethylene concentration using gas

chromatography (GC-17A, Shimadzu, Kyoto, Japan). Activities of ACS and ACO were assayed in accordance with previously established procedures [16].

2.10. Determination of Endogenous H_2S Content

The petals of the cut roses treated with different solutions for 6 d were collected as the plant samples. The frozen samples were ground into powder using liquid nitrogen, and 0.5 g of the powder was accurately weighed and transferred to a pre-cooled centrifuge tube. Then, 2 mL of PBS containing 200 mM AsA and 0.1 mM EDTA- Na_2 (50 mM, pH 6.8) was added and vortexed to mix. The mixture was then centrifuged at 4 °C (12,000 g, 15 min), the supernatant was collected and transferred to a clean test tube and left on ice for later use. Next, 2 mL of a mixed solution containing 100 mM PBS (pH 7.4), 2 mM pyridoxal phosphate (PLP), and 10 mM L-cysteine was added to the supernatant, vortexed to mix, and then incubated at 25 °C in the dark for 30 min. Subsequently, 0.15 mL of 5 mM dimethylphenidine (DMPD) solution and 0.15 mL of a 50 mM ammonium ferric sulfate solution were added to the reaction system, vortexed to mix, and incubated at 25 °C in the dark for 15 min. Finally, the absorbance of the reaction solution at 667 nm was measured. The same volume of ultrapure water without adding zinc acetate was used as a blank control. A standard curve was established using the standard solution, and the content of the target substance in the sample was calculated.

2.11. Determination of Enzyme Activities of LCD and DCD

The cut rose petals collected on the 6th d from different treatments were used as experimental samples. The activities of LCD and DCD were determined via the H_2S colorimetric method. Firstly, the crude enzyme solution was extracted. 0.5 g of frozen rose samples from different treatments were placed in a pre-cooled mortar, and 1 mL of enzyme extraction solution (200 mM PBS, pH 7.5, containing 2 mM EDTA and 1% PVP) was added. A small amount of quartz sand was supplemented, and the mixture was fully ground on ice to obtain a homogeneous suspension. After centrifugation at 4 °C and 4000 g for 20 min, the supernatant was collected as the crude enzyme solution for LCD and DCD determination.

The LCD activity reaction system was 3 mL, containing 100 mM Tris-HCl (pH 9.0), 2.5 mM DTT, 0.8 mM L-cysteine, and an appropriate amount of crude enzyme solution to initiate the reaction. After incubation, the absorbance was measured at 670 nm. One unit of LCD activity ($U \cdot g^{-1} \cdot min$) was defined as the enzyme amount required to produce 1 nmol H_2S per minute.

The DCD activity reaction system was 3 mL, containing 100 mM Tris-HCl (pH 8.0), 2.5 mM DTT, 0.8 mM D-cysteine, and an equal amount of crude enzyme solution to initiate the reaction. The absorbance was determined at 670 nm with the same reaction and colorimetric conditions. The definition of DCD activity unit was consistent with that of LCD ($U \cdot g^{-1} \cdot min$). All samples were determined with three biological replicates to ensure experimental accuracy.

2.12. Statistical Analysis

The data were analyzed using the software SPSS 22.0 (SPSS Inc., Chicago, IL, USA). All datasets were first subjected to a normality test and homogeneity of variance test to verify the prerequisites for one-way analysis of variance (ANOVA). Values were expressed as mean $\pm$ standard error (SE) of three independent experiments, with three biological replicates in each experiment. Multiple comparisons were handled by applying Tukey's honestly significant difference (HSD) test method to determine the significance of the results between different treatments, with the significance level set at $p < 0.05$.

3. Results

3.1. Role of MT on Post-Harvest Preservation of Cut Roses ‘Corolla’

As shown in Table 1, the vase lives of the cut roses treated with different concentrations of MT were longer than that of the control. Especially, the vase life of the cut roses treated with 50 mg·L⁻¹ MT was the longest, ending on the 8.2th d and lasting 2 d longer than the control. In contrast, the 1 mg·L⁻¹ and 25 mg·L⁻¹ treatment groups showed no significant difference in the flowering period of the cut roses, both wilting on the 7th d (Table 1).

Table 1. Effects of different concentrations of MT on vase life of cut roses ‘Corolla’.

Treatments	Vase Life (d)
Control	6.2 ± 0.1 d
1 mg·L ⁻¹ MT	7.7 ± 0.1 bc
25 mg·L ⁻¹ MT	7.8 ± 0.1 ab
50 mg·L ⁻¹ MT	8.2 ± 0.2 a
100 mg·L ⁻¹ MT	6.9 ± 0.1 cd

The experimental data are presented as mean ± standard error, obtained from 3 biological replicates ($n = 3$, with 6 flowering stems measured in each replicate). Significant differences among means were determined by Tukey’s HSD test, and values within the same column marked with different lowercase letters differ significantly at $p < 0.05$.

In order to investigate the regulatory effect of exogenous MT on the post-harvest preservation of cut roses, we treated the roses with different concentrations of MT (0, 1, 25, 50, and 100 mg·L⁻¹). As can be clearly seen in Figure 1, in the later stage of flower opening, the cut roses treated with 50 mg·L⁻¹ MT maintained better conditions, with intact petal morphology and no wilting phenomenon (Figure 1A). In addition, the average maximum flower diameter of the 50 mg·L⁻¹ MT treatment group reached 12.2 cm, which was 43.5% higher than that of the control group. The average maximum flower diameters in the 1 mg·L⁻¹ MT and 25 mg·L⁻¹ MT treatment groups also increased, reaching 9.6 cm and 10.2 cm, respectively. However, compared with the 50 mg·L⁻¹ MT treatment group, they decreased by 21.3% and 16.4%, respectively. (Figure 1B). Compared with the control group, the 1, 25, and 50 mg·L⁻¹ MT treatments significantly promoted the maintenance of relative fresh weight in cut roses, with the 50 mg·L⁻¹ MT showing the best effect, while the 100 mg·L⁻¹ MT treatment did not improve the fresh weight (Figure 1C). Moreover, the fresh weight change rate of cut roses in the 1 and 100 mg·L⁻¹ MT treatments began to decline on the second d, but this rate in the 50 mg·L⁻¹ MT treatment gradually decreased on the third d. Notably, the content of soluble sugar, soluble protein, and Pro in the 50 mg·L⁻¹ MT-treated samples reached their peak, significantly higher than those in the other treatment groups (Figure 1D–F). When compared with the control cut rose samples, we found that the 25, 50, and 100 mg·L⁻¹ MT treatments significantly decreased the MDA content. Specifically, the MDA content in the 50 mg·L⁻¹ MT treatment group was the lowest (Figure 1G). Therefore, 50 mg·L⁻¹ was chosen as the appropriate MT concentration for further study.

Thus, the effects of each treatment group were most pronounced at this time point, with the most significant divergences in plant phenotype and relative fresh weight observed among groups on d 6. Additionally, the differences among different treatments were most distinguishable on the 6th d, which could directly reflect the physiological and molecular changes brought about by the treatments and facilitate comparative analysis between groups. Hence, the 6th d was selected as the sole time point for the subsequent biochemical and molecular index tests.

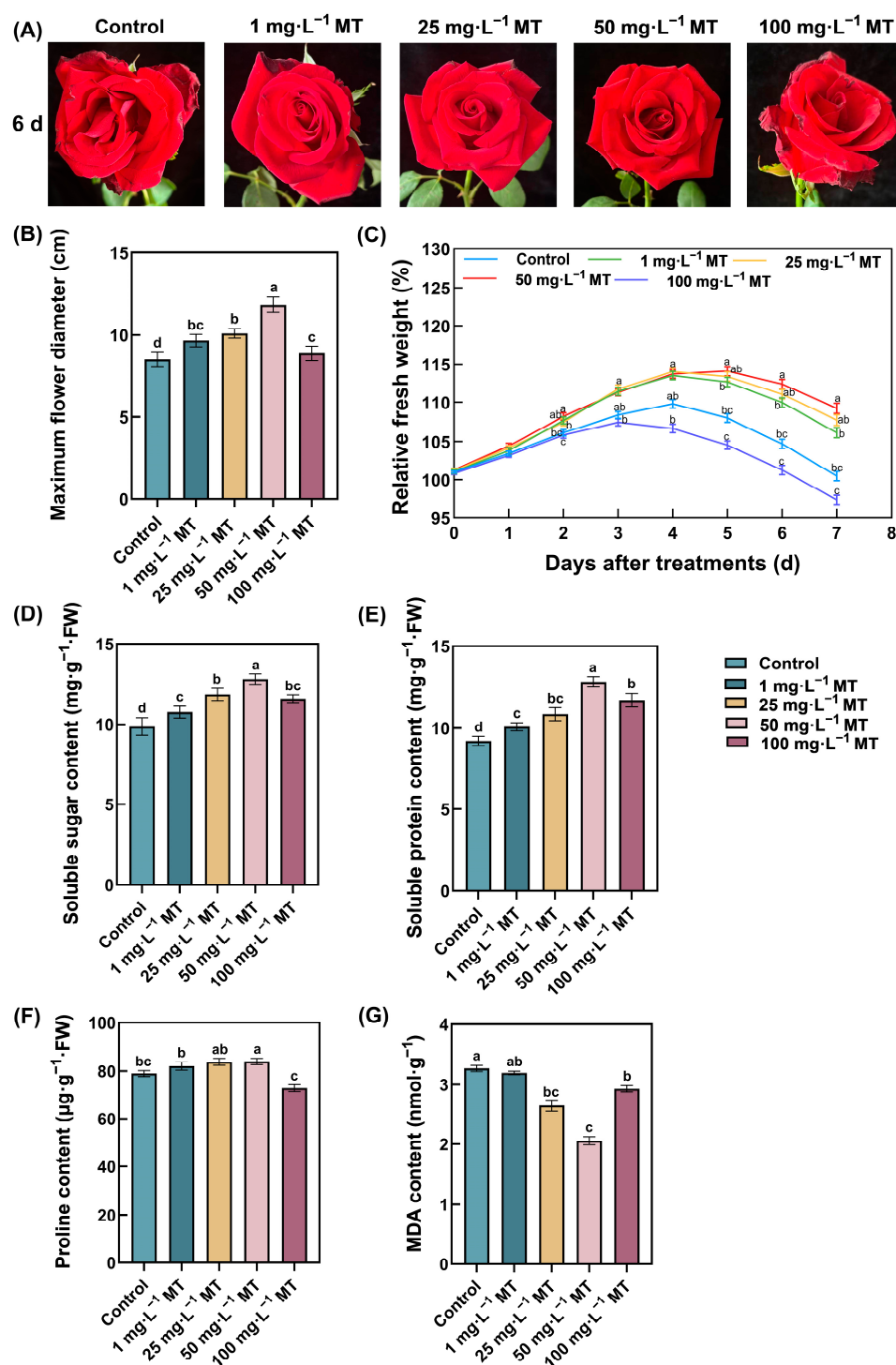


Figure 1. Effects of different concentrations of MT treatment on the post-harvest preservation of cut roses 'Corolla'. (A) Phenotype; (B) Maximum flower diameter; (C) Fresh weight change rate; (D) Soluble sugar content; (E) Soluble protein content; (F) Pro content; (G) MDA content. The values (mean $\pm$ standard error) are the average values of six biological replicate samples ($n = 3$). Bars with different lowercase letters indicate significant differences at $p < 0.05$, as determined by Tukey's HSD test.

3.2. MT and H₂S Promoted Post-Harvest Freshness of Cut Roses 'Corolla'

As shown in Table 2, the cut roses treated with MT, H₂S, and MT + H₂S had longer vase lives compared with the control group. In particular, the cut roses treated with MT + H₂S had a vase life of 8.2 d, which was 2 d longer than the control group (Table 2).

Table 2. The combined treatment of MT and H₂S on the extension of the vase life of cut roses ‘Corolla’.

Treatments	Vase Life (d)
Control	6.2 ± 0.2 cd
MT	7.7 ± 0.1 bc
H ₂ S	7.9 ± 0.1 ab
MT + H ₂ S	8.2 ± 0.1 a

The experimental data are presented as mean ± standard error, obtained from 3 biological replicates ($n = 3$, with 6 flowering stems measured in each replicate). Significant differences among values in the same column were assessed by Tukey’s HSD test, and values marked with different lowercase letters differ significantly at $p < 0.05$.

As shown in Figure 2, the cut roses treated with MT + H₂S exhibited excellent condition, maintaining intact petal morphology without wilting (Figure 2A). In the MT + H₂S treatment group, the average maximum petal diameter was 12.6 cm, a 40.1% increase compared with the control group. Furthermore, the average maximum petal diameter under MT + H₂S treatment was also significantly increased compared with that under the individual use of MT or H₂S treatment (Figure 2B). The relative fresh weight of cut roses in the control group declined rapidly after reaching its peak and dropped below the initial weight by d 3, remaining significantly lower than that in all treatment groups. Among the treatment groups, the MT + H₂S treatment maintained the highest relative fresh weight throughout the experimental period, which was significantly higher than that observed under MT or H₂S treatment alone (Figure 2C). Additionally, the content of soluble sugar, soluble protein, and Pro in the combined MT + H₂S treated group was the highest (Figure 2D–F). However, the MDA content under MT + H₂S treatment was significantly lower than that under MT treatment alone (Figure 2G).

3.3. The Influences of MT and H₂S on ROS Accumulation and Antioxidants in Cut Roses

In order to further investigate the antioxidant effects of MT and H₂S, we measured the enzyme activities of APX, POD, and CAT under MT, H₂S, and MT + H₂S treatments. As shown in Figure 3, the enzyme activities of APX, POD, and CAT in the MT + H₂S treated samples reached the highest values, which were 130.0%, 200.1%, and 78.3% higher than the control. Moreover, MT + H₂S obviously increased the enzyme activities of APX, POD, and CAT compared with MT treatment alone. Among them, the APX enzyme activity in the MT + H₂S treatment was increased by 53.3% and 21.2% when compared with MT and H₂S treatment alone, respectively (Figure 3A–C).

Subsequently, we examined the expression levels of the antioxidant genes *RhAPX*, *RhPOD*, and *RhCAT*. As shown in Figure 3D–F, the expression levels of these genes in all three treatment groups were higher than those in the control group. It is noteworthy that the transcripts of *RhAPX*, *RhPOD*, and *RhCAT* under MT + H₂S treatment reached their peak, all of which were higher than those under MT or H₂S treatment alone (Figure 3D–F). Additionally, MT treatment significantly improved the expression of *RhAPX* and *RhPOD* compared with H₂S treatment (Figure 3D,E).

Based on the measured data, it was found that both the content of H₂O₂ and O₂^{·−} was downregulated by MT, H₂S, and MT + H₂S treatments compared with the control (Figure 3G,H). MT + H₂S treatment significantly reduced the content of H₂O₂ and O₂^{·−} by 61.4% and 20.9%, respectively, compared with the control group. Additionally, the content of O₂^{·−} treated by MT + H₂S was lower than that treated by MT or H₂S alone, with reductions of 11.7% and 9.9%, respectively (Figure 3G,H).

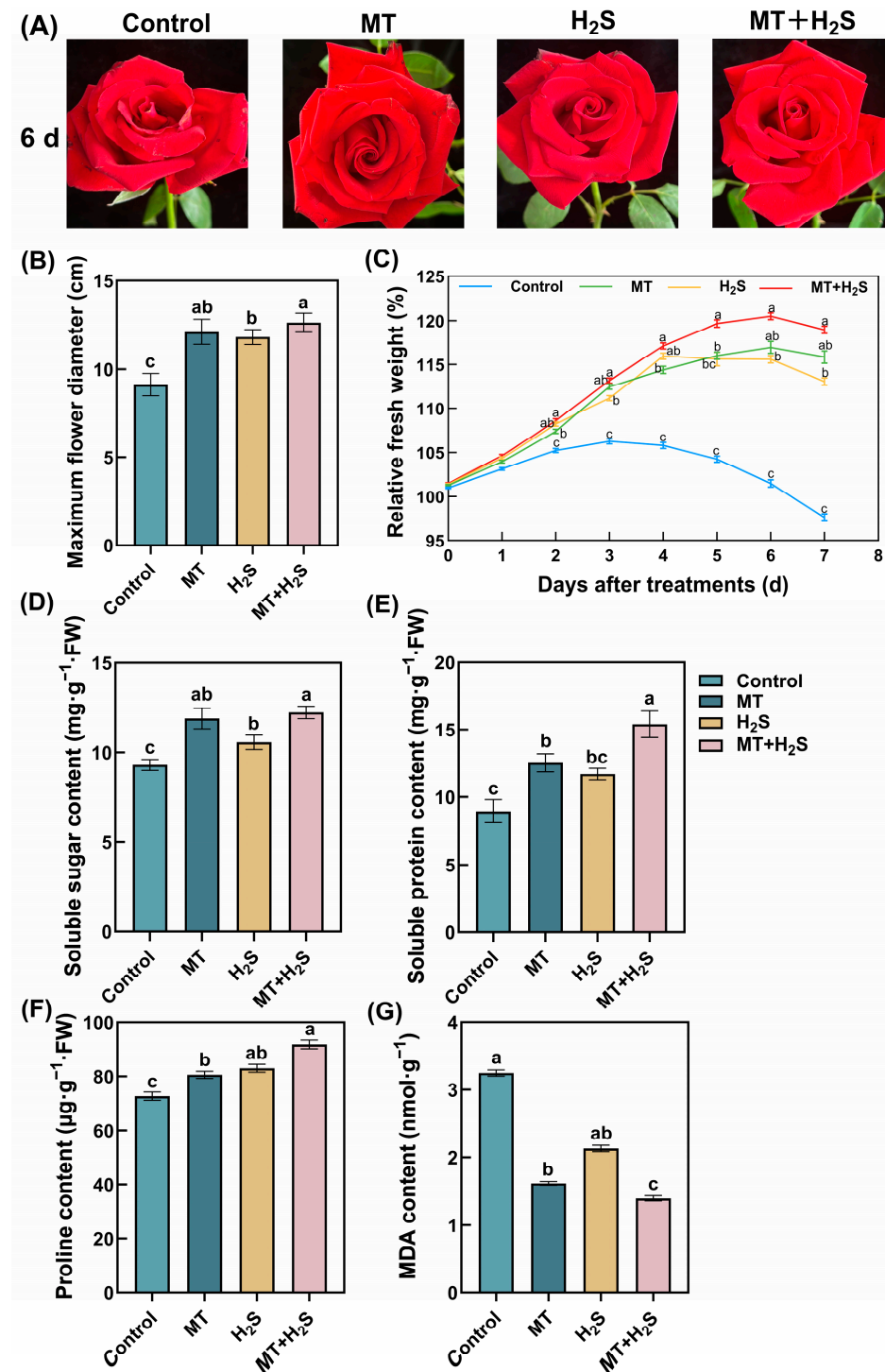


Figure 2. The effects of MT treatment group, H₂S treatment group, and their combined action on the post-harvest preservation of cut roses. (A) Phenotype; (B) Maximum flower diameter; (C) Fresh weight change rate; (D) Soluble sugar content; (E) Soluble protein content; (F) Pro content; (G) MDA content. The values (mean ± standard error) are the average values of six biological replicate samples ($n = 3$). Bars with different letters indicate statistically significant differences ($p < 0.05$) as determined by Tukey's HSD test.

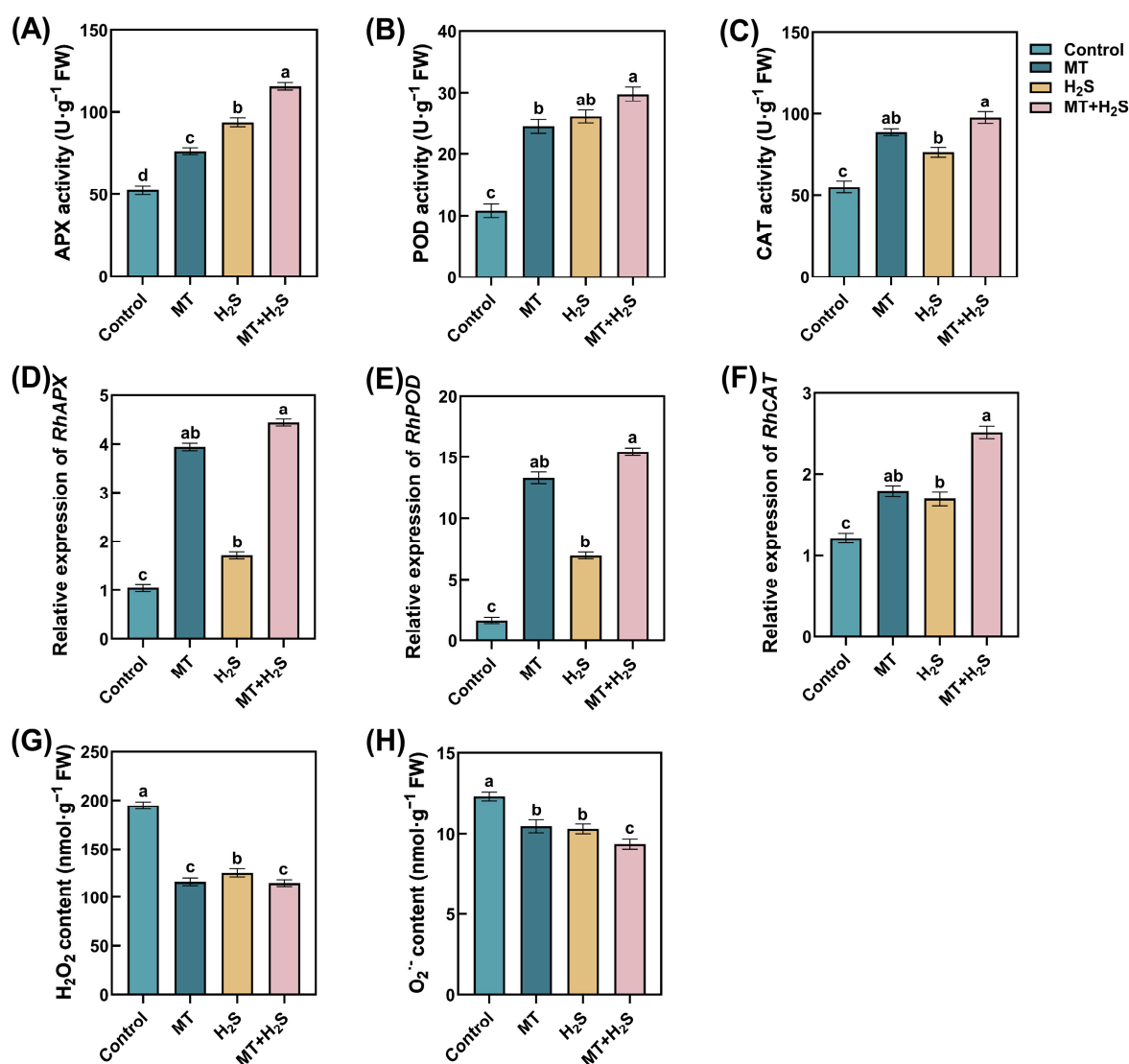


Figure 3. Effects of MT treatment, H₂S treatment, and their combined treatment on the APX, POD, and CAT activities, the gene expression levels of APX, POD, CAT, and ROS content of cut roses. (A) The activity of APX; (B) The activity of POD; (C) The activity of CAT; (D) The relative expression of the *RhAPX*; (E) The relative expression of the *RhPOD*; (F) The relative expression of the *RhCAT*; (G) The content of H₂O₂; (H) The content of superoxide anion (O₂^{·-}). The values (means ± SE) are the averages of three biological replicates ($n = 3$). Bars not sharing the same letters indicate statistically significant differences as determined by Tukey's HSD test ($p < 0.05$).

3.4. MT Activated Up-Stream of H₂S During the Post-Harvest Preservation of Cut Roses

As shown in Table 3, the vase life of cut roses 'Carola' varied significantly across different treatments. Both the MT treatment and the H₂S treatment significantly prolonged the vase life. Between the two, the MT treatment had the most pronounced effect, achieving a vase life of 8.3 d, followed by the H₂S treatment. Notably, the MT + HT treatment group extended the vase life to 7.9 d, significantly longer than the HT treatment group (Table 3).

Table 3. Effects of MT, H₂S, HT, and MT + HT treatments on the vase life of cut roses ‘Corolla’.

Treatments	Vase Life (d)
Control	6.1 ± 0.1 d
MT	8.3 ± 0.1 a
H ₂ S	8.1 ± 0.1 ab
HT	6.2 ± 0.1 cd
MT + HT	7.9 ± 0.1 bc

The data are presented as mean ± standard error (SE) from three biological replicates ($n = 3$, with six stems measured per replicate). Significant differences among treatments were determined using Tukey’s HSD test, and values within the same column marked with different lowercase letters differ significantly at $p < 0.05$.

In order to further investigate the relationship between MT and H₂S, we set up different treatment groups, including control, MT, H₂S, HT, and MT + HT to treat cut roses. As shown in Figure 4, the petals of the HT treatment group were severely withered and had scars, the outer petals of the MT + HT treatment group were curled, and the cut roses in the MT treatment group maintained a better condition, with intact petal shapes and no wilting phenomenon (Figure 4A). The maximum flower diameter in the MT + HT treatment group was significantly lower than that in the MT and H₂S treated groups. Specifically, the average maximum flower diameter in the MT + HT treatment group was 10.1 cm, which was 19.4% lower than that in the MT treatment group. In addition, the maximum flower diameter value of the HT treatment group was the lowest (Figure 4B). Additionally, the MT treatment maintained the highest relative fresh weight throughout the entire period, which was significantly higher than that of the MT + HT treatment (Figure 4C).

From Figure 4, it was observed that the content of soluble sugar, soluble protein, and Pro in the HT treatment group was significantly decreased compared with the MT treatment group. However, the MDA content in the HT-treated petals was significantly increased. In addition, the content of soluble sugar and soluble protein in the MT + HT treatment group was lower than that in the MT treatment group, with decreases of 10.3% and 27.3%, respectively (Figure 4D,E). Regarding the Pro content in Figure 4, the value under the MT + HT treatment was higher than that under the MT treatment alone. Furthermore, when compared with the MT treatment alone, the MDA content in the MT + HT treated plants was raised by 35.1% (Figure 4F,G).

The ACC content of the cut roses began to accumulate before the release of ethylene, reaching its peak value at the 6th d and then decreasing. The HT treatment had a significant effect on the ACC content of cut roses during the blooming period. The ACC content in the HT-treated cut roses started to rise sharply from the 4th d, while the ACC content in the MT, H₂S, and MT + HT treated cut roses presented moderate increases. The ACC content of the cut roses in the HT treatment group was higher than that in the control group throughout the entire treatment process. And on the 6th d, there was a significant difference compared with the control group, being 6.4% higher than the control group. (Figure 3H). Although a similar trend of increased ACC concentration occurred in all samples, lower ACC concentrations were detected in the MT and H₂S treatments in comparison with the controls throughout the whole shelf life, while the MT + HT treatment showed intermediate values. In summary, HT treatment accelerated the accumulation of ACC during the vase life of the cut roses, while MT and H₂S treatments suppressed this increase.

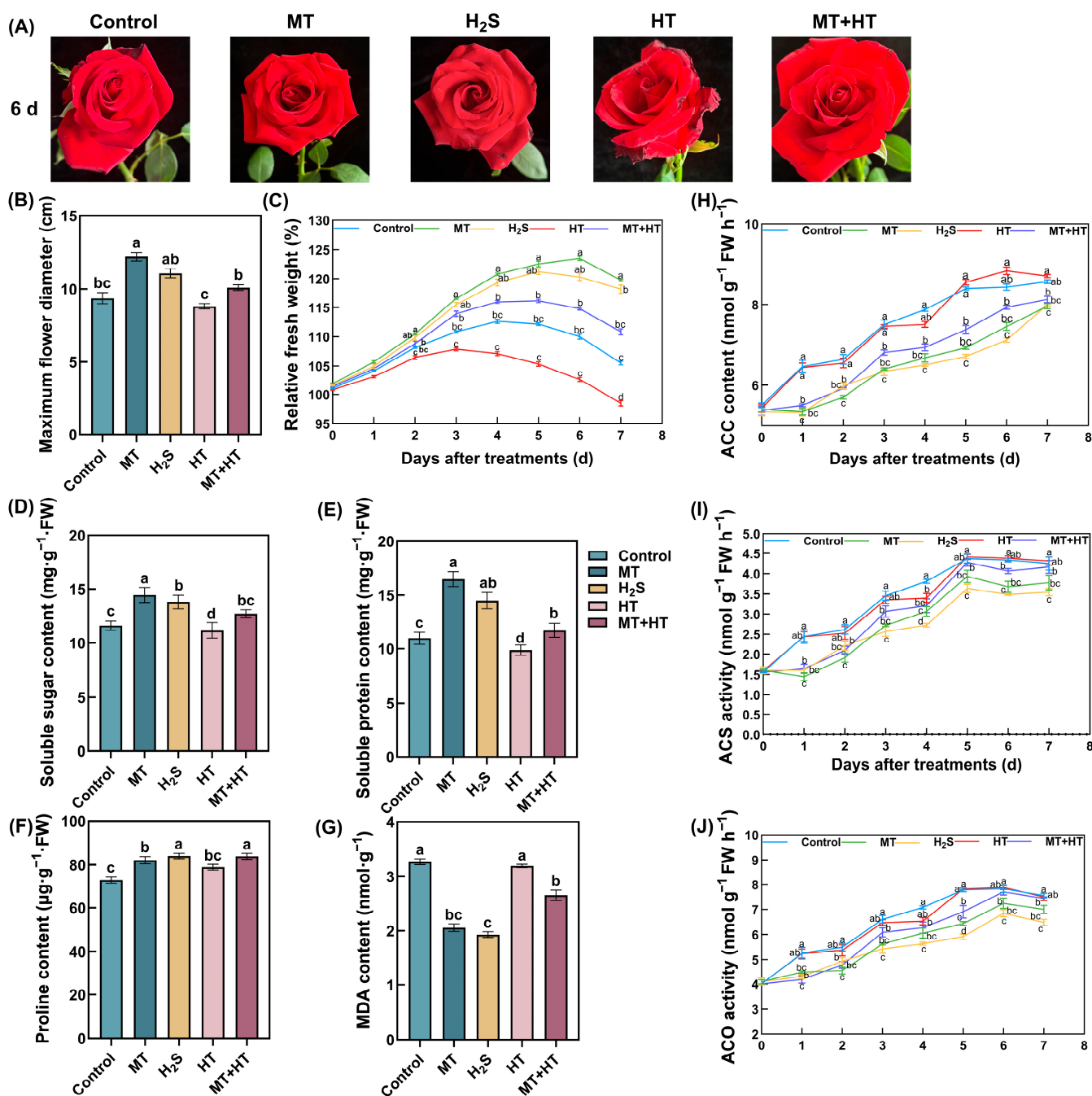


Figure 4. Effects of MT treatment group, H₂S treatment group, HT treatment group, and the combined treatment group of MT and HT on post-harvest preservation of cut roses. The five different treatment groups of cut roses were used for photography and measurement. (A) Phenotype; (B) Maximum flower diameter; (C) Fresh weight change rate; (D) Soluble sugar content; (E) Soluble protein content; (F) Pro content; (G) MDA content; (H) The content of ACC; (I) The activity of ACS; (J) The activity of ACO. The values (means $\pm$ SE) are the averages of three biological replicates ($n = 3$). Bars not sharing the same letters indicate statistically significant differences as determined by Tukey's HSD test ($p < 0.05$).

To further explore the mechanism of different treatments controlling ethylene synthesis in cut roses, we detected the enzyme activities of ACS and ACO. The enzyme activities showed no significant difference between all treated cut roses and the control at 0 d. Subsequently, the ACS activity in the control group and the HT treatment group, respectively, increased on the 6th d and reached its peak, being 4.4 and 4.4. On the 7th d, it slightly

decreased, with a decrease of 4.0% and 2.3% compared to the 6th d. The ACS activity of the MT- and H₂S-treated cut roses showed a similar tendency, but the values were obviously lower than the control cut roses, with the H₂S treatment being the lowest, followed by MT (Figure 4I). The ACO activity showed an analogous variation trend with the ACS activity. Furthermore, from the 4th d to the 7th d, the ACO activity in the MT + HT treatment group was consistently lower than that in the HT treatment group, and the greatest difference was observed on the 5th d, with a decrease of 9.5% compared to HT. Meanwhile, the MT and H₂S treatment groups were significantly lower than the control group and showed a notable decline on 6 d (Figure 4J).

3.5. MT Activated Up-Stream of H₂S to Restrain ROS Accumulation in Cut Roses

As shown in Figure 5, HT significantly decreased the enzyme activities of APX, POD, and CAT compared with the control. Furthermore, the enzyme activities of APX, POD, and CAT in the MT + HT treatment group were all lower than those in the MT treatment group, with reductions of 31.6%, 16.7%, and 38.9%, respectively. Specifically, the activities of APX, POD, and CAT enzymes in the MT + HT treatment group were, respectively, 40.9%, 22.2%, and 38.9% lower than those in the H₂S treatment group alone (Figure 5A–C).

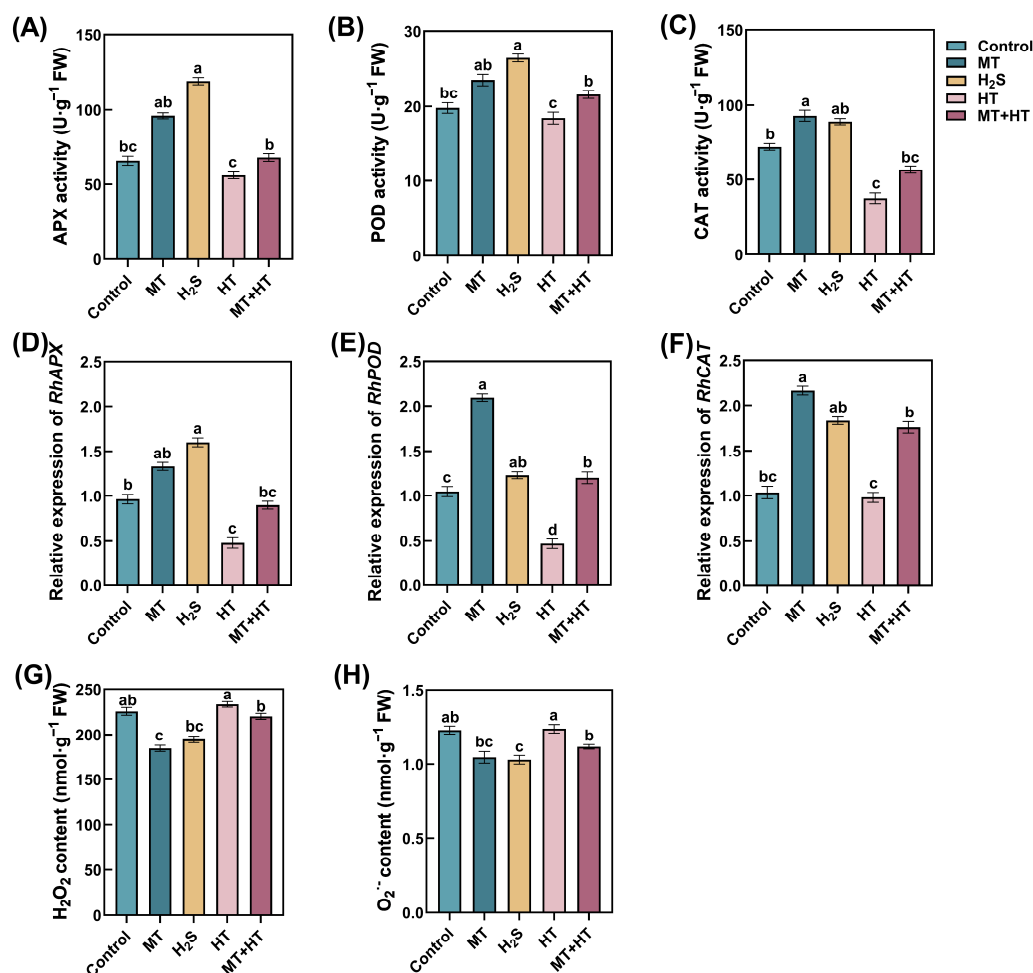


Figure 5. Effects of five treatments on the APX, POD, and CAT activities, the gene expression levels of APX, POD, and CAT, and ROS content of cut roses. (A) The activity of APX; (B) The activity of POD; (C) The activity of CAT; (D) The relative expression of the *RhAPX*; (E) The relative expression of the *RhPOD*; (F) The relative expression of the *RhCAT*; (G) The content of H₂O₂; (H) The content of O₂^{·-}. The values (means ± SE) are the averages of three biological replicates (*n* = 3). Bars not sharing the same letters indicate statistically significant differences as determined by Tukey's HSD test (*p* < 0.05).

Next, we examined the expression levels of the antioxidant genes *RhAPX* and *RhPOD*. As shown in n. 5D–F, the expression levels of these genes in the HT treatment were significantly lower than those in the control group. Moreover, the transcriptional abundances of *RhAPX*, *RhPOD*, and *RhCAT* in the MT + HT treatment group were lower than those in the MT treatment alone, with reductions of 37.0%, 40.5%, and 19.0%, respectively (Figure 5D–F).

Compared with the control group, the MT, H₂S, and MT + HT treatments decreased the content of H₂O₂ and O₂^{•−}. Additionally, the content of H₂O₂ and O₂^{•−} in the MT + HT treatment was lower than that in the HT treatment, with reductions of 4.6% and 7.7%, respectively (Figure 5G,H).

3.6. MT Improved H₂S Production During the Storage of Cut Roses

By measuring the endogenous H₂S content of cut roses, we found that compared with the control group, the application of MT and H₂S significantly increased the H₂S content in cut roses, with increases of 18.6% and 25.4%, respectively. Furthermore, the H₂S content in the MT + HT treatment group was significantly decreased compared with that in the individual treatments of MT and H₂S (Figure 6A).

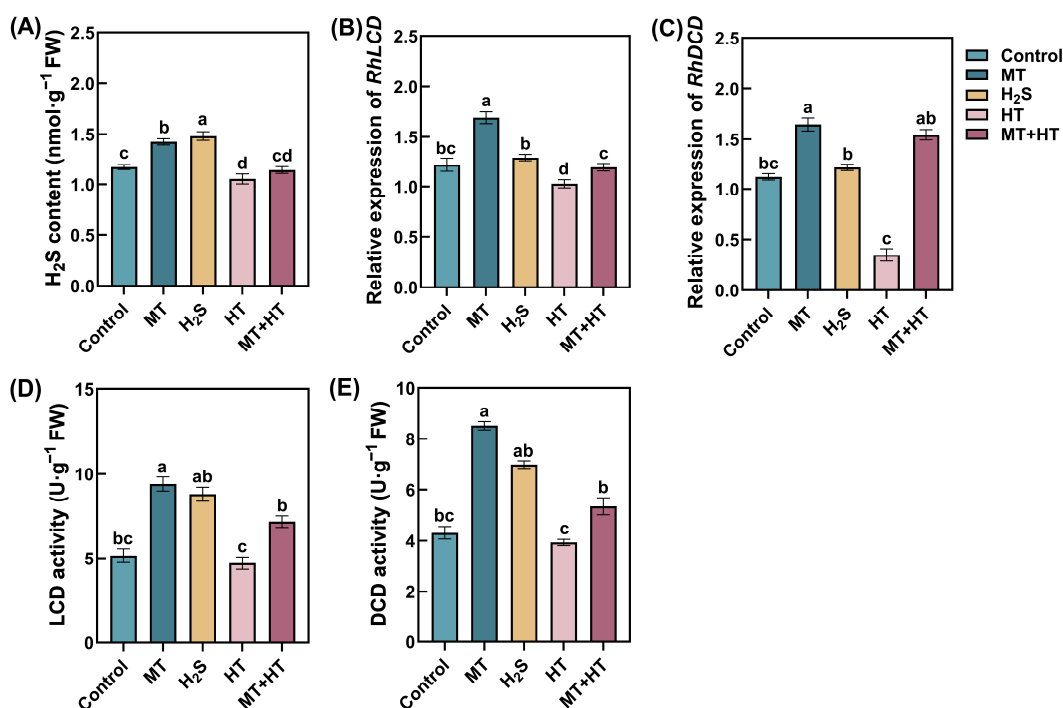


Figure 6. The effects of 5 different treatments on H₂S metabolism in cut rose petals. (A) H₂S content; (B) The relative expression of the *RhLCD*; (C) The relative expression of the *RhDCD*; (D) The activity of LCD; (E) The activity of DCD. The values (means ± SE) are the averages of three biological replicates ($n = 3$). Bars not sharing the same letters indicate statistically significant differences as determined by Tukey's HSD test ($p < 0.05$).

We also measured the expression levels of the related key H₂S synthesis genes. From the data, it was observed that the changes in the expression levels of *RhLCD* and *RhDCD* in the cut rose samples subjected to the different treatments were in line with the changes observed in the H₂S content experiment. The expression levels of the two genes treated with either MT or H₂S alone were significantly higher than those in the control group. Additionally, the expression levels of these genes in the MT + HT treatment group were lower than those in the MT treatment alone, with reductions of 25.1% and 6.3%, respec-

tively. Under HT treatment, the expression levels of *RhLCD* and *RhDCD* were significantly decreased compared with those in the control (Figure 6B,C).

We further analyzed the activities of the key H_2S -synthesizing enzymes, LCD and DCD. As shown in Figure 6D,E, the trends in enzyme activities were consistent with the changes in *RhLCD* and *RhDCD* transcript levels. The activities of both LCD and DCD were significantly enhanced by MT treatment, reaching the highest levels among all groups, followed by the H_2S treatment. On the contrary, the treatment group using HT alone significantly inhibited the activities of these two enzymes, showing the lowest values. Compared with the MT + HT treatment group, it decreased by 37.3% and 29.6%, respectively.

Notably, the combined MT + HT treatment partially alleviated the HT-induced inhibition, maintaining significantly higher LCD and DCD activities than the HT-only group, though they remained lower than those in the MT-alone treatment (Figure 6D,E).

4. Discussion

In recent years, numerous studies have delved deeply into the physiological mechanisms underlying the preservation of cut flower quality after harvest [17,18]. However, there are numerous factors that affect the blooming and aging of cut flowers. For instance, once detached from the mother plant, cut flowers undergo a gradual loss of water, nutrients, and energy, leading to petal wilting and abscission and ultimately resulting in the loss of ornamental value [19]. Previous studies have shown that MT can effectively delay senescence and maintain the postharvest quality of fruits and vegetables. For example, treating postharvest sweet cherry fruits with MT inhibits their decay rate, respiration rate, and mass loss, while enhancing their antioxidant enzyme activities, thereby effectively delaying fruit senescence [20]. This study shows that cut rose flowers treated with $50 \text{ mg}\cdot\text{L}^{-1}$ MT had the longest vase life (Table 1). At the same time, the maximum flower diameter and the fresh weight change rate of this treatment were significantly better than those of the other treatment groups, effectively maintaining petal cell turgor pressure and metabolic activity (Figure 1B,C). This further confirms that the $50 \text{ mg}\cdot\text{L}^{-1}$ MT treatment can extend the flowering period by increasing water absorption and reducing fresh weight loss. Notably, its MDA content showed a decreasing trend (Figure 1G), and the MDA content was the lowest among all treatments. Previous studies also supported the importance of MT concentration selection. For example, in cut carnation research, high-concentration MT treatment led to a significant decrease in vase life and an increase in MDA content [20]. In the study on 'Bartzella' cut flowers, treatment with $100 \mu\text{MMT}$ increased flower diameter and water absorption capacity but did not extend vase life [14]. It reported that $50 \mu\text{MMT}$ significantly extended the vase life and increased the maximum flower diameter of the herbaceous peony cultivars 'Da Fu Gui' and 'Qi Hua Lu Shuang' [21]. The differences in these results may be related to the variety of cut flowers, and they also indicate that an excessively high concentration of MT will reduce the preservation effect of the cut flowers, revealing the importance of the ratio of hormone concentrations. Therefore, in this study, $50 \text{ mg}\cdot\text{L}^{-1}$ was selected as the appropriate MT concentration for the subsequent experiments.

Previous studies reported that some small-molecule compounds had been confirmed to be involved in maintaining the preservation quality of cut flowers and extending their vase life [22]. For example, endogenous H_2S delayed post-preservation senescence in daisies by enhancing antioxidant capacity and maintaining energy status [23]. As a signaling molecule, H_2S is transported within cells via simple diffusion without the need for specific carriers. Therefore, it is widely involved in various physiological processes in plants, including diverse protective mechanisms. For example, It showed that HT treatment attenuated the resistance of postharvest peaches to cold stress by reducing the content

of endogenous H_2S . Similarly, endogenous H_2S enhanced the salt tolerance of alfalfa seedlings by promoting the restoration of redox balance and inhibiting potassium ion efflux [24,25]. In addition, H_2S was confirmed to extend the vase life of cut carnations [8] and to improve the vase life and quality of cut roses and chrysanthemums (*Dendranthema morifolium* Ramat.) [26]. It also prolonged the storage period of fruits by activating the antioxidant system to maintain good appearance and nutritional quality [27,28]. During the senescence of cut flowers, water deficit stress disrupted the normal balance of reactive oxygen species, leading to oxidative stress. Studies showed that the activities of the contents of soluble sugar and soluble protein, were positively correlated with water uptake, water loss, and relative water content [14]. MT influenced flower diameter and fresh weight by affecting water uptake, which in turn affected the activities of the levels of osmotic regulatory substances such as soluble sugar and soluble protein, thereby maintaining the water balance of cut flowers. To date, no study has reported the combined treatment of hydrogen sulfide and melatonin on the preservation of cut roses during production. In this study, MT + H_2S -treated cut roses exhibited a longer flowering period, intact petals, and no wilting (Figure 2A), with significantly better maximum flower diameter and fresh weight change rate than the control (Figure 2B,C). Thus, MT + H_2S effectively delayed senescence and extended vase life. This indicates that H_2S can significantly enhance the MT-induced physiological effects. Through a synergistic action, it further increases the levels of osmotic regulatory substances while more effectively reducing the level of the membrane lipid peroxidation product MDA, thereby exhibiting superior cytoprotective and antioxidant effects compared to MT treatment alone. The observed synergistic interaction between MT and H_2S signals points to a likely bidirectional regulatory relationship of their signaling pathways. MT may function as a potential upstream signal to moderately induce endogenous H_2S biosynthesis. In turn, accumulated H_2S could partially boost MT-dependent antioxidant and osmotic protective responses, which tend to establish a partial positive feedback regulatory pattern within senescing petal tissues to a certain extent. Consistent with previous studies focused on postharvest horticultural crops, the joint action of melatonin and H_2S signaling molecules moderately improves osmotic homeostasis maintenance and partially mitigates oxidative injury to cell membranes in most reported systems, though obvious differences exist in the magnitude and composition of downstream gene regulatory cascades across various ornamental plant varieties [5]. Previous studies have shown that soluble sugars, soluble proteins, and Pro are key substances involved in osmotic regulation in cut flowers, and their content changes are closely related to the osmotic regulatory function of the petals themselves [26]. After the combined treatment with MT and H_2S , the contents of these osmotic regulatory substances in the cut flower petals have significantly increased overall. By increasing the cell solute concentration and optimizing the cell osmotic potential, it effectively alleviates the stress caused by water imbalance and jointly mediates the adaptive response of the petal osmotic regulation system. In summary, MT and H_2S can jointly regulate the accumulation of osmotic regulatory substances and activate the petal osmotic regulation mechanism to improve the water status of cut flowers and achieve post-harvest preservation effects. Furthermore, the role of HT as an H_2S scavenger has been reported in other plants. For example, HT promoted color transition and softening in tomato fruits by reducing the activities of gene expression of chlorophyll synthesis-related enzymes and upregulating the expression of genes related to chlorophyll degradation, softening, and ethylene synthesis [29]. In our research, HT treatment caused severe wilting and scarring of petals, while MT + HT treatment led to curled outer petals (Figure 4A). HT also reduced maximum flower diameter compared to MT (Figure 4B). MT + HT treatment increased soluble sugar, soluble protein, and proline contents and reduced MDA content, thereby delaying senescence (Figure 4D–G). However, HT has removed some

of its protective effects, indicating that MT action partially depends on the H₂S signaling pathway. It should be noted that the application of exogenous HT for exploring signal interactions carries certain inherent limitations within the present experimental system. As a chemical scavenger rather than a specific inhibitor targeting H₂S biosynthesis, HT non-selectively eliminates most cellular free H₂S and may moderately interfere with the homeostasis of other sulfur-containing metabolites. This effect may partially contribute to the abnormal petal phenotypes, such as wilting and scarring, observed in our study. In addition, chemical scavengers hardly achieve fine, gene- or tissue-specific modulation of H₂S signaling, which increases the difficulty in distinguishing direct MT-H₂S interactive responses from the reagent's non-specific adverse side effects. Further genetic assays are therefore required to provide more supportive evidence for the proposed MT-H₂S signaling interaction and make up for the inherent deficiencies of pharmacological experimental approaches. As key components of ethylene biosynthesis, ACC content and the activities of ACS and ACO are closely linked to the onset and progression of flower senescence. In this study, HT treatment significantly accelerated the accumulation of ACC, alongside higher ACS and ACO activities, consistent with its role in promoting ethylene production and hastening senescence. In contrast, MT and H₂S treatments markedly suppressed these ethylene-related parameters, maintaining lower ACC levels and reduced enzyme activities throughout the vase life. These results collectively indicate that MT and H₂S may delay cut roses senescence by downregulating the ethylene biosynthetic pathway, thereby limiting ethylene production (Figure 4H–J).

MT, as an indoleamine compound, scavenged free radicals, conferred antioxidant properties, and inhibited lipid peroxidation and had been widely used in the preservation of cut flowers [30,31]. Membrane lipid peroxidation is one of the important mechanisms leading to the aging of cut flowers [32]. Meanwhile, osmotically active substances protected the organs and cells of cut flowers and enhanced their adaptability to stress [33]. Additionally, previous studies also supported the findings of the present study. It found that in 'Bartzella' Itoh peony cut flowers, exogenous melatonin reduced the content of MDA; significantly increased the activities of SOD, POD, and CAT; and alleviated oxidative stress throughout the entire opening process of the cut flowers [14]. It suggested that treating cut peony flowers with melatonin reduced the MDA content and increased the contents of related components [21]. In the banana variety 'Musa nana', 0.5 mM NaHS reduced the accumulation of MDA, H₂O₂, and O₂, and promoted the activities of antioxidant enzymes, thereby alleviating low-temperature injury in postharvest bananas [34]. This indicates that H₂S plays a significant role in promoting the antioxidant enzyme activities after post-harvest treatment. To ensure that the observed effects were specifically attributable to H₂S rather than to the counterion Na⁺ of the donor compound, we examined the potential interference of Na⁺ released from NaHS. Although NaHS dissociates into Na⁺ and HS[−] in aqueous solution, and elevated Na⁺ levels could theoretically alter osmotic potential or interfere with membrane ion transport, pre-experiments confirmed that at the concentration used (50 mg·L^{−1}), the Na⁺ release is within a physiologically negligible range [35]. Hence, the preservation effects reported herein can be reliably ascribed to H₂S signaling. In this study, the MT + H₂S treatment significantly increased the enzymatic activities of APX, POD, and CAT (Figure 3A–C). Previous studies had found that MT delayed petal browning by upregulating the activities of SOD and CAT [36]. It was further confirmed in this study that MT + H₂S delayed the senescence of cut roses by upregulating the activities of APX, POD, and CAT. Excessive production of reactive oxygen species leads to oxidative damage to plant cell membranes and ultimately results in the death of cells and organisms. Additionally, MT + H₂S significantly reduced the contents of H₂O₂ and O₂, and decreased the electrolyte leakage rate in cut flowers. Similarly, It reported that in two-week-old pepper

seedlings, 0.2 mM NaHS enhanced tolerance to boron toxicity by reducing H_2O_2 content and membrane electrolyte leakage rate [37]. Therefore, these results indicate that MT + H_2S can significantly protect the cell membranes of cut-flower roses. The mechanism lies in promoting the activity of antioxidant enzymes and reducing the accumulation of reactive oxygen species. For example, in postharvest celery (*Apium graveolens* L.), NaHS treatment significantly increased the activities of SOD, POD, and CAT, while HT treatment decreased these activities. The relative expression levels of *AgSOD1*, *AgPOD*, and *AgCAT* showed a similar trend to the enzyme activities [38]. In this study, HT significantly inhibited APX, POD, and CAT activities and the *RhAPX*, *RhPOD*, and *RhPOD* gene expression in cut roses, leading to H_2O_2 and $\text{O}_2^{\cdot-}$ accumulation. It is speculated that HT can significantly reduce the activity of antioxidant enzymes in cut flower petals by inhibiting the biosynthesis of endogenous H_2S in the plants, thus preventing excessive accumulation of H_2O_2 and $\text{O}_2^{\cdot-}$ within the petals. MT, on the other hand, possesses non-enzymatic antioxidant properties and can directly eliminate ROS. Compared with the HT treatment alone group, the accumulation of ROS in the petals of the MT + HT treatment group was significantly reduced (Figure 5G,H). Thus, the effect of MT on ROS accumulation does not only rely on the enzymatic antioxidant system mediated by H_2S . Notably, the combined MT + H_2S treatment outperformed either treatment alone, further confirming that MT enhanced the antioxidant defense system and alleviated oxidative damage via the H_2S signaling pathway.

Regarding endogenous H_2S synthesis, this study revealed a clear upward trend in H_2S content in cut roses (Figure 6A), indicating activation of the biosynthetic mechanism in response to post-harvest storage-induced cell senescence. Meanwhile, HT treatment significantly suppressed endogenous H_2S accumulation, thereby shortening the lifespan of cut roses. H_2S was mainly synthesized by the key enzymes L-cysteine desulphydrase (LCD) and D-cysteine desulphydrase (DCD) [39]. For example, in tomatoes, *SIDCD2* delayed fruit ripening and leaf senescence by producing H_2S to maintain ROS homeostasis and inhibit ethylene synthesis, and its loss of function led to accelerated fruit ripening and premature leaf senescence [40]. Similarly, in the study on *Crataegus pinnatifida*, treatment with NaHS promoted endogenous H_2S accumulation by triggering LCD and DCD enzyme activities and subsequently enhanced ROS scavenging enzyme activities [41]. In addition, HT treatment significantly increased the activities of LCD and DCD and the expression levels of *AgOASTL*, *AgSAT*, and *AgLCD* compared with the control, indicating that endogenous H_2S biosynthesis was activated during storage to counteract cell senescence. Exogenous H_2S treatment further enhanced endogenous H_2S biosynthesis, as evidenced by increased LCD and DCD activities and upregulation of *AgLCD* and *AgDCD* expression [39]. This study showed that exogenous MT significantly upregulated the expression of *RhLCD* and *RhDCD*, the key enzyme genes for H_2S synthesis in cut roses, thereby increasing endogenous H_2S content (Figure 6). This result was consistent with previous reports showing that MT induces H_2S production in various plant species. In contrast, HT treatment significantly weakened the above effects of MT, reducing H_2S content and synthase gene expression to levels below those of the control. Combined with the promoting effects of MT on the antioxidant system and osmotic regulatory substances shown in Figures 4 and 5, we speculated that MT might delay the senescence process of cut roses by activating the endogenous H_2S synthesis pathway, thereby initiating downstream antioxidant defense and osmotic regulation responses. The reversal of MT effects by HT further confirmed, from a reverse perspective, the indispensable mediating role of H_2S in the MT signaling pathway. From a practical perspective, our findings provide a feasible and low-cost preservation strategy for commercial cut flower production and postharvest handling. Different from most previous studies that separately investigated the independent functions of MT or H_2S in regulating flower senescence, this study further advances the current knowledge by uncovering their

internal regulatory cascade and synergistic mechanism in cut roses. A major novelty of the present study lies in the integrated exploration of the cooperative regulatory relationship between MT and endogenous H_2S in cut roses, demonstrating that MT positively modulates endogenous H_2S production to jointly mediate cut rose preservation, which clarifies a new physiological mechanism underlying MT-delayed flower senescence. Exogenous combined MT and H_2S application effectively maintains petal water balance, alleviates oxidative membrane damage, and suppresses petal senescence, significantly extending the ornamental vase life of cut flowers (Figure 7). This simple vase solution treatment avoids complex technical operations and is compatible with conventional postharvest transportation and retail storage systems used in floriculture industries. Therefore, the co-application of MT and H_2S shows great potential to reduce postharvest quality losses, lower commodity waste, and improve the economic value of fresh-cut flowers during commercial circulation. Future research could focus on dynamic and real-time monitoring of H_2S synthesis and ROS accumulation during the preservation of cut roses by MT. This will help us more accurately clarify how MT suppresses ROS through H_2S . Additionally, continuous detection of ethylene release will further reveal the comprehensive functions of MT and H_2S . Further future validations incorporating precise ethylene quantification and systematic molecular approaches are also warranted to consolidate and strengthen the reliability and integrity of the proposed MT- H_2S signaling cascade underlying cut rose senescence regulation. Finally, all experiments in this study were completed under laboratory-controlled conditions, where the temperature, humidity, and light conditions were relatively stable. However, these conditions differ from the complex and variable environmental parameters in commercial post-harvest processing, which may limit the practical application value of the experimental results and make it difficult to directly replicate them in commercial production scenarios.

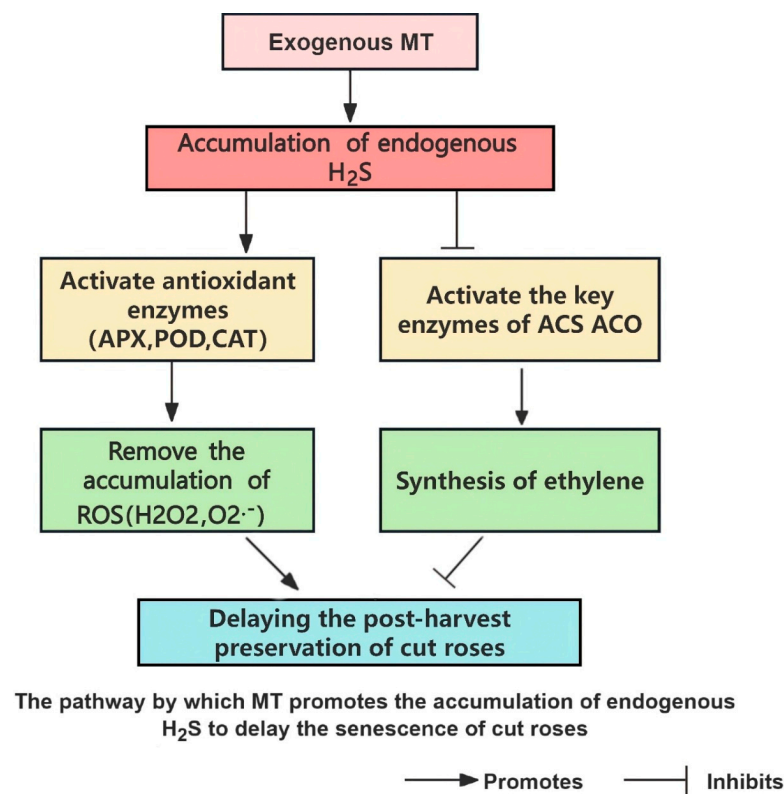


Figure 7. MT-mediated postharvest senescence delay model for cut roses. Exogenous MT treatment induces the accumulation of endogenous H_2S , which acts as a positive downstream signal mediating

cut rose vase life extension. Endogenous H₂S enhances the activities of antioxidant enzymes (APX, POD, and CAT), which eliminate excess ROS, including H₂O₂ and O₂^{·−} to alleviate oxidative injury. Meanwhile, endogenous H₂S suppresses the activities of ethylene biosynthetic key enzymes ACS and ACO, thereby restraining ethylene synthesis. These coordinated physiological responses jointly slow petal senescence, ultimately delaying postharvest deterioration and prolonging the ornamental quality of cut roses. Solid lines indicate regulatory pathways supported by experimental data in this study. Arrows and T-bars represent promotion and inhibition, respectively.

5. Conclusions

Our findings indicate that a concentration of 50 mg·L^{−1} MT is the most appropriate for preserving post-harvest cut roses, which may extend their vase life by 2 d compared with the control. Additionally, H₂S acts as a downstream signaling molecule in the MT-mediated post-harvest preservation, enhancing the antioxidant defense system to scavenge ROS, thereby mitigating oxidative damage and delaying the senescence process of cut roses. Furthermore, MT promotes H₂S synthesis by increasing the expression of *RhLCD* and *RhDCD* during the post-harvest period of cut roses. Meanwhile, MT and H₂S jointly suppressed ethylene biosynthesis by reducing ACC accumulation and the activities of ACS and ACO, further contributing to senescence retardation. This study reveals a novel MT-H₂S regulatory module in cut flower physiology, offering a promising strategy for improving post-harvest preservation. Our simple vase solution offers an affordable, low-cost strategy to slow quality loss and cut economic losses during commercial cut flower transport and retail, with tangible benefits for the flower postharvest industry. All experiments herein used exogenous chemicals and were limited to laboratory conditions. Future work will employ genetic materials to validate this pathway and conduct commercial field trials for industrial implementation.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae12070817/s1>.

Author Contributions: All authors contributed to the conceptualization and design of the study. Conceptualization, C.W.; Fund Acquisition, J.T. and C.W.; Original Writing, J.T. and C.W.; Copy Editing, C.A., J.T., Z.L., R.C. and L.Z.; Character Creation and Adaptation, J.T. and C.W. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the College Students' Innovation and Entrepreneurship Training Program of Gansu Province (S202410733057); the Supporting Funds for Youth Mentor of Gansu Agricultural University (GAU-QDFC-2024-15); the National Natural Science Foundation of China (32460753); and the Key Project of Gansu Provincial Natural Science Foundation, China (No. 23JRR1406).

Data Availability Statement: All the data are included in the article/Supplementary Materials.

Conflicts of Interest: The authors declare no conflicts of interest.

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