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REVIEW ARTICLE

The secret of H_2S to keep plants young and fresh and its products

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ABSTRACT

Recently, accumulating evidence has shown that hydrogen sulphide (H_2S), a newly determined gasotransmitter, plays important roles in senescence, which is an essential biological process for plant fitness and an important agricultural trait that is critical for the yield and quality of farm produce. Here, in this review, we summarize the roles of H_2S in senescence, both before and after the harvesting of agricultural products, and the underlying mechanism is also discussed. During the plant growth process, the function of H_2S in the leaf senescence process has been studied extensively, and H_2S plays roles during the whole process, including the initiation, reorganization and terminal stages. While during the postharvest stage, H_2S can prevents farm products from deterioration resulting from over-ripening, pathogen attack and incorrect storage. The underlying H_2S-related mechanisms during different stages of the senescence process are summarized and compared. The most prominent interaction occurs between H_2S and reactive oxygen species, and the molecular mechanism is explored. Additionally, the conserved action mode of H_2S in different life processes and different species is also discussed. In the future, multi-omics analyses over time will be needed to investigate the detailed mechanisms of H_2S, and a safety attribute analysis of H_2S is also required before it can be used in agricultural production.

INTRODUCTION

Senescence is a critical developmental process during the evolution of plants, and is tightly associated with plant reproduction and survival, as well as adaption to the surrounding environment (Woo *et al.* 2018). As the essential last development stage for annual plants and some organs of perennial plants, a major purpose of this process is to recycle or reallocate valuable nutrients from senescing tissues to newly developing tissues, such as young leaves, stems and roots, as well as reproductive organs and seeds (Thomas 2013). Moreover, under abiotic and biotic stresses, senescence is also a good exit strategy for plants that guarantees their best chance of survival (Bresson *et al.* 2018). During senescence, plants experience a series of metabolic, cellular, physiological and molecular changes, and thus, senescence is closely related to ripening and abscission processes (Bleecker & Patterson 1997; Gan 2018; Zhao *et al.* 2018). Morphologically, the most prominent phenotypes associated with senescence are changes in the colour and texture of leaves and fruits, as well as the vulnerability of the senescing tissues to pathogen attack (Zhao *et al.* 2018). Therefore, senescence is also an important agricultural trait, especially for crop yield improvement and the postharvest storage. Grain yield can be increased by at least an estimated 10% if leaf senescence is delayed in crops, and this would also improve the utilization efficiencies of both carbon and nitrogen (Zhao *et al.* 2018). Additionally, in developing countries, approximately half of vegetable and fruit yields are lost during the postharvest stage (Li *et al.* 2014).

Hydrogen sulphide (H_2S) has been much studied as a gasotransmitter over the last two decades. In plants, it has diverse physiological and molecular effects on developmental processes and responses to both endogenously and exogenously applied stresses (Wang 2012). For senescence, H_2S is generally an inhibitor, no matter in the development stage of the plants or in the postharvest stage (Alvarez *et al.* 2010; Huo *et al.* 2018). Thus, H_2S acts as a potential growth regulator and preservation agent during both agricultural production and storage. In this review, we summarize the mechanisms through which H_2S affects the senescence process and postharvest storage, discuss the limitations that prevent H_2S from being used in agricultural production, and explore the conserved action mode of H_2S among life processes and species to increase our understanding of its mechanisms.

HYDROGEN SULPHIDE INHIBITS SENESCENCE AND SENESCENCE-RELATED PROCESSES IN PLANT DEVELOPMENT UNDER NORMAL AND STRESSED CONDITIONS

Until now, the senescence-related roles of H_2S during plant development have mainly been studied in leaf senescence, fruit ripening and the abscission process (Alvarez *et al.* 2010; Hu *et al.* 2020; Liu *et al.* 2020). Moreover, in other limited work, the effects of H_2S on root and aleurone layer senescence under stressed conditions have also been explored (Xie *et al.* 2014; Luo *et al.* 2020).

Hydrogen sulphide in leaf senescence

Leaf senescence is a representative form of plant senescence, and therefore, a considerable amount of research on the relationship between H_2S and senescence has been performed using leaves. The leaf senescence process is divided into three stages: initiation, reorganization and termination. During the initiation phase, internal factors, such as phytohormones and developmental cues, and external factors, such as environmental cues, interact under the direction of a complex mechanism to determine the developmental age. Then, senescence is initiated when the developmental age reaches a certain threshold. During the reorganization phase, a switch from anabolic to catabolic metabolism occurs, and massive transcriptome changes also occur. Chlorophyll is degraded during this phase, and multiple toxic intermediates and by-products are produced by the catabolic process, which makes detoxification essential. The salvaged nutrients are remobilized, and the characteristic loss of the anti-oxidative capacity occurs. The terminal stage of leaf senescence features the collapse of the remaining cellular components, such as membranes, mitochondria and nuclei, and the integrity and viability of the cell are finally irreversibly lost (Bieker *et al.* 2018).

To date, the reported evidence shows that H_2S acts as an effective inhibitor during the leaf senescence process by functioning at multiple levels (Fig. 1). Before the onset of senescence, as well as in early stages of senescence initiation, the promotive roles of H_2S in nutrient assimilation and photosynthesis help to delay senescence initiation or hinder senescence progression. In soybean, H_2S and rhizobia work synergistically to increase nodule number and leaf chlorophyll content, and eventually delay leaf senescence (Zhang *et al.* 2020). In *Spinacia oleracea* leaves, proteomic data indicate that H_2S treatments increase the expression of enzymes or proteins related to carbon dioxide assimilation and light capture, and hence have positive effects on photosynthesis (Chen *et al.* 2014). During senescence progression, the inhibitory effect of H_2S is mainly achieved by its negative regulation of the autophagy process, as well as by maintaining cell redox homeostasis and mitochondrial performance. The most in-depth understanding of the roles of endogenous H_2S in leaf senescence is of its effects on autophagy. *L-CYS DESULFHYDRASE 1* (*DES1*) encodes an L-cysteine (L-Cys) desulfhydrase that catalyses the desulfuration of L-Cys to generate sulphide, ammonia and pyruvate. In a *des1* mutant, the Cys content increases, whereas the sulphide content decreases. This change is exactly opposite to the change that occurs during natural leaf senescence, in which the Cys content decreases and the sulphide content increases because of the decreased expression of O-acetylserine(thiol)lyase family genes and the increased expression of *DES1* (Alvarez *et al.* 2010). Such changes in *des1* induce the premature leaf senescence evidenced by the upregulation of the senescence-related genes and mediated by the activated autophagy (Alvarez *et al.* 2010, 2012). The autophagy activation in *des1* induces an abnormal hypersensitive response (HR), and this might explain the premature leaf senescence that occurs in this mutant (Gotor *et al.* 2013). A decreased sulphide content is the main inducer of the autophagy activation in *des1*, and the phenotype of this mutant is restored by exogenously applied H_2S. Thus, H_2S functions as a signal molecule in the autophagy process in a manner unrelated to its functions in nutrient deficiency or reactive oxygen species (ROS) (Laureano-Marin *et al.* 2016). Consequently, during natural leaf senescence, the correct sulphide concentration in the cytosol may help restrict the autophagy rate to below a certain level, and this inhibitive effect may benefit the efficient reallocation of nutrients from the leaves to other plant organs or tissues. However, the detailed mechanism behind H_2S's regulation of the autophagy process in leaf senescence still needs further investigation.

Another target of H_2S in the leaf senescence process is the cell redox system. As a critical part of the cell redox system, ROS, notably hydrogen peroxide (H_2O_2), plays important roles in all three major phases of leaf senescence, but particularly in the initiation and reorganization stages. In the leaf senescence process, H_2O_2 is essential for the successful initiation and progression. In natural or age-triggered senescence, exogenously applied H_2S increases antioxidative enzyme activity levels, and helps maintain low levels of H_2O_2 and $O_2^{\bullet -}$ to delay leaf senescence in *Salix matsudana* (Zhang *et al.* 2011). In dark-induced senescence, however, H_2S promotes H_2O_2 generation and increases expression levels of senescence-associated genes (SAGs). It also plays a role in inhibiting chlorophyll degradation in the dark, but this is mainly through the accumulation of pheophorbide (pheide) *a*, which leads to rapid leaf bleaching under light conditions (Wei *et al.* 2017). As a reactive sulphur species, the role of H_2S in thiol redox modifications during the leaf senescence process has also been studied. H_2S treatments can upregulate the expression of glutaredoxin family proteins in spinach leaves, thereby affecting thiol redox homeostasis (Chen *et al.* 2014). Additionally, the cell's thiol redox state may

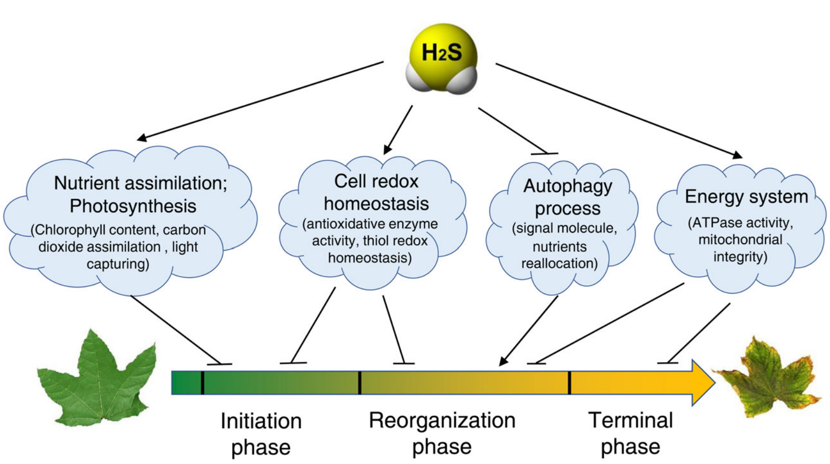

Fig. 1. Main pathways mediating the inhibitory effects of H_2S in leaf senescence. Terms in brackets represent the reported targets of H_2S in each pathway. It should be noted that, in the autophagy process, H_2S functions as a signal molecule and may affect the efficiency of nutrient reallocation through regulating the autophagy rate.

significantly influence the autophagy process (Gotor *et al*. 2013). However, how exactly the thiol redox state affects the senescence process is still unknown. Nevertheless, the persulfidation mediated by H_2S might be the prominent, or at least an important method, through which H_2S exerts its functions. The regulatory effects of exogenous applied H_2S at the transcriptional level result in large numbers of differentially regulated genes compared with non-treated groups (Li *et al*. 2017; Lv *et al*. 2021). Thus, it is easy to speculate that H_2S also plays a role in the massive transcriptional changes that occur in the reorganization phase of senescence.

The role of H_2S in energy production during the leaf senescence process has also been explored. Under drought stress conditions, the overproduced H_2S in the DES1-overexpression plants promotes their ATPase activity levels. The high energy production in these plants might help meet the higher metabolic needs of the DES1-overexpression plants, and thus, delay leaf senescence (Jin *et al*. 2018). In addition, H_2S may also contribute to the structural integrity of the mitochondria, and hence, preserve the activity of these energy factories during both the reorganization and terminal phases (Jin *et al*. 2018).

Hydrogen sulphide in other senescence-related processes during plant development

Fruit ripening is the process of fruit maturation and senescence. The relationship between H_2S and natural fruit ripening has been studied in tomato and sweet pepper. In tomato, when a nuclear-localized cysteine desulfhydrase is mutated, ripening accelerates because of reduced H_2S production. A further transcriptomic analysis indicates that the role of the endogenous H_2S in fruit ripening may be exerted at multiple levels, including gene expression modulation, protein persulfidation modification, as well as interactions with ripening-related hormones (Hu *et al*. 2020). In sweet pepper, the endogenous H_2S content increases during its ripening process, and H_2S can partially regulate the NADPH-generating system through modulating the activities of both NADP-isocitrate dehydrogenase and NADP-malic enzyme (Munoz-Vargas *et al*. 2020).

Abscission is closely related to the senescence process in many cases, although senescence may not necessarily lead to abscission (Bleecker & Patterson 1997). Evidence from tomato, rose and lily indicates that H_2S inhibits the initiation of abscission during leaf abscission, floral organ abscission and the anther dehiscence process (Liu *et al*. 2020). Auxin might be the main mode of action mediating the regulatory effects of H_2S on the abscission process. Exogenously applied H_2S increases the bioactive auxin content of the abscission tissues and, hence, inhibits the initiation of abscission. Moreover, the addition of an H_2S scavenger (HT) accelerates ethylene-induced abscission, indicating that endogenous H_2S plays the same roles in the abscission process as exogenously applied H_2S (Liu *et al*. 2020).

Programmed cell death (PCD) is another term that is closely related to senescence, and in many cases, these two processes are considered to be roughly synchronous (van Doorn & Woltering 2004; Munne-Bosch 2016). The regulatory effect of H_2S on PCD has also been explored in plants. In cadmium-treated cucumber root tips, H_2S inhibits the induction of PCD by reducing ROS accumulation through the increased antioxidative enzyme activity levels and also by inhibiting the release of mitochondrial cytochrome c through the reduced opening of the mitochondrial permeability transition pores (Luo *et al*. 2020). In gibberellic acid-treated wheat aleurone cells, H_2S delays the induced PCD through glutathione homeostasis and haem oxygenase-1 expression (Xie *et al*. 2014).

THE MECHANISM OF H_2S TO MAINTAIN GOOD PERFORMANCE IN POSTHARVEST AGRICULTURAL PRODUCTS

Senescence not only occurs during natural plant development, but it also occurs in organs separated from the main plant body. Senescence in postharvest horticultural products affects their texture, flavour, appearance and nutritional composition. Except for over-ripening, in many cases, postharvest senescence is closely related to the storage conditions and attacks by pathogens. Thus, postharvest decay can be induced in three main ways, over-ripening, improper storage and transport, and pathogen attack, and H_2S plays positive roles in all the three decay-related events (Fig. 2).

Hydrogen sulphide delays postharvest ripening and senescence processes

Regarding the working mechanism of H_2S during the postharvest processes, interaction with ethylene and ROS is the most widely explored aspect. Ethylene is well known as a ripening-promoting hormone. In climacteric horticultural products, an enhanced respiration rate caused by increased ethylene production directly leads to the onset of ripening (Ge *et al*. 2017). During the ripening process, fruit and other farm produce experience a series of dramatic changes at transcriptional, cellular and physiological levels, and these changes may lead to

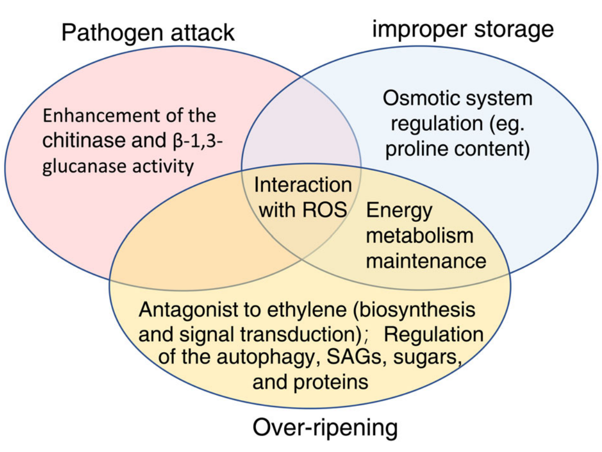

Fig. 2. Current understanding of the roles of H_2S in postharvest storage. H_2S plays positive roles in all the three postharvest decay-related events, namely over-ripening, improper storage and pathogen attack. On mechanism, the interaction between H_2S and ROS occupies a central position. Moreover, to inhibit over-ripening, H_2S also functions as an antagonist to ethylene, regulates the autophagy process, energy metabolism, senescence-associated genes (SAGs), as well as the production of other sugars and proteins. Under improper storage conditions, H_2S also regulates the osmotic system, apart from its roles in the redox and energy systems. Under pathogen attack, H_2S can also increase chitinase and beta-1,3-glucanase activity levels in horticultural products to promote fungal cell wall degradation.

chlorophyll degradation, pigment and sugar accumulation, organic acid reductions, volatile compound increases and fruit softening (Yao *et al.* 2020). In postharvest senescence, H_2S functions as an antagonist to ethylene. Exogenously applied H_2S reverses most of the changes accompanying the ripening process that are induced by ethylene, and H_2S inhibits the production and signal transduction of ethylene during the postharvest stage. In kiwifruit and pak choi, H_2S treatments decrease the production of endogenous ethylene (Zhu *et al.* 2014; Al Ubeed *et al.* 2017). The decrease in ethylene production might be associated with the downregulation of ethylene biosynthesis-related genes. In fresh-cut apple (*Malus × domestica*), exogenously applied H_2S downregulates the transcription of *MdACO1*, which encodes an enzyme that functions in the last step of ethylene biosynthesis (Zheng *et al.* 2016). In banana, both ACS and ACO family genes are downregulated. Additionally, H_2S interferes with the signal transduction of ethylene. In banana, H_2S enhances the expression of ethylene receptor genes, thereby inhibiting the ethylene signalling pathway (Ge *et al.* 2017), whereas in apple and kiwifruit, the transcription of ethylene response factors was disturbed (Zheng *et al.* 2016; Lin *et al.* 2020). Another interesting hypothesis is that H_2S may bind to the same copper ion in ethylene receptors, just like the inhibitor of ethylene perception 1-MCP, thereby inhibiting ethylene signalling (Ziogas *et al.* 2018). This hypothesis is challenged by evidence that H_2S does not inhibit the triple responses caused by ethylene. Thus, the antagonistic relationship between H_2S and ethylene in the senescence process may not be a universal model for interactions between these two signalling molecules (Liu *et al.* 2020).

Generation of ROS during the senescence and ripening process is an inducer of the horticultural product decay (Ge *et al.* 2017). The over-production of ROS usually causes lipid peroxidation, cell membrane damage and energy system collapse. These changes contribute to product quality deterioration, resulting in undesirable textures, flavours and odours, as well as yellowing leaves, withering flowers and the browning of fresh-cut fruit (Ge *et al.* 2017; Liu *et al.* 2017; Huo *et al.* 2018). The effects of H_2S on cell redox homeostasis, especially its relationship with ROS, is the most widely investigated aspect during the postharvest senescence and the ripening process. In the majority of horticultural products (both climacteric and non-climacteric) treated with H_2S, the antioxidative defences are improved through increased antioxidative enzyme activity levels (Huo *et al.* 2018; Ziogas *et al.* 2018). In some species, the expression levels of the antioxidant-encoding genes are also upregulated (Zheng *et al.* 2016; Yao *et al.* 2018). Additionally, H_2S may participate in thiol redox homeostasis, thereby contributing to the total cell redox state (Chen *et al.* 2014).

In addition to ethylene and ROS, other processes or molecules related to the postharvest senescence and ripening are also regulated by H_2S, such as the autophagy process, the energy and respiration status, the senescence-associated genes, as well as other sugars and enzymes (Liu *et al.* 2017; Ziogas *et al.* 2018; Mukherjee 2019). All the above-mentioned molecules are produced during the ripening or senescence process, and some are also key regulators or signals of the postharvest senescence. They may be indirectly regulated by H_2S through the ethylene or ROS pathway, or directly regulated by H_2S through persulfidation modifications (Mukherjee 2019).

Hydrogen sulphide inhibits pathogen-induced postharvest decay by affecting both fungi and fruits

Before being confirmed as a gasotransmitter, H_2S was traditionally viewed as a toxic gas. The H_2S concentration is the key to the gas acting as a signal or a toxin (Wang 2012). In plants and fungi, the tolerance levels to H_2S differ. Fungal growth may be greatly inhibited at a H_2S concentration that has no adverse impact on plants (Huo *et al.* 2018). Recently, H_2S was found to act as a fungistat or a fungicide at different concentrations. When applied at low level (0.5 mM NaHS solution), H_2S inhibits the growth of many fungi, such as *Aspergillus niger* and *Penicillium italicum* (Fu *et al.* 2014). However, when applied at higher levels (2 mM to 50 mM NaHS solution), H_2S acts as a fungicide and prevents fungal infection (Fu *et al.* 2014; Wu *et al.* 2018). The underlying mechanism has been explored. H_2S can also affect the cell redox state of fungi, but in a reverse manner compared with the effects on plants. Exogenously applied H_2S induces ROS accumulation in fungal cells by inhibiting antioxidative enzyme activity levels (Fu *et al.* 2014). Furthermore, H_2S increases chitinase and beta-1,3-glucanase activity in horticultural products and promotes cell wall degradation in fungi, thereby protecting plants from fungal infection (Zhang *et al.* 2014).

Hydrogen sulphide protects postharvest horticultural products in cold-storage conditions

Cold storage at non-freezing temperatures is the most widely used method in postharvest handling to delay the senescence and ripening of horticultural products. However, for some fruits, such as banana, peach and hawthorn, cold-storage conditions may cause injuries, because these fruits are very sensitive to chilling stress (Luo *et al.* 2015; Aghdam *et al.* 2018; Cao *et al.* 2018). H_2S is a good alleviator of chilling stress. In banana, applied H_2S decreases damage to fruit under cold-storage conditions by increasing antioxidant enzyme capacity, proline content and energy metabolism-related enzyme activity levels. This enhanced antioxidative capacity and energy charge level helps to decrease membrane lipid peroxidation and maintain membrane integrity, while the increased proline level helps to maintain osmotic pressure and increase the freezing point (Luo *et al.* 2015; Li *et al.* 2016). In hawthorn under cold-stress conditions, the roles of H_2S in the cell redox system, energy metabolism system, as well as production of osmotic substances, is key. In addition, activity of phenylalanine ammonia lyase is triggered in H_2S-treated hawthorn fruits, and this enzyme is essential for overcoming chilling effects (Siboza *et al.* 2014; Aghdam *et al.* 2018).

PERSULFIDATION, THE CONSERVED MODE OF ACTION OF H_2S UNDERLYING ITS DIVERSE BIOLOGICAL EFFECTS

Current research indicates that H_2S affects the senescence pathways during both developmental and postharvest stages through similar mechanisms, especially its interactions with ROS. In fact, crosstalk between these two signals is universal among life processes and species. In both plants and animals, during normal developmental processes and in responses to environmental stresses, the scavenging effects of H_2S on ROS represent the most prominent mode of action (de Cabo & Diaz-Ruiz 2020; Xuan *et al.* 2020; Zhang *et al.* 2021). In many cases, H_2S functions are only observed under stress conditions, in which the ROS level is elevated (Jin *et al.* 2011; Fang *et al.* 2014). Recently, the persulfidation of protein Cys residues was documented, in which a thiol group (R-SH) is oxidized to a persulfide group (R-SSH), being the main way in which H_2S exerts its numerous effects (Filipovic 2015). Many of the effects caused by H_2S can ultimately be attributed to the persulfidation mediated by H_2S. However, it has been suggested that R-SH may not react directly with H_2S to form R-SSH, because the sulphur atoms of these two substrates cannot be simultaneously oxidized without the involvement of other oxidants (Filipovic *et al.* 2018). Nonetheless, the persulfidation of some proteins is easily obtained *in vitro* with just the purified proteins and the H_2S donor, NaHS (Mustafa *et al.* 2009; Shen *et al.* 2020). The discovery of protein sulfenylation (Cys-SOH) may resolve this confusion. Recent evidence shows that Cys-SOH is a fundamental and reversible post-translational modification oxidized by ROS, and this modification is also a sensor of the cellular redox state (de Cabo & Diaz-Ruiz 2020). Sulfenic acids, like Cys-SOH, further react with either H_2S or ROS to form R-SSH or sulfonate (R-SO_nH), respectively. Then, R-SSH further reacts with ROS to form a series of S-sulfocysteines (R-SSO_nH). The sulfonates are a series of irreversible oxidation products, whereas R-SSH and its derivatives (R-SSO_nH) can be reduced back to thiols by enzymes, such as thioredoxins, to maintain cellular functions (Zhang *et al.* 2021). Thus, the competition between H_2S and ROS for the Cys-SOH sites determines the cell fate in redox homeostasis (Fig. 3).

Regarding the persulfidation of a specific protein *in vitro* in most laboratory systems, it is worth noting that the autoxidation of the H_2S solutions is ignored. In most cases, the H_2S donor solutions (Na_2S or NaHS) in the experiment present as yellow in colour, which indicates that they contain oxygen and trace metals (Filipovic *et al.* 2018). Furthermore, the sulfenylation of a protein may occur simply through the oxygen and trace metal ions present in the buffer (Filipovic 2015). This may partially explain why the *in vitro* persulfidation of a protein is easily obtained without the addition of an oxidant, as the Cys residues may have already been sulfenylated before their persulfidation. Nevertheless, the pathway mediated by sulfenylation may not be the only method to generate persulfide groups. Recently, a proteomic comparation between S-sulfenylation and persulfidation showed that proteins with persulfidation modifications were not completely included in the S-sulfenylation modified proteins (Zhang *et al.* 2021), indicating that other pathways besides the sulfenylation pathway may be involved in the persulfidation of a protein (Fig. 3).

In addition to persulfidation and sulfenylation, the S-nitrosylation (Cys-SNO) of the thiol (-SH) side chains of Cys in proteins occurs through the covalent addition of nitric oxide (NO). Sometimes, persulfidation and S-nitrosylation occur on the same Cys residues, and most of the time, the effects of the persulfidation and S-nitrosylation to a specific protein differ. Thus, competition may exist between H_2S and NO. Nonetheless, in some cases, H_2S and NO work synergistically, such as in increasing ascorbate peroxidase activity (Zhang *et al.* 2021). Therefore, the relationship between persulfidation and

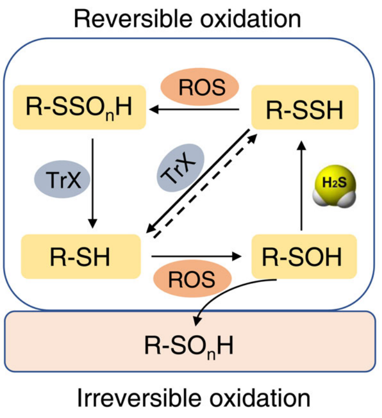

Fig. 3. Crosstalk between H_2S and ROS during post-translational modifications. In the organisms, thiol (R-SH) can be oxidized by ROS to form sulfenic acid (R-SOH). Then, R-SOH can either react with H_2S to form a persulfidate (R-SSH) or be further oxidized by ROS to form sulfonates (R-SO_nH), a series of irreversible oxidation products. Thereafter, R-SSH can further react with ROS to form a series of S-sulfocysteines (R-SSO_nH), and both R-SSH and R-SSO_nH can be reduced back to thiols by enzymes, such as thioredoxins (TrX). In addition, whether R-SH can be directly oxidized to R-SSH (indicated by the dotted arrow) still requires further investigation.

S-nitrosylation on the Cys residue is complicated, and the detailed mechanism requires further investigation.

CONCLUSION AND PERSPECTIVES

In summary, numerous endeavours in the last decade have proved that H_2S has important and positive roles in the senescence process in both natural development and postharvest stages. It is certain that studies around H_2S will help to solve the senescence-related yield limitations and postharvest losses. However, before practical application, the regulatory network behind its biological effects still needs to be completed, and gaps between laboratory research and agricultural implementation should also be resolved.

First, in the natural senescence process, especially in leaf senescence, the relationship between H_2S regulation and nutrient translocation still needs further investigation. This will aid the application of H_2S as a growth regulator and in related breeding processes. Second, before developing H_2S as a preservation agent for the postharvest stage, systematic studies on the treatment methods, adverse effects and safety attributes must be performed. Moreover, the present work has only been carried out on limited farm produce and against very few postharvest pathogens. Extended studies should be launched to obtain a more comprehensive recognition of the roles of H_2S. Third, regarding the action mechanism of H_2S, evidence on the regulatory effects of H_2S at the post-translational level in the senescence process is still lacking, and the regulatory network around H_2S still needs to be resolved. In future, a multi-omics analyses over time may help to elucidate the sequential changes caused by H_2S treatment throughout the whole process and provide the first-hand data needed to assess the critical targets of H_2S. Furthermore, investigating the enzymatic source for protein persulfidation, which is similar to the recent discovery in protein S-nitrosylation (Chen *et al.* 2020), could be another interesting direction in elucidating the mode of action of H_2S.

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