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Nitric oxide and hydrogen sulfide share regulatory functions in higher plant events

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Abstract: Nitric oxide (NO) and hydrogen sulfide (H_2S) are two molecules that share signaling properties in plant and animal cells. NO and H_2S originate two families of derived molecules designated reactive nitrogen and sulfur species (RNS and RSS, respectively). These molecules are responsible for certain protein regulatory processes through posttranslational modifications (PTMs), being the most remarkable *S*-nitrosation and persulfidation, which affect the thiol group of cysteine residues. NO and H_2S can also exert regulatory functions due to their interaction through the iron present in proteins that contain heme groups or iron-sulfur clusters, as reported mainly in animal cells. However, the available information in plant cells is still very limited thus far. In higher plants, NO and H_2S are involved in a myriad of physiological events from seed germination to fruit ripening, but also the mechanism of response to biotic and abiotic stress conditions. This viewpoint manuscript highlights the functional regulatory parallelism of these two molecules which also interact with the metabolism of reactive oxygen species (ROS) in plant cells.

Brief Historical Perspective

Nitric oxide (NO) and hydrogen sulfide (H_2S) are two gaseous molecules that were initially considered dangerous because they were associated with some detrimental effects on animal and plant cells. However, this perspective underwent a drastic change of mind when it was found that these molecules were endogenously generated in animal cells (Kolluru *et al.*, 2013). There was a gap of about 10 years between the initial research works that described the signaling functions of either NO or H_2S in living organisms. Accordingly, key research on NO, published in 1987 (Palmer *et al.*, 1987), and on H_2S in 1996 (Abe and Kimura, 1996) in animal systems provided the first pieces of evidence showing that these molecules exerted diverse signaling roles in the cardiovascular and nervous systems, respectively. Years later, plant biologists also found that these molecules were also endogenously generated in plant cells where they are involved in almost all of the stages of plant development including seed germination, root development, plant growth, stomata movement, senescence, flowering and fruit ripening (Leshem *et al.*, 1998; Lamattina *et al.*, 2003; Simontacchi *et al.*, 2004; Corpas *et al.*, 2004; Corpas *et al.*, 2006; Corpas *et al.*, 2008; Zhou *et al.*, 2018; Chen *et al.*, 2019; González-Gordo *et al.*, 2019; Mukherjee and Corpas, 2020; Zuccarelli *et al.*, 2021). And both compounds were also linked to the mechanisms of response against adverse environmental conditions triggered by either abiotic or biotic agents (Corpas, 2019; Kharbech *et al.*, 2020; Iqbal *et al.*, 2021). Fig. 1 illustrates the key functions in which NO and H_2S have been shown to participate in higher plants.

Chemistry and Biochemistry of NO and H_2S

Although NO and H_2S are very simple molecules, their (bio) chemistry is more complex than it could be thought (Stamler *et al.*, 1992; McCleverty, 2004; Hughes, 2008; Kabil and Banerjee, 2010; Filipovic *et al.*, 2018; González-Gordo *et al.*, 2020). NO is a colourless gas that belongs to the free radical-type molecules because it has an unpaired electron in the π orbital of the nitrogen atom, what is usually indicated with a dot in the chemical formula ($^{\bullet}NO$). Some of the NO and H_2S physical and chemical properties are: (i) Solubility of NO is 1.9 mM in aqueous solutions at 1 atm pressure, whereas the solubility of H_2S is 100 mM at the same pressure; (ii) Their *in vivo* lifetime is relatively short,

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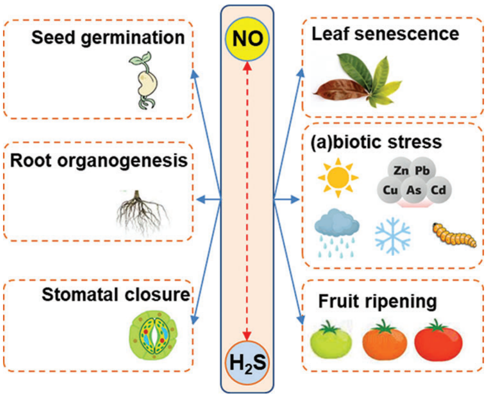

FIGURE 1. Main processes where both nitric oxide (NO) and hydrogen sulfide (H_2S) are involved in higher plants.

less than 10 seconds for NO and between second to few minutes for H_2S. Therefore, they can trigger regulatory functions in cellular loci far from their production sites; (iii) NO and H_2S are lipophilic molecules and they can diffuse across cell membranes; and, (iv) Both molecules can react with thiol groups from peptides and proteins affecting the function of the target molecules.

Likewise, NO and H_2S can also interact with iron containing-proteins, where the metal is present as either heme group or as part of the iron-sulfur cluster. Thus, there are multiple examples in higher plants where either NO and H_2S, or both, can modulate, through their interaction with the cysteine thiol groups, the functions of proteins such as cytochrome *c* oxidase, catalase, Fe-superoxide dismutase, ascorbate peroxidase, ferredoxin(Fd)-NADP reductase, glutaredoxin, Fd-dependent glutamine:2-oxyglutarate aminotransferase (Fd-GOGAT) or phytoglobins, (Ramirez *et al.*, 2011; Aroca *et al.*, 2017; Bahmani *et al.*, 2019; Palma *et al.*, 2020; Niu *et al.*, 2019; Corpas *et al.*, 2021). These proteins are involved in essential plant processes including photosynthesis, respiration, antioxidant system, nitrogen and sulfur assimilation, which remarks the physiological relevance of these signaling molecules. However, in higher plants, the available information about the direct interaction of NO and H_2S with the iron side of protein is still scarce. In addition to reactions that can originate the respective families of molecules derived from nitric oxide and hydrogen sulfide (RNS and RSS, respectively), NO and H_2S can interfere with the biosynthesis of each other and also produce novel species through their chemical interaction, thereby expanding the network of interactions that can affect to macromolecules (Kolluru *et al.*, 2013; Scuffi *et al.*, 2014; Kolluru *et al.*, 2015; Hancock and Whiteman, 2016; Iqbal *et al.*, 2021).

S-nitrosation and Persulfidation: Two Protein PTMs that Exert Redox Control of Thiol Groups

The amino acid cysteine (Cys) can play relevant roles in proteins such as a structural function through disulfide bonds, but it could also have implications on redox reactions by means of its thiol group (–SH). Thus, depending on the conditions surrounding the thiol group in the protein, Cys can be found in its anionic form, designated as thiolate (RS^-), which is a stronger nucleophilic agent than its protonated form (Netto *et al.*, 2007). Fig. 2A depicts the different oxidation states of sulfur which range from thiol (–2) to sulfonic acid (+4). Among these states, NO or H_2S can interact with the thiolate form through either *S*-nitrosation or persufidation (Fig. 2B), also known previously as protein *S*-nitrosylation and *S*-sulfhydration, respectively (Aroca *et al.*, 2018; Wolhuter *et al.*, 2018; Corpas *et al.*, 2019, Corpas *et al.*, 2021). For that reason, Cys is considered as a redox switch in the protein metabolism because it is the main target of these two PTMs, and this could affect significantly the biological activity of the corresponding protein, either positively or negatively.

Ascorbate Peroxidase (APX) in Plant Cells: A Case Study of NO and H_2S Target

In-plant systems, the number of identified proteins that undergo PTMs mediated by either NO or H_2S has progressively increased thanks to the efforts of many researchers focused on this biochemical area (Lindermayr *et al.*, 2005; Tanou *et al.*, 2009; Fares *et al.*, 2011; Begara-Morales *et al.*, 2013; Kato *et al.*, 2013; Chen *et al.*, 2014; Aroca *et al.*, 2015; Liu *et al.*, 2019). Moreover, the analyses of these modified proteins have revealed that many of them can be the simultaneous target of both PTMs and, by *in vitro* assays, it has been also proven the relevance of these two regulatory molecules to modulate the biological activity of the affected proteins (Muñoz-Vargas *et al.*, 2018, 2020; Palma *et al.*, 2020; Corpas *et al.*, 2021). Among the different plant proteomic studies focused on the identification of the potential targets of PTMs mediated by either NO or H_2S, it has been found that ascorbate peroxidase (APX) is one of those shared targets.

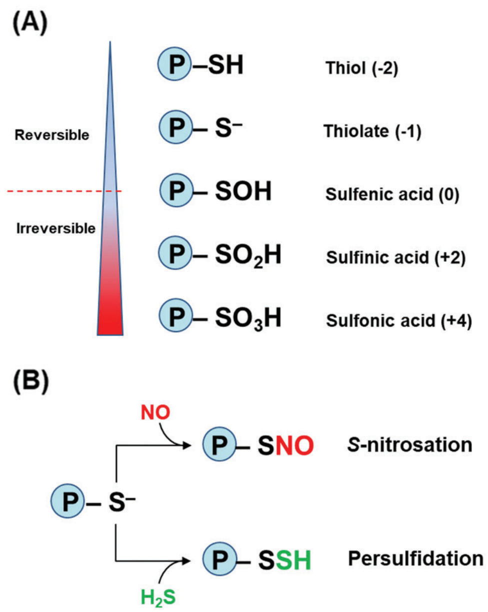

FIGURE 2. (A) Oxidation states of sulfur (S) in proteins from thiol (–2) to sulfonic acid (+4) forms. Under cellular oxidant conditions, the oxidation from sulfenic acid becomes irreversible. The numbers in parenthesis represent the different oxidation states of S in the protein. (B) Protein thiol modifications mediated by NO (*S*-nitrosation) and H_2S (persulfidation).

APX is a key antioxidant enzyme that is part of the ascorbate-glutathione cycle, which is an essential system to modulate the mechanism of response against (a)biotic stress environmental conditions (Shigeoka *et al.*, 2002; Asada, 2006; Maruta and Ishikawa, 2018). APX is a hemoprotein that controls the cellular level of hydrogen peroxide (H_2O_2) according to the following reaction:

$$\text{L-ascorbate} + H_2O_2 \rightarrow \text{dehydroascorbate} + 2H_2O$$

This enzyme system is composed of different isozymes located in almost all subcellular compartments including cytosol, chloroplasts, mitochondria and peroxisomes (Asada, 1992; Yamaguchi *et al.*, 1995; Bunkelmann and Trelease, 1996; Jiménez *et al.*, 1998; Yoshimura *et al.*, 1999; Maruta *et al.*, 2016; Chin *et al.*, 2019). This molecular and location diversity suggests the great relevance of APX in cell signaling under physiological and stressful conditions, which is consolidated by its regulation through both *S*-nitrosation and persulfidation, as indicated above. Furthermore, it was identified by mass spectrometric analyses that the Cys32 is the residue that underwent *S*-nitrosation and persulfidation (Begara-Morales *et al.*, 2014; Yang *et al.*, 2015; Aroca *et al.*, 2015) and, in both cases, the APX activity was positively regulated. This mimicking biochemical regulation provides a clear connection between NO and H_2S with the metabolism of reactive oxygen species (ROS) (Rodríguez-Ruiz *et al.*, 2017), thus indicating the biochemical link among all these families of molecules.

Conclusions and Future Perspectives

The previous perception of NO and H_2S as harmful molecules to plant cells has drastically changed and, nowadays, they are key signal molecules that regulate a myriad of biochemical and physiological processes. These two gases have also families of derived molecules designated as reactive nitrogen and sulfur species (RNS and RSS, respectively). They have a wide range of biochemical implications, being *S*-nitrosation and persulfidation two representative examples of their cellular relevance which compete molecularly to modulate protein functions through the reaction with the thiol group of cysteines. Additionally, NO and H_2S have been started to be considered as molecules with biotechnological properties since when they are applied exogenously they can exert beneficial effects on crops (Corpas *et al.*, 2019; Corpas and Palma, 2020; Corpas *et al.*, 2020). Therefore, it could be concluded that NO and H_2S behave as competing but mimicking molecules that they can reinforce each other in their signaling properties.

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