Hydrogen Sulfide Alleviates Dark-promoted Senescence in Postharvest Broccoli

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Abstract. Broccoli (*Brassica oleracea* var. *italica*) is an important vegetable crop rich in vitamins and sulforaphane. However, the floral heads of broccoli experience rapid postharvest senescence. Here we found that hydrogen sulfide (H₂S) treatment alleviated dark-promoted senescence in broccoli florets. H₂S delayed the symptoms of senescence and maintained higher levels of chlorophyll and Rubisco and lower protease activity compared with water control. Gene expression analysis showed that H₂S down-regulated the expression of chlorophyll degradation-related genes *BoSGR*, *BoNYC*, *BoCLH1*, *BoPPH*, and *BoRCCR*. Expression of lipoxygenase gene *BoLOX1* and the genes involved in the ethylene synthesis pathway, *BoACS2* and *BoACS3*, were also down-regulated by H₂S. The reduced expression level in cysteine protease gene *BoCP3* and aspartic protease gene *BoLSC807* suggested the role of H₂S in alleviating protein degradation during broccoli senescence. H₂S up-regulated the expression of sulfur metabolism genes *BoSR* and *BoOASTL*, and the antioxidant gene *BoCAT*. These results show that H₂S plays a vital role in alleviating broccoli senescence through a broad regulation on gene expression of reactive oxygen species (ROS) metabolism genes, ethylene synthesis genes, and protease genes.

Broccoli (*Brassica oleracea* var. *italica*) is an important vegetable of high nutritional value and a common component of the human diet. Floral heads of broccoli are harvested during the immature stage when florets are composed of male and female reproductive structures surrounded by immature petals and

enclosed with chlorophyll-containing sepals (Chen et al., 2008). Postharvest broccoli florets experience senescence similar to those seen in developmental leaf senescence at biochemical and transcriptional levels, involving decline in photosynthesis and degradation of macromolecules such as proteins, lipids, and chlorophyll (Buchanan-Wollaston, 1997; Smart, 1994). Production of ethylene plays an important role in regulating the yellowing of florets after harvest, because chlorophyll loss is associated with an increase in ethylene synthesis in florets (Tian et al., 1994). Dark treatment has been shown to accelerate leaf senescence (Quirino et al., 2000). In many plant species, dark treatment causes chlorophyll and protein degradation, which also occurs in developmental senescent leaves (Biswal and Biswal, 1988). When stored in darkness, broccoli florets experienced more rapid deterioration in quality compared with those under light (Li et al., 2014). To extend the shelf life of

broccoli, various methods have been developed, including modified atmosphere packaging, ultraviolet-C and light irradiance, and appropriate refrigerated storage (Chen et al., 2008).

H₂S has been shown to play various physiological roles in animal cells (Wang, 2002). Recently, more evidence has demonstrated that H₂S plays multiple roles in plants, including seed germination, root organogenesis, abiotic stress response, and photosynthesis (Jin et al., 2011, 2013; Lisjak et al., 2013; Zhang et al., 2008, 2009). More recently, H₂S was found to prolong postharvest shelf life of strawberry, kiwifruit, and broccoli by acting as an antioxidant (Gao et al., 2013; Hu et al., 2012; Li et al., 2014). Previously we reported that H₂S fumigation effectively alleviates postharvest senescence of broccoli through modulation of the endogenous antioxidant system. In this article we investigate accelerated senescence of broccoli florets in darkness using biochemical and molecular analysis and the interplay of H₂S with sulfur metabolism, the ethylene pathway, and protein degradation.

Materials and Methods

Plant materials and treatment. Fresh broccoli (*Brassica oleracea* var. *italica*) heads were kindly supplied by Anhui Academy of Agricultural Sciences, Hefei, Anhui province, China. Broccoli heads having similar size and maturity and florets without physical damage or microbial infection were used in this study. The H₂S donor, sodium hydrosulfide (NaHS·3H₂O; Sigma, St. Louis, MO) was used in aqueous solutions (150 mL at concentrations of 0, 0.24, 0.48, 0.72, 0.96, and 1.2 mmol·L⁻¹) and applied to florets in sealed containers (volume 3 L). Six pieces of fresh-cut broccoli florets (16 ± 2 g per piece) from six different broccoli heads allocated on board with pores that separated florets and NaHS solution were fumigated with H₂S in the sealed containers at 25 °C with 85% to 90% relative humidity and stored in darkness. NaHS solutions were renewed every 24 h. Florets were sampled from different broccoli for RNA extraction and other experiments at the time points indicated in figure legends. Each experiment was repeated three times.

Visual evaluation of postharvest broccoli. The appearance of fresh-cut broccoli florets fumigated with H₂S gas was evaluated subjectively by 20 people using five indices, including freshness, yellowness, texture, flavor, and rotting rate (Takamiya et al., 2000). Each index was divided into four ranks and the score was 0, 1.0, 1.5, and 2.0 with lower numbers indicating lower quality. A sum of the five indices ranging from 0 to 10 was used for visual evaluation.

Determination of chlorophyll content. Chlorophyll content of broccoli florets was determined according to the method of Lichtenthaler and Wellburn (1983) with minor modifications. Broccoli florets (0.5 ± 0.01 g) were homogenized using a pestle and mortar in the presence of ice and then incubated in

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10 mL of 80% acetone for 24 h in darkness at 4 °C. The supernatant was measured at 663 and 645 nm, respectively. Chlorophyll content was calculated using the following formulas: total chlorophyll (Chl) ($\text{mg}\cdot\text{g}^{-1}$) = $(20.2A_{645} + 8.02A_{663}) \text{ V/W}$; Chl a ($\text{mg}\cdot\text{g}^{-1}$) = $(12.7A_{663} - 2.69A_{645}) \text{ V/W}$; Chl b ($\text{mg}\cdot\text{g}^{-1}$) = $(22.9A_{645} - 4.68A_{663}) \text{ V/W}$. Each analysis was repeated three times and the results were expressed as $\text{mg}\cdot\text{g}^{-1}$ fresh weight.

Determination of endoprotease and Rubisco. Protease activity was assayed according to the method of Reimerdes and Klostermeyer (1976). Frozen broccoli florets ($0.50 \pm 0.01 \text{ g}$) were homogenized with a cold mortar and pestle in 5 mL of 50 $\text{mmol}\cdot\text{L}^{-1}$ Tris-HCl buffer (pH 7.5) containing 1 $\text{mmol}\cdot\text{L}^{-1}$ EDTA, 15 $\text{mmol}\cdot\text{L}^{-1}$ β -mercaptoethanol, and 1% (w/v) polyvinyl pyrrolidone. The homogenate was centrifuged at 10,000 g at 4 °C for 30 min and the supernatant was used for protease activity and ribulose-1,5-bisphosphate carboxylase (Rubisco) determination. One unit of protease activity was defined as an increase of 0.1 OD at 278 $\text{nm}\cdot\text{h}^{-1}$. Rubisco was analyzed by sodium dodecyl sulphate-polyacrylamide gel with Coomassie R-250 staining according to the method of Ono et al. (2013).

RNA extraction and reverse transcription-polymerase chain reaction. Broccoli florets were ground in liquid nitrogen, and RNA was extracted by the RNAiso Plus according to the manufacturer's instructions (Takara, Kyoto, Japan). The cDNA was synthesized from total RNA using a reverse transcription kit (Prime Script™ RT Master Mix; Takara) and was used as template for polymerase chain reaction (PCR) with Easy Taq™ DNA Polymerase (TransGen, Beijing, China). The actin

(AF044573) gene was used as a control. All primers used for reverse transcription-PCR are listed in Table 1. The primer sequences of Chl degrading genes were designed according to Hasperue et al. (2013). Three independent extractions of RNA were carried out and similar results were obtained.

Statistics. Statistical significance was tested by one-way analysis of variance, and the results are expressed as the mean values $\pm$ SD of three independent experiments. Fisher's least significant differences were calculated following a significant ($P < 0.01$ or $P < 0.05$) t test.

Results

H₂S alleviates dark-induced senescence in broccoli. Broccoli florets stored in darkness were fumigated with different concentrations of NaHS. As shown in Figure 1A, H_2S effectively alleviated dark-induced senescence in broccoli florets in a dose-dependent manner. Control broccoli yellowed quickly after 3 d of storage, whereas NaHS at an optimal concentration of 0.96 $\text{mmol}\cdot\text{L}^{-1}$ postponed yellowing process until Day 5. Florets exposed to 0.96 $\text{mmol}\cdot\text{L}^{-1}$ NaHS showed the highest visual value according to the five indices of freshness, yellowness, texture, flavor, and rotting rate (Fig. 1B).

Effects of H₂S fumigation on content of chlorophyll, activity of protease, and degradation of Rubisco. We determined the effect of H_2S fumigation on Chl degradation, which is the principle symptom of broccoli senescence. As shown in Figure 2A–C, total Chl, Chl a, and Chl b in both control and H_2S -fumigated broccoli decreased gradually

during the whole storage time. However, H_2S fumigation maintained significantly higher Chl level than a water control (Fig. 2A–C).

Senescence-associated proteases, which are accumulated in the vacuole, play a vital role in protein degradation during plant senescence (Li et al., 2014). As expected, protease activity in control samples increased steadily during the first 4 d of storage followed by a decrease on Day 5 (Fig. 2D). In contrast, H_2S sustained stable and lower levels of protease activity in broccoli (Fig. 2D). During leaf senescence, Rubisco is gradually degraded for recycling within the plant. As shown in Figure 2E, Rubisco was degraded gradually during dark senescence in control broccoli florets, whereas H_2S fumigation alleviated the degradation of Rubisco, especially on Days 4, 5, and 6.

Effects of H₂S on expression of chlorophyll degrading genes. To understand the role of H_2S in alleviating Chl degradation, we assayed the expression of Chl degrading-related genes in broccoli. Stay-Green (SGR) is required for destabilization of light harvesting complex to Chls (Hörtensteiner and Kräutler, 2011). The enzymes named, non-yellow coloring 1 (NYC1), chlorophyllase (CLH), pheophytinase (PPH), pheophorbide a oxygenase (PaO), and RCC reductase (RCCR), serially degrade Chl b to "primary" fluorescent Chl catabolite (Hörtensteiner and Kräutler, 2011). As shown in Figure 3, the expression of *BoSGR*, *BoCLH1*, *BoCLH2*, *BoPPH*, *BoPaO*, and *BoRCCR* was activated after 1 d of storage, suggesting the ongoing Chl degradation. H_2S obviously repressed the expression of *BoSGR*, *BoCLH1*, *BoCLH2*,

Table 1. Primers used in reverse transcription-polymerase chain reaction.

Gene name	Forward primer (5' → 3')	Reverse primer (5' → 3')	Accession number
<i>BoACT</i>	CCAGAGGTCTTGTTCACGCCATC	GTTCACCACTGAGCACAATGTTAC	AF044573
Chlorophyll degrading genes			
<i>BoSGR</i>	TTCCGACAACCGAAGTAA	GTGAGCGTATAAGTTCTTGG	DK463677
<i>BoNYC</i>	TTACATCTCGCAGTTCTGA	GCAATACCAACTACCTTAGC	EH428873
<i>BoCLH1</i>	AGACCCATCCATCAAGTTTTCAGC	AGATTTCCGGGATCGGTTCTTATGC	AF337544
<i>BoCLH2</i>	AGATGCCTGTCTAGTTATTGG	CACGCTGGACCTTGACATTC	AF337545
<i>BoPaO</i>	GCGAAATTCCCGTCCAGAGTCTC	TTATCTCCGCGGTGCTCTTCTTC	AM388844
<i>BoPPH</i>	AGAGGTTATCGGTGAGCCA	GACGAGATGAGGATGGG	OL386R
<i>BoRCCR</i>	CCTCCCTCATCGCAAAGACCTAG	AGCAGAGCGGACAAAGAGAGAC	AB470927
Ethylene synthesis and signaling associated genes			
<i>BoACS1</i>	TGCGAACATGGACTACCAG	CACGAACAAGCGGAGAAGG	X82273
<i>BoACS2</i>	CCAACCCATCAAACCCATTA	CCAACTCGGAAACCAGGAA	AF338651
<i>BoACS3</i>	GACTGGAGCCAATGAAACG	TTTGATGGGTTGGTGAAGA	AF338652
<i>BoACO1</i>	ACCTTCGTCTATCTCCCTCAGTC	GATAGTTGCTCACCTTAGTCCC	X81628
<i>BoACO2</i>	TCAAAGGGCTTAGGGCTCA	ATCTCCGCATCACTTCCAG	X81629
<i>BoACO3</i>	CTATCCACCTTGCTCTAAACCG	CACTCTATGCTCCACGCTCTT	AF252853
<i>BoERS</i>	CCCGAGGTTGTTTCTGTCC	TCACGAGCGTGCTTGTATT	AF047477
<i>BoETR1</i>	CCCACCTCATCAACCTAT	CTACTCAGCGTGCTTCTA	AF047476
<i>BoETR2</i>	AAAAGATGAACACCGAAGG	CGAAGCGTATTTCAGTT	AB078598
Sulfur metabolism-related genes			
<i>BoSR</i>	CCACCAGACAAACCTTCC	ACTGCTCACCATCAACCC	DK483402
<i>BoOASTL</i>	GGGTATTGGACTTGCGTTTA	TCTTTGGATTGGCAGGGT	DK510396
<i>BoLCD</i>	GGAGGACACTGTGGTGATGT	CGTTCGCTTACCTTTCTTT	DK509015
Other genes			
<i>BoLOX</i>	TCTCCACGAATTTCTGGGAAACAAAG	AGTCTCTGAGTGTGTAGCCTTTC	BT010358
<i>BoCAT</i>	TCCTTTACACCAGAGAGGC	GAATCTCGTTCACTCGTTT	GQ500124
<i>BoAPX</i>	GGTGTGTGGCTGTGAA	CCTATCTTGTGGCATCTT	HQ871864
<i>BoGR</i>	GGGAGATGCTACAAACCG	TCCTGCCGTCCAGAAATG	AB125639
<i>BoCP1</i>	CGCCGATCTGACCAACGAA	CCGCTCCAATCGCAGAAAA	AF454956
<i>BoCP3</i>	TGGCAAAGACTATTGGATTG	ACGGGAGATGGAGGTGAAG	AF454958
<i>BoLSC807</i>	GGAGTTTATTGAGGCAACCAAG	AAGTTCACCACCTTCTTCATCCT	X80067

Fig. 1. Effects of hydrogen sulfide (H_2S) application on the senescence and visual evaluation of broccoli florets stored in darkness. Broccoli florets were treated with different concentrations (0, 0.24, 0.48, 0.72, 0.96, and 1.20 $\text{mmol}\cdot\text{L}^{-1}$) of NaHS aqueous solutions for 0 to 7 d, respectively, and the experimental treatments were shown in lower right part of A. Broccoli were fumigated with H_2S in sealed containers at 25 °C with 85% to 90% relative humidity and stored in darkness. (B) Visual evaluation of broccoli florets.

BoPPH, and *BoRCCR* especially on Day 1 of storage, whereas the expression of *BoPaO* and *BoNYC* was not significantly changed by H_2S fumigation.

Effects of H_2S on expression of ethylene-related genes. Because ethylene plays a crucial role in floret yellowing of broccoli (Tian et al., 1994), we studied the effect of H_2S on ethylene biosynthesis and signal transduction genes. As shown in Figure 4, *BoACS2*, *BoACS3*, *BoERS*, and *BoETR1* increased markedly in control broccoli during storage time, whereas *BoACS1*, *BoACO1*, and *BoACO2* remained stable, and *BoACO3* and *BoETR2* were not detectable. In contrast, H_2S fumigation repressed the expression of *BoACS2* and *BoACS3*, suggesting the antagonistic role of H_2S in ethylene biosynthesis.

Effects of H_2S on expression of sulfur metabolism genes. We determined the effect of H_2S on sulfur metabolism through the expression assay of O-acetylserine(thiol)lyase (*OASTL*) gene *BoOASTL*, *BoSR* (sulfite reductase), and *BoLCD* (L-cysteine desulfhydrase). As shown in Figure 5A, H_2S fumigation induced higher expression of *BoOASTL* and *BoSR* on Day 3 of storage compared with the water control, suggesting accelerated sulfur metabolism induced by exogenous H_2S application.

Effects of H_2S on expression of antioxidant enzyme genes. The expression of genes encoding enzymes involved in redox metabolism such as lipoxygenase (LOX), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) were studied in broccoli

florets. As shown in Figure 5B, the expression of *BoLOX* was induced in water controls on Day 3, whereas H_2S fumigation sustained lower expression level. Besides, H_2S application induced higher expression of *BoCAT* on Day 3 compared with water control. In contrast, the expression levels of *BoAPX* and *BoGR* were not significantly changed in H_2S -fumigated broccoli.

Effects of H_2S on the transcription of protein degrading genes. Genes involved in protein degradation and in amino acids recycling from degraded protein are thought to be up-regulated in senescing tissues (Chen et al., 2008). Expression of senescence related protease genes *BoCPI*, *BoCP3*, and *BoLSC807* were assayed in broccoli (Fig. 5C). *BoCP3* was activated on Days 1 and 3 of storage, whereas H_2S fumigation significantly decreased *BoCP3* expression (Fig. 5C). Meanwhile, expression of *BoLSC807* (aspartic protease encoding gene) was also repressed by H_2S fumigation on Day 3 of storage, suggesting the role of H_2S in inhibiting protein degradation (Fig. 5C).

Discussion

Dark exposure is used as a conventional method to induce plant senescence (Smart, 1994). Our results suggest that dark treatment induces broccoli senescence by Chl and protein degradation, and H_2S effectively alleviates the senescence symptom (Fig. 1). Chlorophyll content decreases dramatically in control broccoli during storage, whereas H_2S application sustains higher Chl content (Fig. 2A–C). Consistently, H_2S application was found to delay Chl degradation under normal light storage conditions (Li et al., 2014). However, we find that broccoli stored in darkness becomes yellow quickly compare with broccoli under normal light conditions, suggesting that dark treatment is effective in triggering broccoli senescence (data not shown).

Chlorophyll degradation is the first visible symptom of senescence exhibited by a loss of green color and shortening of shelf life (Chen et al., 2008). SGR, a chloroplast-located protein, destabilizes light harvesting complex subunits to release Chls. During Chl degradation, transformation from Chl b to Chl a is catalyzed by a Chl(*ide*) b reductase [non-yellow coloring 1 (NYC1)] (Hörtensteiner and Kräutler, 2011). The enzyme, PPH, is involved in dephytylation of pheophytin to generate pheophorbide (Hörtensteiner and Kräutler, 2011). PaO catalyzes the opening of the porphyrin ring of pheophorbide and generates red chlorophyll catabolites (RCC) followed by the reduction of RCC by RCC reductase (RCCR) (Hasperue et al., 2013). In this work, we analyze the expression of Chl degradation-related genes (*BoSGR*, *BoCLH1*, *BoCLH2*, *BoPPH*, *BoRCCR*, *BoPaO*, and *BoNYC*), and H_2S is able to sustain a lower expression level of *BoSGR*, *BoCLH1*, *BoPPH*, and *BoRCCR* in broccoli. *BoCLH1* and *BoCLH2* are involved in ethylene-induced Chl degradation (Chen et al., 2008). The activation of *BoCLH1* and *BoCLH2* expression in control broccoli suggests that their

Fig. 2. Effects of hydrogen sulfide (H₂S) on the contents of total chlorophyll (A), chlorophyll a (B), and chlorophyll b (C). Effect of H₂S fumigation on protease (D) and Rubisco degradation (E). Broccoli florets were fumigated with H₂S released from 0.96 mmol·L⁻¹ NaHS aqueous solution (T) with water as the control (CK) for 0 to 6 d. The proteins of broccoli samples were separated by sodium dodecyl sulphate–polyacrylamide gel and then stained with Coomassie Brilliant Blue R-250. Data are presented as means ± SD (n = 3). * and ** in this figure and following ones stand for significant difference between CK and T at *P* < 0.05 and *P* < 0.01, respectively.

expression might be induced by endogenous ethylene generated during senescence (Fig. 3).

Ethylene plays an important role in floret yellowing of broccoli (Tian et al., 1994). 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) and ACC oxidase (ACO) are key enzymes for ethylene production, which catalyze the conversion of S-adenosylmethionine to ACC and ACC to ethylene, respectively (Hamilton et al., 1991; Lin et al., 2009). In our experiment, increased expression of ethylene synthesis and signaling related genes *BoACS2*, *BoACS3*, *BoERS*, and *BoETR1* during storage suggests the key role of the ethylene

pathway in inducing broccoli senescence (Fig. 4). However, we first present evidence that H₂S application effectively blocks the ethylene synthesis pathway through reducing the transcripts of *BoACS2* and *BoACS3* (Fig. 4), suggesting the role of H₂S in counteracting ethylene synthesis. The lowered ethylene synthesis in H₂S treatment will also help to reduce Chl and protein degradation (Chen et al., 2008). Similarly, nitric oxide has been shown to transcriptionally repress the expression of genes of ethylene biosynthesis enzymes, suggesting a similar mechanism in decreasing ethylene synthesis (Manjunatha et al., 2012).

Fig. 3. Effects of hydrogen sulfide (H₂S) on the expression of chlorophyll degrading genes, including *BoSGR* (stay-green protein), *BoNYC* (chlorophyll b reductase), *BoCLH1* (chlorophyllase 1), *BoCLH2* (chlorophyllase 2), *BoPaO* (pheophorbide a oxygenase), *BoPPH* (pheophytinase), and *BoRCCR* (RCC reductase). Broccoli florets were fumigated with H₂S released from 0.96 mmol·L⁻¹ NaHS aqueous solution (T) with water treatment as the control groups (CK) for 0, 1, and 3 d. *BoACT* (Actin, AF044573) in this figure and following were used as a control gene.

Fig. 4. Effects of hydrogen sulfide (H₂S) treatment on the expression of ethylene related genes, including 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (*BoACS1*, *BoACS2*, and *BoACS3*), ACC oxidase (*BoACO1*, *BoACO2*, and *BoACO3*), and ethylene receptors (*BoERS*, *BoETR1*, and *BoETR2*) in postharvest broccoli florets. Broccoli florets were fumigated with H₂S released from 0.96 mmol·L⁻¹ NaHS aqueous solution (T) or water as the control groups (CK) for 0, 1, and 3 d.

Previous study shows that exogenous H₂S affects sulfur metabolism on enzymatic activity and gene expression levels (Lai et al., 2014). In the present study, the expression of *BoOASTL* and *BoSR* is up-regulated by H₂S fumigation, implicating the accelerated sulfur metabolism induced by exogenous H₂S.

Reactive oxygen species overproduction and oxidative damage are essential events in the senescence of postharvest vegetables (Li et al., 2014). LOX catalyze the hydroperoxidation of polyunsaturated fatty acids and contribute to membrane deterioration (Gomez-Lobato et al., 2012). However, plants have developed the ability to eliminate ROS with an efficient ROS-scavenging system (Mittler et al., 2004).

Fig. 5. Effects of hydrogen sulfide (H_2S) fumigation on the expression of sulfur metabolism-related genes, including *BoSR* (sulfite reductase), *BoOASTL* [O-acetylserine(thiol)lyase], and *BoLCD* (L-cysteine desalhydrase) (A). Effects of H_2S fumigation on the expression of *BoLOX* (lipoxygenase), *BoCAT* (catalase), *BoAPX* (ascorbate peroxidase), and *BoGR* (glutathione reductase) (B). Effects of H_2S fumigation on the relative expression of *BoCP1* (cysteine protease 1), *BoCP3* (cysteine protease 3), and *BoLSC807* (encoding an aspartic protease) in broccoli florets stored in darkness (C).

Here we find that H_2S can up-regulate the expression of *BoCAT* and down-regulate *BoLOX* expression, which might help to reduce ROS accumulation and alleviate broccoli senescence. Similar to the antioxidant role of H_2S in plants, H_2S was found to protect cells from oxidative stress and premature senescence in animal cells through S-sulfhydration of Keap1, antioxidant gene transcription, and glutathione production (Yang et al., 2013).

Protein degradation processes rapidly during postharvest senescence of broccoli through the activities of cysteine proteases or aspartic proteases (Buchanan-Wollaston, 1997). In the present study, H_2S inhibits the activity of protease during broccoli senescence (Fig. 2D). Consistently, reverse transcription-PCR results show that H_2S reduces gene expression of *BoCP3* (cysteine protease) and *BoLSC807* (aspartic protease) (Fig. 5C). Cysteine proteases have been found to degrade the large subunit of ribulose-1,5-bisphosphate carboxylase (Rubisco) (Mae et al., 1984). Consistent with the lower protease activity and gene expression, Rubisco degradation is partially alleviated by H_2S treatment (Fig. 2E).

In conclusion, we provide evidence that H_2S delays dark-promoted senescence in broccoli by inhibiting Chl and protein degradation, and this observation offers the prospect of H_2S application in managing post-harvest storage of horticultural products. The gene expression assays confirms the multiple roles of H_2S in regulating Chl and protein degrading genes and ethylene synthesis pathway. Senescence is a complex, highly regulated process in plants (Buchanan-Wollaston, 1997; Smart, 1994), which involved ethylene, H_2O_2 , cytokinins, jasmonic acid, etc. Thus, more works regarding the interplay between H_2S and other signals are required to elucidate the senescence regulator role of H_2S .

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