Plant in Challenging Environments 5

Tariq Aftab
Francisco J. Corpas *Editors*

Gasotransmitters Signaling in Plants under Challenging Environment

Springer

Plant in Challenging Environments

Volume 5

This book series provides recent advancements in wide areas related to higher plants and how they adapt / evolve under environmental changes in a scenario of climate change. It investigates plants under the complementary point of views, including agronomy aspects (vegetables and fruits), nutrition and health (food security), "omics," epigenetics, contamination by heavy metals, environmental stresses (salinity, drought, high and low temperatures), interaction with beneficial or pathogenic microorganisms, and application of exogenous molecules (nitric oxide, melatonin, chitosan, silicon, etc.) to palliate negative effects. It also includes changes due to climatic condition (high/low rainfall) taking into account that the climate change is often the reason why plants evolve in a challenging environment.

This book series also covers molecular-/cellular-level responses of plants under different climatic reasons. Families of molecules derived from hydrogen peroxide (H2O2), nitric oxide (NO) and hydrogen sulfide (H2S) designated as reactive oxygen, nitrogen and sulfur species (ROS, RNS and RSS, respectively) are included since, depending on the production level, they function both as signal molecules and as a mechanism of response against adverse/changing environmental conditions that can produce multiple cellular damages, alter the redox state or even trigger cell death. During these ensued metabolic processes, some anti-oxidative/oxidative enzymes are also disturbed or triggered abruptly, but there are adequate mechanisms of regulation/homeostasis in the different subcellular compartments to keep these enzymes under control.

In the last decades, the progression in this field has been enormous, but still there is so much in this field to understand the plethora of phenomena behind.

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ISSN 2730-6194 ISSN 2730-6208 (electronic)
Plant in Challenging Environments
ISBN 978-3-031-43028-2 ISBN 978-3-031-43029-9 (eBook)
https://doi.org/10.1007/978-3-031-43029-9

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Preface

Abiotic stressors, such as drought, extreme temperature, heavy metals, or high salinity, are causing huge crop losses worldwide. These abiotic stressors are expected to become more extreme, less predictable, and more widespread in the near future. With the rapidly growing human population and changing global climate conditions, it is critical to prevent global crop losses to meet the increasing demand for food and other crop products. The harm of abiotic stresses includes the disruption of cellular redox homeostasis, reactive oxygen species (ROS) overproduction, and oxidative stress damages of cellular plant components. Plants have different mechanisms to fight stress, and these mechanisms are responsible for maintaining the required homeostasis in plants. Recently, the study of gasotransmitters in higher plants has attracted much attention, especially for abiotic stress. Various signalling molecules have pivotal roles in the regulation of plant growth and development. Additionally, they emerged as cellular signaling molecules with key functions in the regulation of responses to various abiotic and biotic stressors. Their signaling pathways are interconnected in a complex network, which provides plants with an enormous regulatory potential to rapidly adapt to their environment and utilize their limited resources for growth and survival in a cost-efficient manner.

Gasotransmitters are gaseous molecules that are generated by organisms and transmit biological signals. Research on gasotransmitters is rapidly expanding and knowledge regarding the potential of gasotransmitters in biology and medicine is accumulating. Gasotransmitters, such as hydrogen gas (H_2), hydrogen sulfide (H_2S), nitric oxide (NO), carbon monoxide (CO), and methane (CH_4), are unique and regulate specific biological functions. Over the past few decades, the roles of these signaling molecules, especially NO and H_2S, have been extensively studied for their application in plants. Recently, the emissions of endogenous gasotransmitters in plants have been widely studied and analysed, thereby providing information to facilitate our understanding of new gasotransmitters signaling pathways.

Given the multidimensional role of these signaling molecules, research over the past decades in mitigating abiotic stresses in plant biology and from an agriculture point of view, we bring forth a comprehensive volume *Gasotransmitters Signaling in Plants Under Challenging Environment*. The volume comprises chapters from diverse areas dealing with biotechnology, molecular biology, postharvest technology, and metabolomics among others.

Moreover, we are highly grateful to all our contributors for accepting our invitation for not only sharing their knowledge and research but for venerably integrating their expertise in dispersed information from diverse fields in composing the chapters and enduring editorial suggestions to finally produce this venture. We also thank the Springer-Nature team for their generous cooperation at every stage of the book production.

Lastly, thanks are also due to well-wishers, research students, and editors' family members for their moral support, blessings, and inspiration in the compilation of this book.

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Chapter 1
Role of Gasotransmitters in Hormonal Responses of Plants to Abiotic Stress

Irina F. Golovatskaya and Nikolay I. Laptev

Abstract The hormonal regulation system refers to remote control systems of plant processes. It coordinates the functioning of various tissues and organs and maintains the relationships of the processes by internal rhythms, and also coordinates all the processes with the changes in the environment. Gasotransmitters (nitric oxide, hydrogen sulfide, carbon monoxide) are involved as secondary messengers in the transmission of hormonal signals. These gaseous compounds are components of the net of signals that regulate plant functions, and the implementation of their effects depends on the concentration and direction of action. Under stress conditions, the hormonal system through gasotransmitters triggers compensatory-adaptive mechanisms that prevent the disturbance of homeostasis of the plant organism. In this chapter, we have summarized information about the interaction between gasotransmitter molecules and their regulatory functions in phytohormone-controlled growth and stress-responses.

Keywords Gasotransmitters · Hormonal regulation · Adaptive mechanisms · Stress-responses

1.1 Initial Considerations

Due to their sessile mode of life, plants are exposed to impact of numerous stressors. This leads to the development of different strategies by the plants for responding to various stressors (Aftab and Roychoudhury 2021). The stress signal of the

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T. Aftab, F. J. Corpas (eds.), *Gasotransmitters Signaling in Plants under Challenging Environment*, Plant in Challenging Environments 5,
https://doi.org/10.1007/978-3-031-43029-9_1

external environment is transmitted through the plant using the hormonal system, which is represented directly by phytohormones, as well as hormone-like substances (polyamines, neurotransmitters-like and gasotransmitters) and is involved in the formation of defense reactions. Each of the hormones triggers its own signaling chain that is consistent with the functioning of other signaling pathways in the plant. As a result, a whole signaling network is created focused on maintaining the vital activity or survival of the plant under stressful conditions. Thanks to the nodes of interaction of chains, in which gasotransmitters (GTs) can participate, changes occur at the genomic and postgenomic levels, leading to a change in the whole metabolism. The latter determines the redistribution of energy and metabolic resources available to the body, and the switching of the growth and development program to the protection program.

It has now been shown that GTs play the role of messengers in the transduction of phytohormone signals. It is believed that a molecule can become a signal messenger if it meets several criteria. Messengers must be synthesized in a specific place and at a certain time, when there is a need for it. The molecule must be recognized by other molecules to convey specific information and must be deleted when there is no need to translate (Hancock 2017). Modern research confirms compliance with the criteria for nitric oxide (NO), hydrogen sulfide (H_2S) and carbon monoxide (CO) molecules. In addition, these molecules are combined into the GTs group of plant cells, since they are characterized by similar properties: gaseous state; free migration across membranes; functioning at physiological concentrations; the existence of specific systems of enzymatic (NO, H_2S and CO) and non-enzymatic (NO and CO) synthesis; lack of classic receptors; interaction with functional target groups of macromolecules; the temporary nature of the increase in the content of GTs under the influence of factors with their subsequent binding or transformation into other compounds (He and He 2014; Kolupaev et al. 2019). Since high concentrations of GTs have a negative effect on cell function, processes aimed at maintaining the dynamic equilibrium of the levels of these molecules provide one of the mechanisms of plant survival.

1.2 General Characteristics of Gasotransmitters

1.2.1 General Characteristics of Nitric Oxide (NO)

NO, playing the role of GT, regulates various processes in organisms. In higher animals and many insects, NO formation is caused by oxidation reactions of L-arginine to L-citrulline under the NO synthase (NOS) action (Montfort et al. 2017). Several pathways for NO synthesis have been suggested in plants (Fig. 1.1a). The most studied mechanisms include nitrite reduction by NIA1 and NIA2 (NITRATE REDUCTASE 1 and 2, NR1 and NR2, respectively). In contrast, the functioning of an arginine-dependent pathway involving NOS-like synthase in

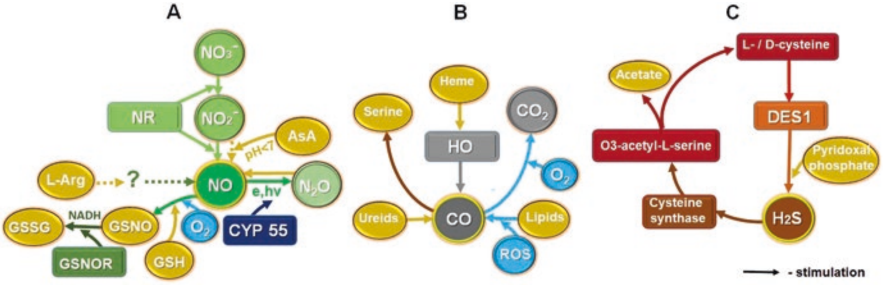

Fig. 1.1 Scheme of synthesis and utilization of gasotransmitter molecules in plants. (**a**) *NO* nitric oxide, (**b**) *CO* carbon monoxide, (**c**) H_2S hydrogen sulfide (See description in text). *AsA* ascorbate, CO_2 carbon dioxide, *CYP55* cytochrome P450 NO-reductase, *DES1* L/D-cysteine desulfhydrase, *e* electron, *GSH* glutathione, *GSNO* S-nitrosoglutathione, *GSNOR* S-nitrosoglutation reductase, *GSSG* glutathione disulfide, *HO* heme oxygenase-1, *hv* light, *L-arg* L-arginine, *NADH* nicotinamide adenine dinucleotide reduced form, N_2O nitric oxide, NO_2^- nitrite, NO_3^- nitrate, *NR* nitrate reductase, O_2 oxygen, *ROS* reactive oxygen species

plants remains controversial. However, NO formation in peroxisomes is carried out by a protein similar to animal NOS (Astier et al. 2018; Corpas et al. 2019a, b). Nonenzymatic NO formation during the conversion of nitric oxides (e.g., N_2O) and reduction of NO_2^- at pH < 7 in the presence of reducing agents such as ascorbate (AsA) has also been suggested.

NO homeostasis is maintained by coordinated processes of its synthesis and destruction. NO removal occurs in different ways. In particular, the green microalga *Chlamydomonas reinhardtii* performs light-dependent reduction of NO to N_2O at the expense of electrons from the ETC of photosynthesis using the flavodiiron protein, while the dark reaction is mediated by the cytochrome P450 NO-reductase (CYP55) (Burlacot et al. 2019).

Directed transport of gaseous compounds is difficult; therefore, S-nitrosothiols (SNOs), which are formed by S-nitrosation reactions of thiols and proteins, act as spare and transport forms of NO. S-nitrosation refers to one of the significant NO-mediated posttranslational modifications of proteins (Jahnová et al. 2019) that serve as components of NO signaling pathways. Significant SNOs include GSNO (S-nitrosoglutathione), which is formed in O_2-dependent interactions between reactive nitrogen species (RNS) and glutathione (GSH). The enzyme GSNOR (S-nitrosoglutatione reductase; EC 1.1.1.1) reduces GSNO to GSSG (glutathione disulfide) in the presence of NADH (Sakamoto et al. 2002). Thus, the enzyme GSNOR, by removing GSNO, controls NO homeostasis and is involved in many plant development and defense programs (Barroso et al. 2006; Lee et al. 2008; Leterrier et al. 2011; Guerra et al. 2016). The latter include responses to injury and jasmonic acid (JA)-dependent responses (Díaz et al. 2003; Espunya et al. 2012).

The use of exogenous sources of NO shows that nitrates ($NaNO_3$) cause a stronger and more prolonged increase in NO than L-arginine in the roots of seedlings of *Triticum aestivum* L. The accumulation of NO is inhibited by an NR inhibitor (sodium tungstate) or an animal NOS inhibitor (methyl ester of N^G-nitro-L-arginine), which allows us to discuss two pathways of NO biosynthesis (Karpets et al. 2018). These pathways are antagonistic to each other, since a combination of NO donors causes a weaker effect than individual donors.

In the lichen *Ramalina farinacea*, NO synthesis occurs during rehydration (lipid peroxidation) and reduction (NR functioning). In addition, NADPH-diaphorase activity was detected, indicating a manifestation of NOS-like activity (Expósito et al. 2019). However, it is assumed that each of the symbionts included in the lichen may have its own enzymes NR and NOS with specific characteristics that increase resistance to environmental stress factors.

Using mutant (*nia1 nia2*) and inhibitory (N^G-monomethyl-L-arginine) methods on *Arabidopsis thaliana* leaves, NOS-like activity enhanced by mechanical stress has been shown (Garcês et al. 2001). NR has also been shown to be involved in NO synthesis in guard cells in response to abscisic acid (ABA) and *Verticillium dahlia* toxins in *Arabidopsis* leaves (Desikan et al. 2002; Shi and Li 2008). NO is involved in the regulation of various processes: stomatal movement, root growth, plant development (seed germination, flowering, senescence) and stress resistance (Vidhyasekaran 2014).

The signaling function of NO is carried out through the cGMP-dependent pathway or the cGMP-independent pathway based on S-nitrosylation of protein molecules (Gupta et al. 2020). The first pathway also involves calcium and reactive oxygen species (ROS) in NO signaling; the second pathway involves nitrosation reactions (modification of protein thiol groups) (Lindermayr et al. 2005; Vidhyasekaran 2014). Nitric oxide can oxidize and interact with metals.

The use of NO donors (either nitrate or L-arginine) causes changes in plant metabolism. First of all, the content of phytochelatin, AsA, GSH and activity of the antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol peroxidase (POD) and others increases (Karpets et al. 2018; Nahar et al. 2018). Nitric Oxide prevents the damaging effect of NaCl on the growth of *Brassica juncea* L. plants, activating the accumulation of proline and the intracellular supply of Na^+, Cl^-, and K^+ (Kholodova et al. 2011).

1.2.2 General Characteristics of Carbon Monoxide (CO)

One of the ways of CO formation is the degradation of heme during its oxidation by the plastid enzyme heme oxygenase (HO) (Fig. 1.1b). At the same time, other sources of CO have been proposed for legumes (soybeans): lipid peroxidation and ureide metabolism (Wang and Liao 2016). Air can also be another CO source. The uptake of CO by plant leaves occurs in the light (Bidwell and Bebee 1974) and depends on the leaf age. The absorbed CO can be oxidized to CO_2 (corn) or reduced by being incorporated into serine (beans). Brahm (1986) annotated the ability of C3 and C4 herbaceous plants to absorb and release CO. It has been suggested that the CO uptake pathway is not related to photosynthesis because the magnitude of the process is similar in different plant species. However, the release of CO by plants depends on light and the CO_2/O_2 ratio in the environment. Since CO release is stimulated with increasing O_2 concentration, it has been suggested that CO in C3 plants is a byproduct of glycolate metabolism (Fischer and Lüttge 1978; Lüttge and Fischer 1980).

It was shown that the direction of CO action in the plant depends on its concentration. At low concentrations, CO acts as an important signaling molecule that regulates the physiological activity of plants; at the same time, it also has a toxic effect at high concentrations. CO inhibits mitochondrial cytochrome c oxidase and chloroplastic monooxygenase P450 enzymes (Muneer et al. 2014). With an increase in CO concentration, its negative effect on photosynthesis increases. CO and nitrogen oxides (NOx) treatment increases the ROS level. Depending on the CO and NOx level, different effects were revealed: under the action of low oxides concentrations, detoxification of oxidative damage occurs by increasing the antioxidant enzymes activity, whereas under the action of high GTs concentrations, the enzyme activity is reduced and AsA-GSH pathways are disrupted.

1.2.3 General Characteristics of Hydrogen Sulfide (H_2S)

Plants use a variety of reactions to maintain H_2S homeostasis (Fig. 1.1c). H_2S formation occurs by catalytic decomposition of L/D-cysteine in the presence of the coenzyme-5′-pyridoxalphosphate by L/D-cysteine desulfhydrase (DES1; EC 4.4.1.1 and EC 4.4.1.15), or in the presence of hydrogen cyanide by cyanoalanine synthase (EC 4.4.1.9). In addition, the sulfite reduction reaction with sulfite reductase (EC 1.8.7.1) in the presence of ferredoxin can be a source of H_2S. Meanwhile, H_2S binding is performed by cysteine synthase (EC 4.2.99.8) through its incorporation into cysteine (Li 2013; Liu et al. 2017; González-Gordo et al. 2020; Gautam et al. 2021).

The intensity of H_2S release by plants and their organs depends on the intensity of metabolic activity and environmental factors (Wilson et al. 1978; Rennenberg 1983; Muñoz-Vargas et al. 2022). This process enhances the activity of sulfite and sulfate metabolism. The functions of H_2S in the plant are dose-dependent: at low concentrations, H_2S is used as a sulfur source, whereas at high concentrations and prolonged exposure it impairs growth processes (Li 2013; Li et al. 2016; Hancock 2017; Huo et al. 2018) and reduces plant productivity. H_2S is an inhibitor of electron transfer in the mitochondrial chain and thus suppresses ATP production (Dorman et al. 2002).

However, at low concentrations, H_2S is considered a signaling molecule involved in many plant development and stress tolerance processes caused by excess heavy metal salts, drought, high and low temperatures, and pathogens (Li et al. 2016; Corpas 2019). For example, H_2S treatment slows fruit ripening and aging (Hu et al. 2014; Siddiqui et al. 2021; Molinett et al. 2021), prevents heavy metal accumulation (Corpas and Palma 2020; Arif et al. 2021; Raza et al. 2021), and suppresses oxidative stress (Arif et al. 2021). The latter function of H_2S is not related to direct oxidant uptake because low concentrations of H_2S are usually noted (Li and Lancaster Jr 2013). The most likely molecular mechanism that uses H_2S as a gasotransmitter is post-translational modification of cysteine residues of persulfide-forming proteins (RSSH) (Corpas et al. 2021).

The inhibitory effect of exogenous H_2S on pericarp aging in harvested litchi has been shown (Siddiqui et al. 2021). H_2S reduces the accumulation of quinone, ROS ($O_2^{\cdot -}$ and H_2O_2) and electrolyte leakage through the membrane, and maintains high levels of anthocyanins, ascorbic acid, the sum of phenols and antioxidants. In addition, H_2S increases the activity of phenylalanine ammonia lyase (PAL) and inhibits the activity of peroxidase and polyphenol oxidase, which may indicate the effectiveness of H_2S in slowing down metabolic activity.

Fu et al. (2013) found that H_2S acts as a signaling molecule under cold stress in *Vitis vinifera* L. Cold stress increases H_2S levels and *L/DCD/DES1* gene activity and expression. At the same time, exogenous H_2S (NaHS) increases SOD activity and expression of *VvCBF3* and *VvICE1* genes, but reduces the intensity of lipid peroxidation (LPO) and cell membrane permeability.

1.3 Impacts of Cross-Talk Between Gasotransmitters and Phytohormones on Plant Development and Stress Resistance

Hormonal regulation system refers to remote control systems of plant processes. It coordinates the functioning of various tissues and organs and maintains the correlative relationships of the processes occurring in them in accordance with internal rhythms, and also coordinates the processes with changes in the environment. Gasotransmitters (NO, H_2S, and CO) are involved as secondary messengers in the transmission of hormonal signals. These gaseous compounds act as components of interference in many control systems of the plant organism under normal conditions and during the adaptation of plants to unfavorable environmental conditions. Changes in the level of GTs are accompanied by transformations in the hormonal status of plants (Banerjee et al. 2018). The integration of ecological and hormonal signaling pathways, including GTs, sheds light on unique adaptive strategies in plants and expands the ways of influencing plant life.

1.3.1 Nitric Oxide-Phytohormones Cross-Talk under Abiotic Stress

The ability of a plant to mobilize defense reactions depends on its physiological status and stage of its development. Metabolism and resistance to stress factors also change when the availability of nutrients to the plant is limited. NO along with phytohormones is involved in the regulation of plant activity when the nitrogen level in plants is reduced, because NO homeostasis is caused by nitrogen metabolism. It was shown that during the formation of plant immunity *Medicago truncatula* in relation to the oomycete *Aphanomyces euteiches*, there is a cross-interaction between nitrogen metabolism and NO-signaling (Thalineau et al. 2016), which is controlled by hormones. These interactions are involved in the redistribution of nutrients in favor of growth reactions rather than defense. In particular, nitrogen-deficiency causes the salicylic acid (SA) accumulation by the plant leaf. At the same time, JA and ethylene altered the expression of NO_3^--transporter genes (*NRT1.8* and *NRT1.55*) by activating the former and inhibiting the latter (Acosta and Farmer 2010). Such regulation of NO_3^--transporters function was responsible for redistribution of NO_3^- into the roots and activation of their growth (Fig. 1.2a).

In the case of P-starvation, numerous signaling compounds are involved in the signal transduction pathway: phosphorus, inositol polyphosphate, miRNAs, cytokinins (Ck), photosynthetic products, and Ca^{2+} (Ruffel 2018). Under conditions of phosphorus deficiency, the H^+-ATPase of the plasma membrane is activated, causing "acidic growth" of the cells. This process is associated with the interaction of NO with IAA (Fig. 1.2b). During plant root formation, auxins cause an increase in NO levels (Pagnussat et al. 2002) that increases membrane-bound enzyme activity and modifies the auxin receptor TIR1 through the S-nitrosation reaction (Terrile et al. 2012; Berleth et al. 2004). The reaction enhances the interaction between

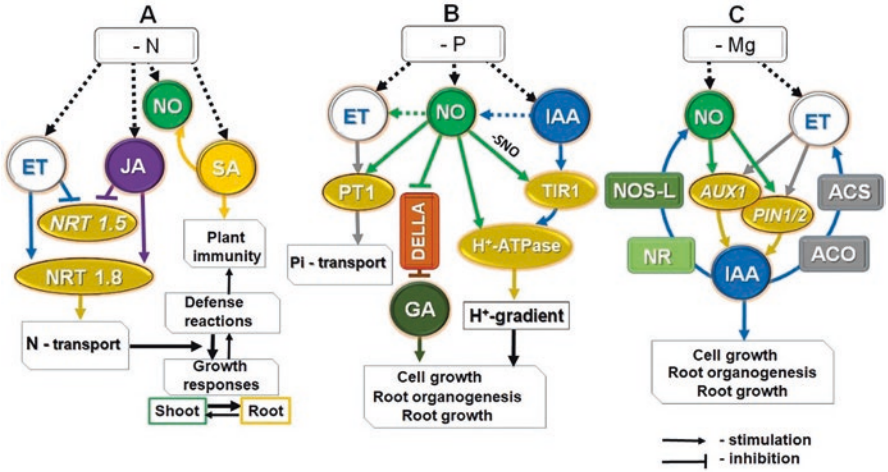

Fig. 1.2 Scheme of cross-interaction between phytohormones and gasotransmitters with a deficiency of macronutrients (N, P, Mg) in the environment and plant (See description in text). *ACO* 1-aminocyclopropane-1-carboxylate (ACC) oxidase, *ACS* ACC synthase, *AUX1* auxin influx transporter, *DELLA* gibberellin signaling negative regulators, *ET* ethylene, *GA* gibberellins, *IAA* indole-3-acetic acid, *JA* jasmonic acid, *NO* nitric oxide, *NOS-L* NO synthase-like, *NR* nitrate reductase, *NRT1.5* bidirectional nitrate-transporter, *NRT1.8* nitrate-transporter, *PIN1/2* peptidylprolyl cis/trans isomerase 1/2, *PT1* inorganic phosphate (Pi) transporter 1, *SA* salicylic acid, *-SNO* S-nitrosation, *TIR1* protein transport inhibitor response 1

SCF^{TIR1} and Aux/IAA, promoting degradation of the latter and activation of gene expression (Feng et al. 2013).

With P-deficiency in rice, there is an interaction between ET and NO. Following an increase in NO production is followed by a sharp jump in ET levels. It is expected that both molecules can be involved in optimizing the transport and efficiency of using the deficient Pi. During aging of the petunia flower, ET reactivates phosphorus turnover by regulating its transport due to the increased expression of the transporter *PhPT1* gene (Chapin and Jones 2009). During P-starvation, NO counteracts the stimulatory effect of GA in the regulation of the *Arabidopsis* primary root (PR). P-deficiency reduces GA biosynthesis and increases NO in the pericycle, endoderm cells and the buds of new plant roots, which promotes the formation of lateral roots (LK). Whereas upon inhibition of the growth of primary roots, NO turns on the pathway of degradation of the negative regulator of GA signaling DELLA (Wu et al. 2014; Jiang and Fu 2007). An interaction of NO with hormones, such as ethylene, GA, and auxins during P deficiency have been proposed in some key acclimation responses (Galatro et al. 2020).

With Mg deficiency, ET and NO increase the auxin level in *Arabidopsis* roots (Liu et al. 2018), inducing the expression of the AUX1 (AUXIN-RESISTANT1), PIN1, and PIN2 transporters (Fig. 1.2c). In turn, auxin stimulates the production of ET and NO by activating the activity of ACO [ACC (1-aminocyclopropane-1-carboxylate) oxidase], ACS (ACC synthase), NR and NOS-L (NO synthase-like). These processes form a NO → ET-auxin feedback loop.

The participation of hormones and NO in the regulation of growth processes in a plant has been shown (Fig. 1.3a). In particular, IAA and NO modulate the structure of plant shoots and leaves (Sánchez-Vicente et al. 2021). In NO-deficient mutants, the IAA level increases, which determines the participation of NOS in new NO production. Polar auxin transport supported by the PIN1 transport protein is known to determine leaf shape. In turn, NO modulates the level of IAA-transporter during IAA-mediated leaf development. Joint participation of NO and auxin in the activation of cell division and embryogenesis in alfalfa leaf cell cultures was shown (Otvös et al. 2005). The NO activity is confirmed by the data on the stimulating effect of the NO-donor and the inhibitory effect of the NO-acceptor and NO synthesis inhibitor on auxin-dependent cell division. NO is also considered to mediate the auxin action root hair growth, as NO-donor-treated lettuce plants increase their number and length (Lombardo et al. 2006). A cross-interaction between the root growth regulators auxins and NO was also established on cucumber explants. NO modulates the expression of genes that regulate the cell cycle and the formation of LR buds in the pericycle.

Under saline conditions, the levels of IAA and zeatin (Z) decrease and the level of ACC (an ethylene precursor) increases. The use of sodium nitroprusside (SNP) as a NO donor may attenuate the negative influence factor (Campos et al. 2019). A negative correlation was shown between IAA and Z levels and the Na^+ content in *L. sativa* leaves, while a positive correlation was noted for ACC (Fig. 1.3b). Such a dynamics of hormones in the leaves makes possible to increase the plant sensitivity to stress and redistribute resources for plant protection. Following changes in

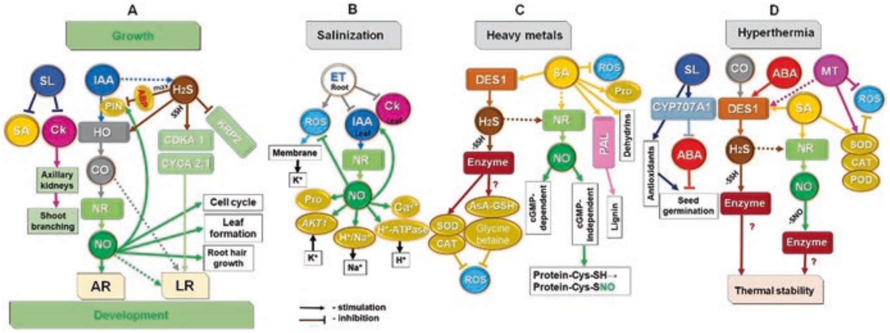

Fig. 1.3 Scheme of cross-interaction between phytohormones and gasotransmitters in the regulation of root and shoot growth (**a**) and under the influence of salinization (**b**), heavy metals (**c**), and hyperthermia (**d**) (See description in text). *ABA* abscisic acid, *ABP* actin binding protein, *AKT* serine/threonine kinase, *AR* adventitious root, *AsA-GSH* ascorbate-glutathione, *CAT* catalase, *CDKA1* cyclin-dependent kinase 1, *cGMP* cyclic guanosine monophosphate, *Ck* cytokinine, *CO* carbon monoxide, *CYCA2;1* Cyclin a2;1, *CYP707A1* Cytochrome P450 family 707, *DES1* L/D-cysteine desulfhydrase, *ET* ethylene, *HO* heme oxygenase-1, *H_2S* hydrogen sulfide, *IAA* indole-3-acetic acid, *JA* jasmonic acid, *KRP2* Kip-related protein2, *LR* lateral roots, *MT* melatonin, *Na^+/H^+* Na^+/H^+-antiporter, *NO* nitric oxide, *NR* nitrate reductase, *PAL* phenylalanine ammonia-lyase, *PIN*, auxin transporter protein, *POD* peroxidase, *Pro* proline, *ROS* reactive oxygen species, *SA* salicylic acid, *SL* strigolactones, *SOD* superoxide dismutase, *-SNO* S-nitrosation, *-SSH* S-persulfidation

hormone levels in response to salinity, NO is produced, which, using feedback mechanisms, restores the hormone concentration. During salinity, NO provides stabilization of ionic exchange: increases Ca^{2+} concentration, increases Na^+ outflow by stimulating the expression of transporter genes (H^+-ATPase, Na^+/H^+-antiporter) and inducing the expression of K^+-channel gene (AKT1-type), which is the main way of K^+ uptake by roots. NO increases proline (Pro) accumulation.

Under conditions of osmotic stress and Cd toxicity, the interaction of SA and NO has been shown (Fig. 1.3c). The addition of SA and/or NO (SNP) in the presence of Cd restores rice leaf growth. The action of these signaling molecules limits Cd uptake and accumulation, decreasing ROS-induced Cd accumulation and stabilizing redox status by maintaining AsA and GSH levels and antioxidant enzyme (SOD, CAT) activity. The restoration of growth was associated with the action of SA and NO on the protection of photosynthetic pigments and maintenance of the water level in the leaves. The combined use of SA and NO was more effective than the action of the factors separately, which indicates the intersection of SA and NO signaling in defense reactions (Mostofa et al. 2019).

When plants are injured, the interaction of NO, ROS and auxin is noted (Piacentini et al. 2020). Among the early reactions, a short-term release of NO occurs, which is accompanied by an increase in ROS. Stress products modulate the redox status of the cell. At the same time, increased NO reduces the level of endogenous IAA. With a decrease in ROS and NO, homeostasis of auxin is gradually restored, which, together with NO, regulates the process of plant wound healing (Casalongué et al. 2012).

1.3.2 Carbon Monoxide-Phytohormones Cross-Talk Under Abiotic Stress

It was found that CO is involved in the regulation of tomato root structure (Guo et al. 2008). The CO treatment promotes the formation of LR (Fig. 1.3a), which is also consistent with the activity of the CO biosynthesis enzyme heme oxygenase-1 of tomato. Exposure to CO increases the intracellular generation of NO in the roots and increases the total levels of IAA in various tissues of tomato. In addition, the action of CO is blocked by N-1-naphthylphthalamic acid (an inhibitor of auxin transport) and cPTIO (a NO acceptor). In this regard, we can talk about the interaction of IAA-, NO-, and CO-mediated pathways of formation of LR. In the regulation of adventitious root (AR) development in plants, a relationship between the gas transporter and hormones has also been shown. Xuan et al. (2008) found that CO and the enzyme HO-1 are involved as components of the signaling system in the IAA-induced AR pathway of *Cucumis sativus*. At the same time, HO-1 regulates cucumber AR growth induced by H_2S (Lin et al. 2012).

Salt stress causes water deficiency and decreased SOD and CAT activity in *Arabidopsis* and *Solanum tuberosum* plants (Fig. 1.3b) (Efimova et al. 2018; Shkliarevskyi et al. 2021b). Treatment with a CO donor has a protective effect in wild-type *Arabidopsis* plants after stress induction, but it is absent in mutants *coi1* (*coronatine insensitive 1*) and *jin1* (*jasmonate insensitive 1*). This indicates the participation of the JA in the adaptive processes induced by exogenous CO (Shkliarevskyi et al. 2021b).

The ABA signaling chain that induces stomatal closure also involves CO and the downstream components NO and cGMP (Cao et al. 2007) (Fig. 1.4). CO may be involved in the H_2S signaling system, which plays a cytoprotective role in suppressing the reactions responsible for programmed cell death, usually induced by GA (Xie et al. 2014). It follows from this that CO can induce various IAA-, ABA-, JA- and GA-dependent growth and defense responses in plants.

1.3.3 Hydrogen Sulfide-Phytohormones Cross-Talk Under Abiotic Stress

H_2S plays the role of a signaling mediator in the implementation of the protective effect of phytohormones during abiotic stress of plants. H_2S is used as GT to suppress oxidative stress caused by pollution with heavy metal salts (Fig. 1.3c). In particular, under stress conditions caused by the action of Pb and Cd, SA promotes the accumulation of endogenous H_2S in leaves (Zanganeh et al. 2018; Kaya 2020). In addition, the cross interaction between SA and H_2S reduces the phytotoxicity of heavy metals by reducing their accumulation associated with increased signaling of glycine-betaine and NO, as well as with the activation of enzymes involved in the

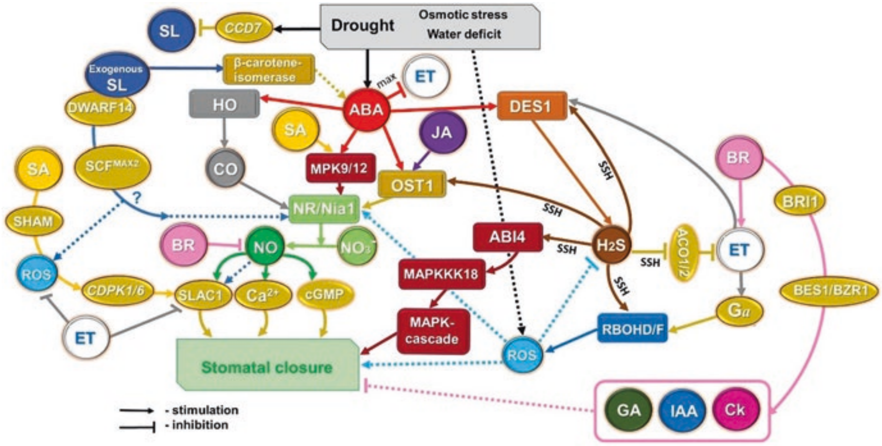

Fig. 1.4 Scheme of cross-interaction between phytohormones and gasotransmitters in the regulation of root and shoot growth (**a**) and under the influence of salinization (**b**), heavy metals (**c**), hyperthermia (**d**) (See description in text). *ABA* abscisic acid, *ABI1* protein phosphatase, *ABI4* transcription factor ABA insensitive 4, ABA signaling pathway repressor, *ACO* 1-aminocyclopropane-1-carboxylate (ACC) oxidase, *AR* adventitious root, *BR* brassinosteroid, *BRI1* brassinosteroid insensitive 1, brassinosteroid receptor, leucine-rich receptor-like protein kinase, *BZR1* protein brassinazole-resistant 1, brassinosteroid signaling positive regulator, *CAT* catalase, *CCD7* carotenoid cleavage dioxygenases, CD*PK1/6* calcium-dependent protein kinases 1/6, *CDKA1* cyclin-dependent kinase 1, *cGMP* cyclic guanosine monophosphate, *Ck* cytokinine, *CO* carbon monoxide, *CYCA2;1* Cyclin a2;1, *DES1* L/D-cysteine desulfhydrase, *DWARF14* α/β-hydrolase, strigolactone receptor, *ET* ethylene, *Gα* heterotrimeric G-protein subunit, *HO* heme oxygenase-1, H_2S hydrogen sulfide, *IAA* indole-3-acetic acid, *JA* jasmonic acid, *KRP2* Kip-related protein2, *LR* lateral roots, *MAPK-cascade* mitogen-activated protein kinase-cascade, *MAPKKK18* mitogen-activated protein kinase kinase kinase 18, ABA signaling pathway, *MAX2* more axillary growth2, *MPK9/12* mitogen-activated protein kinases 9/12, *MT* melatonin, *NO* nitric oxide, *NR/Nia1* nitrate reductase, *OST1* open stomata protein kinase 1, *PAL* phenylalanine ammonia-lyase, *POD* guaiacol peroxidase, *RBOHD/F* respiratory burst oxidase homologs D/F, *ROS* reactive oxygen species, *SA* salicylic acid, *SCF* Skp1–Cullin–F-box, *SHAM* salicylhydroxamic acid, *SL* strigolactones, *SLAC1* slow anion channel-associated 1, *SOD* superoxide dismutase, -*SNO* S-nitrosylation, -*SSH* S-persulfidation

ascorbate-glutathione cycle. SA and H_2S increase the water potential and proline (Pro) content in the leaves.

The treatment of *Triticum aestivum* L. seedlings with SA increases H_2S levels and SOD, CAT, and POD activities in roots, favoring resistance to hyperthermia (Fig. 1.3d). Inhibitors of H_2S synthesis (hydroxylamine, potassium pyruvate) in part eliminated the effects of SA, whereas application of NaHS (H_2S donor) replicated the effect of SA, and combined application of the hormone and H_2S donor further increased these parameters (Karpets et al. 2020).

The role of melatonin in reducing oxidative stress in *T. aestivum* L. during hyperthermia by reducing the intensity of lipid peroxidation (LPO) and H_2O_2 and increasing the antioxidant enzyme activities was shown (Iqbal et al. 2021) (Fig. 1.3d). Melatonin also increases photosynthesis in the plant to provide energy and metabolites under stress. H_2S is involved in melatonin signaling, since the administration of the inhibitor of its synthesis hypotaurin reverses the positive effect of the hormone.

1.3.4 Cross-Talk Between Gasotransmitters in Hormone-Dependent Growth Responses and Responses to Abiotic Stress

Root formation is a convenient model for studying the interaction of GTs and hormones (Fig. 1.3a). The H_2S donor promotes the formation (number and length) of AR of *Ipomoea batatas* L., mediated by NO and IAA, because an increase in H_2S is followed by an increase in NO and IAA (Zhang et al. 2009). H_2S-mediated root formation is reduced by an inhibitor of IAA transport and an NO scavenger, suggesting an action of H_2S upstream of the IAA and NO signaling pathways. Similar phenomena in the H_2S-dependent organogenesis of roots are observed in the shoots of *Salix matsudana* var. Toruosa Vilmak and in the seedlings of *Glycine max L.* Like auxin, H_2S can induce LR formation in seedlings of *S. lycopersicum* L. (Fang et al. 2014). It has been shown the NaHS- and NAA-induced modulation of regulatory genes of the cell cycle, in particular, upregulation of Cyclin-dependent kinase 1 (*SlCDKA 1*) and *SlCYCA2; 1* (Cyclin a2;1) and downregulation of Kip-related protein2 (*SlKRP2*). H_2S may influence the capacity of a component of the auxin-signaling pathway, regulating formation of LR. The relationship between the signaling pathways of auxin and H_2S and actin-dependent development of the root system in *Arabidopsis* has been shown (Jia et al. 2015). High concentrations of H_2S inhibit IAA transport through changes in the polar distribution of PIN transporters in cells via changes in the expression of actin binding proteins (ABP) and vesicle transport. ABP is considered as downstream effectors of the H_2S signal, regulating the assembly and depolymerization of F-actin in root cells. GSNOR regulates growth through changes in the functioning of the hormonal auxin and abscisic acid signaling pathways. As an example, in the *A. thaliana gsnor1–3* mutant, reduced GSNOR activity causes reduced basipetal IAA transport and its signal transduction, leading to impaired growth (Shi et al. 2015b).

Phytohormones are directly involved in plant adaptation to living conditions through coordination of endogenous processes according to environmental factors. In nature, very often several abiotic stress-factors act simultaneously. The phenomenon of cross-adaptation was discovered, which is a process of increasing the body's resistance to this factor as a result of adaptation to an agent of a different nature (Li et al. 2016). The mechanism of adaptation to different factors, controlled by several hormones, involves many interconnected secondary messengers (Ca^{2+}, cAMP, cGMP, MAPK-cascade, H_2O_2, H_2S and NO and others) participating in a complex signaling network. Cross-adaptation manifests itself through increased activity of defense systems (enzymatic and non-enzymatic antioxidants, osmolytes, stress proteins) and optimization of nutrient composition. Regulating the vital processes of plants, H_2S, NO, and CO are involved in cross-adaptation of plants, often realizing the functions of a large number of phytohormones.

During the formation of plant resistance to hyperthermia, cross paths of CO and H_2S, ABA and H_2S, SA and H_2S signals were established (Li et al. 2015; Li and Gu 2016; Li and Jin 2016). Pretreatment with CO, ABA and SA increases the thermal stability of the cells of the *Nicotiana tabaccum* L. suspension *in vitro* and of maize seedlings. At the same time, H_2S can play its signaling role downstream of CO-, ABA- and SA-induced thermal stability, since CO, ABA and SA increases the activity of the enzyme DES1 (Fig. 1.3d). In addition, as evidence for the involvement of H_2S in the SA and ABA signaling pathways are experiments with the addition of its donor and an inhibitor of its biosynthesis. Hyperthermia resistance is enhanced in the first case and inhibited in the second case.

A study of the induction of heat tolerance in *T. aestivum* L. seedlings showed a cross-interaction between NO and CO (Fig. 1.3d). NO acts as a signaling molecule in the anti-stress CO mechanism at high positive temperature, regulating ROS formation (Shkliarevskyi et al. 2021a).

The interaction of the SA and MeJA signaling pathways is known. MeJA stimulates senescence of leaf, whereas SA acts as an antagonist in the regulation of this process by engaging the NO signal controlled by the NOS enzyme (Ji et al. 2016).

NO and H_2S donors increase the salt tolerance of *A. thaliana* by reducing the intensity of LPO and permeability of membrane as well as by increasing the activity of SOD and CAT (Fig. 1.3b). JA and components of its signal transduction pathway (JIN1 transcription factor and COI1 protein that removes repressor proteins) have been implicated in the protective effect of GT in salinity (Yastreb et al. 2020).

SA protects wheat plants *T. aestivum L.* from stress caused by the action of heavy metals (in particular, Cd), activating PAL, which is involved in the synthesis of lignin, that in turn reduces the uptake of Cd (Fig. 1.3c). On the other hand, SA reduces PAL and electrolyte leakage, stabilizes the level of IAA, Ck and ABA, which contributes to an increase in the content of low molecular weight dehydrins, thus reducing Cd toxicity (Shakirova et al. 2016).

The interaction of signaling compounds NO, H_2S and ROS with each other with the formation of signaling molecules of the next level is shown. In particular, $O_2^{\cdot-}$ and NO react to form $ONOO^-$(peroxynitrite), NO and H_2S react to form nitrothiols (Klotz 2005; Whiteman et al. 2006), and ROS and H_2S also form products that act downstream of the signaling pathway (Li and Lancaster Jr 2013). In addition, NO and H_2S alter the levels of antioxidants in cells, decreasing the level of ROS. H_2S treatment increases the glucose-6-phosphate dehydrogenase enzyme activity, causing the accumulation of ROS (Li et al. 2013). The convergence points of the ROS and NO, ROS and H_2S signaling molecule pathways can be the activity of the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and MAP kinases (Hancock et al. 2005; Wang et al. 2010). Thus, NO and H_2S can interfere with ROS signaling.

The stomata movement under drought conditions is a good model to illustrate the interaction of a large number of hormones through ROS and NO (Fig. 1.4). NR-mediated NO formation is involved in the regulation of stomatal closure in the leaf of *A. thaliana*, controlled by ABA (Desikan et al. 2002; Kotchoni and Gachomo 2006). Brassinosteroid (BR) negatively affects the production of ABA-induced H_2O_2 and NO (Ha et al. 2016). The regulation of the expression of genes encoding proteins required for the biosynthesis of H_2O_2 and NO in plants has been shown. BR and ABA induce the expression of *AtrbohD* and *AtrbohF* genes encoding catalytic subunits of NADPH oxidase producing ROS in guard cells. However, when ABA and BR are treated together, the levels of transcripts of these genes and ROS production are lower than when treated with the phytohormone alone. NO production is also controlled by BR. Individual treatment with BR and ABA hormones increases the expression of the *NIA1* and *NIA2* nitrate reductase genes, but co-treatment with hormones decreases the expression of these genes (Desikan et al. 2002). The observed effects of the hormones indicate an antagonism of BR and ABA in the regulation of genes involved in the production of H_2O_2 and NO. Consequently, co-treatment with these hormones can suppress stomatal closure.

There is much evidence to suggest that stomatal closure is controlled by abscisic acid (ABA). However, it has been shown that treatment with the JA precursor 12-oxo-phytodienoic acid (OPDA) can have ABA-dependent and ABA-independent effects on the process. Drought tolerance in plants associated with ABA-dependent stomatal closure is also formed under the influence of SA. SA initiates SHAM (salicylhydroxamic acid)-sensitive ROS signal transduction pathway that activates calcium-dependent protein kinases 1 and 6 (CDPK1/6), which activate SLAC1 (SLOW ANION CHANNEL-ASSOCIATED 1) (Prodhan et al. 2018). MAP kinases (MPK9/12) are also involved in the SA signaling pathway, whereas OST1 (open stomata protein kinase 1) is involved for ABA and JA signaling.

The ET role in the stomata movement regulation depends on the strength of the acting stress factor. Severe water deficiency in plants increases the level of ABA, but decreasing ET biosynthesis. Normally, ET keeps the stomata semi-open, allowing CO_2 to enter the leaf and photosynthesis to take place. To achieve this function, ET inhibits ABA and JA signaling pathways at the level of anion channels and ROS production. At the same time, BP triggers the mechanism of stomata closure through increase in ET and Gα protein levels, activation of NADPH-oxidase channels (RBOHD/F, respiratory burst oxidase homologs D/F) triggering ROS production and subsequent Nia1-dependent production of NO (Shi et al. 2015a).

Among the ABA-independent processes of stomatal closure regulation are strigolactones (SLs)-mediated mechanisms based on ROS and NO production and SLAC1 activation (Lv et al. 2018). Meanwhile, stomatal opening is supported by growth-stimulating hormones: auxins and Ck (Müller and Munné-Bosch 2021).

Under osmotic stress, ABA treatment significantly increases enzymatic antioxidant activity and H_2S content compared with osmotic stress alone. In turn, H_2S increases the activity of ascorbate-glutathione (AsA-GSH) pathway (Shan et al. 2017) supported by ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, and glutathione reductase enzymes, which plays an important role in ROS detoxification in the cell (Hasanuzzaman et al. 2019). The interaction of H_2S and ET signaling pathways under osmotic stress in the guard cells of *S. lycopersicum* has been established (Jia et al. 2018). H_2S is involved in

ET-induced stomatal closure and feedback that controls ET biosynthesis (Chang 2003). ET in turn induces the production of H_2S.

Administration of H_2S scavengers or inhibitors of H_2S biosynthesis eliminates the influence of ET or osmotic stress on stomatal closure. These facts suggest H_2S as a link in the signal transduction chain of ET and osmotic stress. However, H_2S, while inhibiting ET synthesis in a dose-dependent manner, suppresses *ACO* gene expression and inhibits ACO1/2 (1-aminocyclopropane-1-carboxylic acid oxidase 1/2) enzyme activity by persulfation. This indicates the possibility of a feedback loop between ET-induced H_2S and ET through regulation of ET biosynthesis.

Scuffi et al. (2014) proposed a model of the intersection of ABA, H_2S, and NO signal transduction pathways in the regulation of stomata movement. According to this model, ABA enters the cell via ATP-binding cassette transporters and interacts with the ABA-receptor (PYR/PYL/RCAR). The resulting complex, which binds to protein phosphatases (PP2Cs: ABI1 and ABI2), releases SnRK and lower-level signaling elements. Specifically, Ca^{2+}, K^+, guanylate cyclase/cyclic ADP ribose, H_2O_2, and NO are framed among the secondary messengers of the ABA-dependent signaling network. ABA causes an increase in intracellular Ca^{2+} concentration by inhibiting K^+ transport. This is followed by the outflow of negative ions. As a result, membrane depolarization occurs. Simultaneously, ABA or the hormone-receptor complex activates the DES1 enzyme, increasing the formation of H_2S, which, through NR activation, promotes endogenous NO levels. NO controls the interaction of ABI1 with the ABA-receptor, this suggesting to be a co-receptor (Rodriguez et al. 2019; Miyazono et al. 2009; Raghavendra et al. 2010).

An important component of ABA signaling in guard cells is SnRK2.6 (nonenzymatic sucrose 1-dependent protein kinase 2.6) (Wang et al. 2015). At present, a large variety of SnRK forms has become known. For example, the barley *HvSnRK* gene family is represented by 3 subfamilies and 50 genes (Chen et al. 2021). Regulatory elements (LRE, ABRE, and others) have been established as promoters of these genes, suggesting the existence of complex networks involving the interaction of various signals, including light and hormone signals.

SLs play an important role in the induction of stomatal closure, which prevents water loss and determines plant resistance to stress caused by negative environmental factors (Lv et al. 2018). For the perception and transmission of SL signals, the SL receptor, represented by the hydrolase DWARF14 (D14), and MAX2 (MORE AXILLARY GROWTH2), which is a constituent member of the SCF E3 ligase complex, are required. During the interaction of the hormone with the receptor, SL hydrolyzes D14, causing its conformational changes that provide binding to SCF^{MAX2}, subsequent labeling by ubiquitin, and destruction of the downstream signaling chain.

A comparison of the ABA- and SL-mediated signal transduction pathways in the regulation of stomata movement shows common messengers, in particular H_2O_2, NO, and SLAC1. A feature of the ABA pathway was the connection of MRK 9/12 and OST1 kinases (Lv et al. 2018). Other relationships between SL and ABA in the regulation of resistance have been observed. During drought, the SL level in *S. lycopersicum* decreases due to downregulation of *SlCCD7* (*Carotenoid Cleavage*

Dioxygenases), the gene for SL biosynthesis, while SL GR24 treatment increases stress resistance. This is due to an increase in the chlorophylls content and photosynthesis intensity, and a decrease in the ROS content and PAL activity. SL induces stomatal closure by regulating ABA biosynthesis, which is linked through the *D27* gene encoding β-carotene isomerase, thus increasing plant resistance to drought (Bhoi et al. 2021).

1.4 Concluding Remarks

Currently, the participation of GTs in the signal transduction of hormones and abiotic stress-factors (mineral nutrition, temperature, drought, salinity, heavy metals) has been convincingly established. GTs play not only a signaling role (Ivanovic-Burmazovic and Filipovic 2019; Mishra et al. 2021), but also a regulatory one, since they change the cell redox homeostasis, the activity of enzymes and plant growth and development (Gupta et al. 2019; Huang et al. 2021; Mukherjee and Corpas 2020; Xuan et al. 2020). GTs participate in a feedback loop with hormones through the regulation of their synthesis and transport. Numerous intersections of hormone signaling pathways have also been identified, the nodes of which can be GTs. At the same time, H_2S and NO can carry out persulfidation and S-nitrosation reactions, respectively, acting on the same proteins (Corpas et al. 2022). In addition, NO and H_2S have an effect on the intracellular content of each other. Depending on the nature of the stress factor and regulated processes, NO in signaling chains can be located both upstream and downstream H_2S. Interaction occurs between GTs: they influence the generation of each other, absorb each other, reducing intracellular concentrations, and thus reducing or neutralizing their effects. Thus, GTs regulate growth processes and the formation of defense responses to many negative environmental factors. The study of phytohormones signaling and their relationships with gasotransmitters make possible to find ways to influence the vital activity of plants, what is of no little importance for increasing plant resistance to the increasingly unpredictable dynamics of the environment associated with current climate change. This will ensure an increase in plant productivity, and, consequently, the maintenance or a high level of food security for the population of our planet.

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Chapter 2
Understanding the Involvement of Gasotransmitters in the Regulation of Cellular Signalling and Adaptive Responses Against UV-B Mediated Oxidative Stress in Plants

Sayanti De, Mehali Mitra, and Sujit Roy

Abstract Due to their sessile and immobile nature, plants continuously encounter multifarious abiotic stress factors including solar UV-radiation, changing temperature conditions, desiccation, soil salinity, re-hydration, heavy metal toxicity, etc. All these abiotic stress factors severely affect plant growth and development at both vegetative and reproductive levels eventually leading to compromised crop yield. These stressors damage plants at the physiological, cellular, and molecular levels by inducing direct or indirect oxidative stress due to over-production of Reactive Oxygen Species (ROS) and disruption of the redox homeostasis within the cell. Plants counterbalance these adverse effects by activating stress-responsive mechanisms via signal perception, transduction and ultimately activating complicated defense pathways. Concerning these stress factors especially in abiotic stress tolerance, the study of gasotransmitters in plants and animals has emerged in the past couple of years. Gasotransmitters (GTs) are endogenously generated small gaseous molecules that play a crucial role in transmitting biological signals and induce physiological or biochemical changes in response to stress. Nitric oxide (NO), carbon monoxide (CO), hydrogen sulfide (H_2S), methane (CH_4) and hydrogen gas (H_2) are considered to be the most important classes of gasotransmitters. Production of gasotransmitters is induced when exposed to abiotic stress factors. However, recent studies have demonstrated that these gasotransmitters play key role in the enhancement of the functional activity of several antioxidant enzymes which help plants to cope up with these abiotic stress factors by decreasing the effects of oxidative damages. Therefore, deciphering the mechanisms of action of these gasotransmitters and their interactions with each other may greatly contribute to the improvement of crop yield and increase their acceptance in agriculture in near future. In this chapter, we

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T. Aftab, F. J. Corpas (eds.), *Gasotransmitters Signaling in Plants under Challenging Environment*, Plant in Challenging Environments 5,
https://doi.org/10.1007/978-3-031-43029-9_2

have mainly highlighted the role of gasotransmitters in mediating cell signaling and their adaptive aspects in the context of UV-B mediated oxidative stress responses in plants.

Keywords Abiotic stress · Oxidative stress · Reactive oxygen species (ROS) · Gasotransmitters · Nitric oxide (NO) · Hydrogen sulfide (H_2S) · Carbon monoxide (CO) · Hydrogen gas (H_2) · Methane (CH_4) · Stress tolerance · UV-B radiation

2.1 Introduction

Urbanization and industrialization to cope up with the excessive population growth are contributing to drastic and lethal global climate change. As plants are sessile, they cannot migrate being exposed to diverse environmental stressors like high and low temperature, dehydration, rehydration, UV radiation, salinity, heavy metal toxicity, etc. These stressors hamper the normal growth and development of plants by affecting their size, changing leaf morphology, and stomatal aperture which ultimately affects agricultural crop productivity and yield under these unfavourable conditions (Kul et al. 2019). Yield potential of plants becomes strongly affected when these stress factors act in combination. Among these stress factors, UV-B is one of the major environmental stressors that can result in certain physiological changes in plants, like reduction in biomass and size, disruption of the photosynthetic machinery, and also aiding in the accumulation of UV-absorbing compounds (Brosché and Strid 2003; Frohnmeyer and Staiger 2003). Likewise, exposure to high UV-B radiation also causes the overproduction of reactive oxygen species (ROS) and subsequently induce oxidative stress (Zhao et al. 2015). To ameliorate the adverse effects resulting from UV-B exposure, plants have developed diverse strategies to repair the damages. Plants respond through an interactive network of biochemical and molecular mechanisms including selective ion absorption, compartmentalization of ions into vacuoles, ion exclusion, organic solute accumulation, etc. (Miller et al. 2009; Zandalinas et al. 2019). ROS production is considered to be the most common response to abiotic stress. Several previous studies have shown that the redox environment in a cell is maintained by the balance between ROS production and its antioxidant capacity (Alleman et al. 2014). Plants have developed a well-organized antioxidant defense system to combat damages caused by environmental stresses (Hasanuzzaman et al. 2018a). To adapt to these stress conditions plant also synthesizes some secondary metabolites, hormones, and signaling molecules that transmit biological signals. These small gaseous molecules are termed gasotransmitters.

Gasotransmitters (GTs), such as nitric oxide (NO), carbon monoxide (CO), hydrogen sulfide (H_2S), methane (CH_4) and hydrogen gas (H_2) are widely involved in the regulation of specific biological functions (Peers and Lefer 2011). Other than these, ammonia (NH_4^+) and ethylene (C_2H_4) also fulfil the criteria to be considered gasotransmitters. Several previous studies have reported that these gasotransmitters are usually produced as a response to abiotic stress tolerance (Abdulmajeed et al. 2017; Cui et al. 2017; Jia et al. 2018). Over the past few decades, the function of gasotransmitters has been characterized well in mammalians and more recently the involvement of gasotransmitters in mediating the abiotic stress responses in plants has also been taken into interest. Recent studies involving GTs have facilitated our understanding of GT-mediated signaling pathways and their role in enhancing plant tolerance (Yao et al. 2019). GTs play a pivotal role in the regulation of seed germination, growth, and development, cell cycle as well as senescence in plants (Vasil'eva 2010; del Giudice et al. 2011). It has been reported that GTs are involved in most of the phytohormone signaling pathways and changes in their concentration can also affect hormonal activity (Jin et al. 2016; Banerjee et al. 2018). Moreover, GTs can also interact with different biologically active proteins and modulate their activities along with their interactions with phytohormones like auxin (AUX), gibberellins (GAs), cytokinin (CKs), abscisic acid (ABA), salicylic acid (SA), jasmonic acid (JA), and brassinosteroids (BRs). (Bhuyan et al. 2020). In this chapter we have summarized the production of gasotransmitters in plants under adverse abiotic conditions, their role in the amelioration of UV-B mediated oxidative stress, and cross-talk between several gasotransmitter-mediated signaling pathways.

2.2 Synthesis of Major Gasotransmitters in Plant Cells Under Adverse Conditions

In 2002, Rui Wang first reported the term 'Gasotransmitter' referring to a gaseous messenger molecule that is involved in signaling pathways. The signaling molecules exhibit a very wide range starting from large proteins, lipids, and peptides to amines, amino acids, and gaseous molecules depending on their chemical natures (Mustafa et al. 2009). Gasotransmitters or neurotransmitters are basically those signaling molecules whose property variation is based on their chemical nature. These gasotransmitters are considered to be a subfamily of endogenous gaseous signaling molecules (Li et al. 2011). As they are freely permeable to the cell membrane, they do not require any receptor for their activation and, in response to any stimulation, their synthesis is very rapid unlike other signaling molecules (Wang 2004; Allan and Allan and Morris 2014). NO exocytosis takes place upon release of any gasotransmitters or no endocytosis is required for them to enter the cell. The gasotransmitters are endogenously produced and enzymatically regulated and they have their own significant biological roles at different concentration levels. At first nitric oxide (NO) was discovered as a gasotransmitter which triggered the discovery of other possible gasotransmitters like carbon monoxide (CO), hydrogen sulfide (H_2S), hydrogen gas (H_2), and methane (CH_4) (Wang 2002). The discovery of these gasotransmitters has provided new insights into biological sciences and many other gases like acetaldehyde, ammonia, sulfur dioxide, and dinitrogen oxide are still under investigation to understand whether they too act as endogenous mediators. In the past couple of years, gasotransmitters have been a rapidly expanding topic of research (Wang 2018). Previously, several studies have reported that these gasotransmitters are produced in plant cells due to the presence of abiotic stress factors and those studies have also shown that gasotransmitters play pivotal roles in plants in response to the stressors (Jin et al. 2017; Abdulmajeed et al. 2017, Jia et al. 2018; Maryan et al. 2019; Xu et al. 2017a, b).

2.2.1 Nitric Oxide (NO)

Nitric oxide (NO) is the first gasotransmitter that was discovered in 1987 in animal cells (Palmer et al. 1987) having major function as one of the signaling molecules (Sukmansky and Reutov 2016). The research regarding the presence of NO in plant cells started almost two decades later (Durner et al. 1998). The studies on the synthesis of plant NO are considered to be the oldest but still popular topic of research among scientists (Astier et al. 2017). But the synthesis of NO is still largely unknown and assumed to be much more complicated in plants as it occurs in multiple ways. At first, Klepper (1979) observed the NO production in herbicide-treated soybean (*Glycine max*). The NO production was also found to be triggered under drought stress in marigold plants (Liao et al. 2012). Other studies indicated that among heavy metals, cadmium and aluminium induce NO production in *Arabidopsis*, lichen, peanut and wheat (Han et al. 2014; Kováčik et al. 2019; Sun et al. 2018; Faria-Lopes et al. 2019; He et al. 2018).

In plants, NO is generated by reductive and oxidative pathways involving both enzymatic and non-enzymatic systems (Kolbert et al. 2019). In animal cells, the biosynthesis of NO is catalyzed by NO synthase (NOS), a small family of multidomain hemeproteins which is basically an enzyme that exists in multiple isoforms. NOS catalyzes the oxidation of L-arginine to N^G hydroxy-L-arginine, then to L-citrulline plus NO. This catalytic cycle of NOS occurs through two oxygen-dependent mono oxygenation reactions which include one stable intermediate N^G-hydroxy-L-arginine (Santolini 2011). In this oxidation process of NO generation, a total of five electrons are required among which two are from O_2 and three are contributed by nicotinamide adenine dinucleotide phosphate (NADPH). FAD (flavin adenine dinucleotide), FMN (flavin mononucleotide), and tetrahydrobiopterin (BH_4) are responsible for the regulation of NOS enzymes, and phosphorylation of the enzyme is dependent on several serine kinases.

On the other hand, the discussion and research are advancing regarding the synthesis of NO from L-arginine in plants and as a result, it has been assumed that the L-arginine dependent NO synthesis in plants is quite similar to the process in animal cells. Also, the NOS homologs of animals have been found in green algae (Roszer 2014). Although the homologs have not yet been detected in higher plants, it is assumed that particular proteins using L-arginine as a substrate can produce NO, and consequently, it has been designed as a NO-like synthase (Astier et al. 2017). Numerous data on the inhibition process of NO-synthesis or NO-dependent pathways by NOS inhibitors supported the presence of L-arginine-dependent NO formation in plants (Crawford 2006). The activity of polyamine oxidase decreases in the presence of L-NAME, a NOS inhibitor, which might be associated with the enzyme catalysis of NO formation (Flores et al. 2008). Previously, it has been shown that, in plant leaves, L-arginine is converted to polyamines which are considered as potential sources of NO (Rosales et al. 2011). Also, several studies have revealed that there is a clear increase in NO production with the application of exogenous L-arginine indicating clear evidence of NO generation by the oxidation of the amino acid (Table 2.1). Under drought conditions in wheat, the production rate of endogenous NO was increased by the exogenous NO and arginine application (Hasanuzzaman et al. 2018b). Subsequently, NO formation was also triggered via exogenous CH_4 and sodium nitroprusside under osmotic stress conditions (Zhang et al. 2018). In wheat seedlings, nitrate-reductase-dependent NO formation was observed after treatment with L-arginine (Astier et al. 2017). Likewise, NO has been shown to be produced under salinity stress conditions in *Arabidopsis* and tobacco and it was also found that under stress conditions the increase in NO formation in tobacco leaves occurs due to the induction of nitrate reductase which indicated that the plant nitrate assimilation is closely associated with NO formation (Da Silva et al. 2017; Liu et al. 2015). The activity of nitrate reductase is stimulated by inhibitors of the L-arginine dependent NO formation pathway L-NAME and D-arginine, what shows the functional interaction of the nitric oxide synthesis pathways in plants (Rosales et al. 2011). NO formation was also found to be increased by phytohormones and signaling molecules like indole-3-butyric acid and 1-methylcyclopropene (Liao et al. 2011, 2013).

Apart from the synthesis pathways of NO, the total content of NO also depends on the activity of utilization mechanisms in plants (Corpas et al. 2008). Nitric oxide reacts with glutathione (GSH) to form S-nitrosoglutathione (GSNO) which is considered the most important reservoir of NO as well as a NO donor in the plant cells. The enzyme S-nitrosoglutathione reductase regulates the GSNO content in plants as it can reduce GSNO to glutathione disulphide (GSSG) and NH_3 (Gupta et al. 2011). The amount of NO which is not required for plant cells is converted to nitrite or peroxynitrite ($ONOO^-$)by binding to its nonsymbiotic hemoglobin forms or with the superoxide anion radical ($O_2^{\cdot -}$) respectively (Corpas et al. 2008; Freschi 2013).

2.2.2 *Carbon Monoxide (CO)*

Back in the year 1959, scientists got an indication of plants' ability to produce carbon monoxide (Wilks 1959). At first, Wilks (1959) discovered the formation of CO in plants. It was also noticed that abiotic stress factors trigger CO production in plants. Zilli et al. (2014) reported that the leaves and roots of soybean produce CO under NaCl stress. Subsequently, CO production was detected under heavy metal cadmium stress in the root tissue of *Medicago sativa* (Han et al. 2008). Also, it was found that sunlight-exposed lima beans directly emit CO (Tarr et al. 1995). It has

Table 2.1 Synthesis of major gasotransmitters under different abiotic stress conditions

Gasotransmitter	Studied plant	Type of stress	Reference
Nitric Oxide (NO)	Soybean	Herbicide treatment	Klepper (1979)
	Marigold, Wheat	Drought stress	Liao et al. (2012) and Hasanuzzaman et al. (2018b)
	Arabidopsis, Lichen, Peanut, Wheat	Heavy metal stress: Cadmium and Aluminum	Han et al. (2014), Kováčik et al. (2019), Sun et al. (2018), Faria-Lopes et al. (2019), and He et al. (2018)
	Arabidopsis, Tobacco	Salinity stress	Da Silva et al. (2017)
Carbon Monoxide (CO)	Leaves and roots of Soybean	Salinity stress	Zilli et al. (2014)
	Roots of *Medicgo sativa*	Cadmium stress	Han et al. (2008)
	Lima beans	Sunlight exposure	Tarr et al. (1995)
	Arabidopsis	Light induced stimulation of plant pigment B	Wang and Liao (2016)
Hydrogen Sulfide (H_2S)	Wheat	Exogenous abscisic acid, Osmotic stress	Ma et al. (2016) and Corpas et al. (2019)
	Arabidopsis thaliana	Drought stress	Jin et al. (2011, 2016)
	Bermuda grass	Cadmium stress	Shi et al. (2014)
	Cauliflower	Lead stress	Chen et al. (2018)
	Zucchini	Nickel stress	Valivand et al. (2019)
	Grape, Cucumber, Poplars	High temperature stress	Fu et al. (2013), Liu et al. (2019), and Cheng et al. (2018)
	Hawthorn fruit	Cold stress	Aghdam et al. (2018)
Hydrogen gas (H_2)	Lettuce seeds	Bright light condition	Renwick et al. 1964
	Rice	Salt stress, low temperature condition, drought stress, aluminum stress	Xu et al. (2013, 2017a, b)
	Alfalfa	Salt stress, exogenous application of methyl viologen	Xu et al. (2013) and Jin et al. (2013)

(continued)

Table 2.1 (continued)

Gasotransmitter	Studied plant	Type of stress	Reference
Methane (CH_4)	Canola	Blue light condition	Martel and Qaderi (2019)
	Rice	Aerobic conditions	Keppler et al. (2006)
	Poplar	Low light condition	Brüggemann et al. (2009)
	Pea leaves	High temperature condition	Abdulmajeed et al. (2017)
	Alfalfa	Salt stress; heavy metal exposure: cadmium, copper or aluminum	Zhu et al. (2016), Gu et al. (2018), Samma et al. (2017), Cui et al. (2017)
	Maize	Polyethylene glycol (PEG)	Han et al. (2017)
	Tobacco, Citrus fruits	UV-radiation	McLeod et al. (2008)

also been found that in *Arabidopsis*, light-induced stimulation of plant pigment B results in CO formation (Jia et al. 2018). Previously, many studies have shown that the production of CO occurs by the enhanced heme oxygenase (HO) activity in plants of different taxa. The release of CO along with iron occurs through the stereospecific cleavage of heme to BV-IX2α, a process that is catalyzed by HO in the presence of reducing agents (Gisk et al. 2010). A small family of HOs has been detected so far in *Arabidopsis* which is represented by four members belonging to two HO subfamilies: HO-1 and HO-2. There are three members in the HO-1 subfamily which are the HO-1 (HY1), the HO-3, and the HO-4, whereas there is only one member in the HO-2 subfamily which is the own HO-2, although this enzyme is not considered to be a true heme oxygenase (Emborg et al. 2006). HO-1 enzyme is induced by several factors like stress conditions, signal transduction, and phytohormones' regulation which can be noticed by the increased content of the protein (Jin et al. 2016). NADPH functions as an electron donor at the time of disintegration of heme by members of the HO-1 subfamily in plants (Jin et al. 2016). CO can also be produced through a non-enzymatic pathway during the destruction of heme-methylene bridges (Zilli et al. 2008). The excess CO produced in plants may get inactivated by binding with leghaemoglobin which is a similar characteristic of hemoglobin (Stetzkowski et al. 1985).

2.2.3 Hydrogen Sulfide (H_2S)

Hydrogen sulfide as a gasotransmitter was discovered after NO and CO. Reportedly, H_2S is produced by many abiotic stress factors like drought, heavy metal stress, and temperature (Hancock 2019). Many researchers are currently showing interest in the studies regarding H_2S, as it is thought to be one of the signaling molecules in plants (Hancock and Whiteman 2014). In wheat seedlings under drought stress, exogenous abscisic acid application induces an increase in endogenous H_2S

formation (Ma et al. 2016). Although wheat seedlings noticeably release H_2S under osmotic stress (Zhang et al. 2010). In *Arabidopsis thaliana*, drought stress promotes H_2S production (Jin et al. 2011, 2016). As well as some heavy metals like cadmium treatment in Bermuda grass (*Cyanodon dactylon*) have been shown to increase endogenous H_2S production significantly (Shi et al. 2014). Lead and nickel increased H_2S production in cauliflower and zucchini, respectively (Chen et al. 2018; Valivand et al. 2019). It has been shown that temperature variation in several plants plays a major role in H_2S production in plants. Reportedly, high-temperature stress induces a rapid release of H_2S in grapes, cucumbers, and poplars (Fu et al. 2013; Liu et al. 2019; Cheng et al. 2018) (Table. 2.1). Interestingly, it has also been found that under cold stress, treatment with exogenous H_2S promotes the release of endogenous H_2S in hawthorn fruit (Aghdam et al. 2018). To date, six enzymatic pathways have been detected which are most capable of H_2S biosynthesis (Li 2015; Rudenko et al. 2015). It has been suggested by Jost et al. (2000) that L-cysteine produces H_2S which is catalyzed by β-cyanoalanine synthase in the presence of hydrogen cyanide in plants. L-cysteine is converted to pyruvate which takes place by the activity of L-cysteine desulfhydrase and thus H_2S and ammonia are released (Romero et al. 2013; Li 2015). There is also a possibility of H_2S formation from D-cysteine by the action of D-cysteine desulfhydrase (Li 2013). Apart from this, there are several other enzymatic sources in plants from which H_2S can be produced like cysteine synthase or carboanhydrase. Cysteine synthase catalyzes the formation of O-acetyl-L-serine and H_2S by the reversible reaction between L-cysteine and acetate, and the cysteine synthase enzyme is found in the cytosol, mitochondria as well as in chloroplast (Wirtz and Hell 2006; González-Gordo et al. 2020). β-cyanoalanine synthase is a mitochondrial enzyme that catalyzes the condensation of cyanide and L-cysteine with H_2S release but mostly its activity increases to control the cyanide content in the cell as cyanide acts as a potent inhibitor of the mitochondrial respiratory chain (Li 2015; 2016) (Table. 2.1). Carboanhydrase catalyses the carbonyl sulfide decomposition into CO_2 and H_2S (Yamasaki and Cohen 2016). There is another enzyme in plants namely O-acetyl-serine lyase which has been detected as promoting the degradation of H_2S (Lisjak et al. 2013). H_2S mainly promotes antioxidant defence systems, and also plays a key role in the interaction network with other molecules like NO, reactive oxygen species (ROS), phytohormones etc. (Raza et al. 2021). Although several enzymatic pathways for H_2S synthesis in plants have been detected so far, the mechanism of H_2S production under several abiotic stress factors is yet to be established.

2.2.4 Hydrogen Gas (H_2)

At the beginning of the twentieth century, H_2 gas was first detected in bacteria and subsequently in green algae and higher plants (Stephenson and Stickland 1931; Sanadze 1961). Scientists suggested that bacteria can produce H_2 due to the presence of endogenous hydrogenase. In the past few years, studies have revealed that abiotic factors like light, salt, temperature, and heavy metals can promote H_2 production in plant cells. Thus, in lettuce seeds H_2 production has been shown during germination under bright light conditions (Renwick et al. 1964). In rice, H_2 was reportedly produced by salt stress, low-temperature condition, drought stress, or aluminium stress (Xu et al. 2013, 2017a, b) (Table 2.1). H_2 production was also induced by several phytohormones like ethylene, abscisic acid, and jasmonate acid (Zeng et al. 2013). In alfalfa, H_2 production is promoted by salt stress as well as by the exogenous application of methyl viologen (paraquat) which increases endogenous H_2 production in plants (Xu et al. 2013; Jin et al. 2013). However, the investigation to date has detected the production of H_2 in plants due to several abiotic stress factors but any clear mechanism of the H_2 production pathway is yet to be established.

2.2.5 *Methane (CH_4)*

At first, the production of CH_4 was detected in rice seedlings (Nouchi et al. 1990). Among abiotic stress factors, salt, drought, heavy metals, and UV radiation play a major role in producing CH_4 in plants. Also, under blue light conditions CH_4 is produced in canola (Martel and Qaderi 2019). Under aerobic conditions, rice (Keppler et al. 2006), low light, poplar (Brüggemann et al. 2009), high-temperature, pea leaves (Abdulmajeed et al. 2017), and salt stress, alfalfa produce methane (Zhu et al. 2016). Alfalfa also produces CH_4 under heavy metal exposure such as cadmium, copper, or aluminium (Cui et al. 2017; Gu et al. 2018; Samma et al. 2017). Reportedly, polyethylene glycol (PEG) induces dehydration in plants and increases methane formation in maize plants (Han et al. 2017). In several plants including tobacco, methane is produced under UV radiation (McLeod et al. 2008) (Table 2.1). In citrus fruits, the mechanism of CH_4 production upon UV irradiation has been somewhat established by Messenger et al. (2009). They reported that, under UV radiation, UV reacts with plant photosensitizer and produces hydroxyl radicals which eventually form CH_4 from the pectin methyl group.

2.3 Role of Gasotransmitters in Alleviating UV-B Mediated Oxidative Stress

As plants are photoautotrophic organisms, they require sunlight to carry out their basic physiological processes like photosynthesis, respiration, growth, and development. On the other hand, due to this obligatory dependence on sunlight, plant leaves also absorb damaging UV rays specifically UV-B (280–320 nm) rays which account to nearly about 0.5% of the total UV radiation. Though it represents a very marginal portion of the solar radiation it is enough capable of causing severe damage to living organisms. In recent years, due to the gradual depletion of the protective ozone layer by anthropogenic activities like the production of chlorofluorocarbons (CFCs), the proportion of UV-B absorption is likely to increase in plants (Caldwell et al. 2003; McKenzie et al. 2011). Overexposure to UV-B radiation has been shown to have deleterious impacts on plant cells including disruption of the thylakoid membrane, knocking down of the chlorophyll and carotenoid pigments, reduced photosynthetic rate, and decreased protein synthesis due to indirect induction of oxidative stress via generation of ROS all of which eventually led to programmed cell death (Booij-James et al. 2000; Lytvyn et al. 2010; Krasylenko et al. 2012).

The main sources of ROS in such stressful conditions mainly include augmentation in photorespiration, NADPH oxidase (NOX) activity, and impairment of the electron transport chains of mitochondria and chloroplasts (Frohnmeyer and Staiger 2003). To cope with this adverse situation plants have developed a well-organized and highly developed antioxidant defense system having both enzymatic [e.g., ROS detoxifying enzymes like superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) etc.] and non-enzymatic components such as ascorbic acid (ASC) and reduced glutathione (GSH) that are ubiquitously present in almost all of the subcellular compartments (Cassia et al. 2019). As a general protective response under UV-B stress several UV-protective secondary metabolite production pathways are activated such as the phenylpropanoid pathway (Hollósy 2002; Kovács and Keresztes 2002). Gasotransmitters have been found to play major roles in UV protective mechanisms. It has previously been reported that NO, a well-known gasotransmitter, is involved in UV-protective responses in plants through abscisic acid-mediated steps (An et al. 2005; Qu et al. 2006; Tossi et al. 2009). NO orchestrates a wide range of events for maintaining the redox equilibrium in a plant cell by regulating the ROS concentration. Whatever may the sources be, ROS concentration must be adequately regulated to avoid the cellular damages due to their overproduction. When ROS are produced in a considerable amount within the cell, NO induces the transcription of several genes regulating the production of enzymatic antioxidants like SOD, APX, and CAT (Cassia et al. 2019). It can also directly act as a ROS scavenger as it possesses unpaired electrons. Additionally, it can mitigate the formation of hydroxyl radicals ($^{\cdot}OH$) by scavenging Fe or $O_2^{\cdot -}$ (Lamattina et al. 2003). The reaction between NO and ROS produces reactive nitrogen species (RNS), and excess accumulation of RNS leads to nitrosative stress (Kohli et al. 2019). NO also regulates GSH concentration within the cell. It forms GSNO which serves as the cellular reservoir of NO and the main source for S-nitrosation (Corpas et al. 2013). Several previous studies have reported the involvement of NO-like synthase and nitrate reductase activities under UV-B stress conditions. The interaction between NO and ROS signaling pathways plays a key role in the protective mechanism against UV-B stress (Yemets et al. 2015). It has also been shown that there is an interaction between Gα protein, NO, and H_2O_2 at the time of UV-B induced stomatal closure in the leaves of Arabidopsis (He et al. 2013). Recent studies regarding its mechanism of action have suggested that under UV-B exposed conditions, NO induces the transcriptional activation of phenylpropanoid pathway genes *chalcone synthase* (*CHS*) and *chalcone isomerase* (*CHI*) which leads to an increase in secondary metabolite production (Tossi et al. 2011). Some authors have indicated that UV-B mediated flavonoid production and nitrate reductase mediated NO generation may be interlinked (Zhang et al. 2011). It has also been considered that H_2O_2 and NO together can reduce UV-B mediated damages by regulating the stomatal closure under UV-B exposed conditions (Tossi et al. 2014). Previous studies have shown that NO synergistically interacts with ROS and play a role during the augmentation of hypersensitive cell death in soybean (*Glycine max*) cells (Durner et al.,1998). To confirm the involvement of endogenous NO in UV-B stress responses, the *Arabidopsis NOD* transgenic line expressing the inducible bacterial NO dioxygenase (NOD) has been studied and results have shown that UV-B-irradiated mutant plants exhibited more symptoms of UV-B mediated damage as compared to the wild type (Cassia et al. 2019).

Apart from NO, other gasotransmitters like CO and H_2S are also thought to be involved in UV-B mediated stress responses. In plants, CO is mainly derived from the lipid peroxidation of biofilms, automatic oxidation of phenols and HO mediated enzymatic reactions. According to previous studies, in the process of ABA-mediated stomatal closure, ABA activates HO to increase the CO production and also involves NO/cGMP mediated signaling cascade to induce stomatal closure as reported in *Vicia faba* (Tossi et al. 2014). Another report on *G. max* has indicated that, in the case of UV-B irradiated plants, HO shows upregulated action that confers them protection against UV-B mediated oxidative damages (Yannarelli et al. 2006; Noriega et al. 2007). Among other gasotransmitters, H_2S is closely connected with NO and its signal transduction pathway does not always work independently. Under adverse conditions, NO reacts with CO to regulate the activity of the antioxidant enzyme system in the plant cell. Other than these gasotransmitters, another gaseous plant growth regulator, ethylene, also plays a pivotal role in abiotic stress responses. Ethylene also possesses several characteristics similar to that of GTs. Along with NO, ethylene production also significantly increases under UV-B radiation as reported in several plant species (Mackerness et al. 2001; Wang et al. 2006; Vanhaelewyn et al. 2016). Moreover, some reports also claimed that, under UV-B stress conditions, NO could promote the accumulation of ET in maize leaves (Wang et al. 2006). Taken together, available reports have suggested that gasotransmitters are released under different adverse conditions in plant cells to enhance plant tolerance to these environmental stimuli by lowering oxidative stress and lipid peroxidation, and enhancing the activity of antioxidant enzymes with the maintenance of ion and GSH homeostasis.

2.4 Crosstalk Between Major Gasotransmitter-Mediated Signaling Pathways Under UV-B Stress

Several environmental stresses like drought, flood, salt, UV-radiation, heat, heavy metal toxicity, etc. interfere with the normal physiological processes of the plant. Previous studies have reported that plants usually produce GTs to enhance their tolerance against these adverse abiotic stress conditions. These gaseous molecules transmit environmental stimuli and subsequently interact with several extra and intracellular pathways that regulate the biological processes in a synergistic or antagonistic way (Yao et al. 2019).

2.4.1 NO Mediated UV-B Stress Response and Crosstalk with H_2S and CO Signaling

Previous studies regarding the involvement of NO in UV-B stress response have reported that exogenously added NO alleviates the ROS mediated damages caused by exposure to high UV-B irradiation (Shi et al. 2005; Zhang et al. 2007). It was also reported that under UV-B exposed conditions accumulation level of both NO and phenylpropanoid takes place. Different reports have also indicated endogenous accumulation of NO and H_2S in plants under different adverse conditions which ultimately confer them better stress tolerance. Both NO and H_2S enhance the anti-oxidative defense system in plants by reducing excess production of ROS and also decreasing the lipid peroxidation (Hasanuzzaman et al. 2018a; Bhuyan et al. 2020). Several approaches have been made to determine the role of NO and H_2S under adverse conditions and reports involving the exogenous application of a H_2S donor have claimed that it influences the biosynthesis of NO as well as upregulates several enzymatic antioxidants like APX, SOD, CAT, etc. (Da Silva et al. 2017) (Fig. 2.1). It was also reported from previous studies that ABA plays a pivotal role in the NO-mediated signaling cascade under UV-B exposure as found in maize (Zhang et al. 2007), Arabidopsis (Chen et al. 2013), and *Vitis* (Berli and Bottini 2013). Under an oxidative stress environment, NO and H_2S may lead to the formation of reactive nitrogen and sulfur species (RNS and RSS, respectively) which jointly regulate the vital physiological processes during abiotic stress tolerance (Yamasaki and Cohen 2016; Corpas et al. 2019). The interaction between NO and H_2S produces an intermediate known as persulfide (Lisjak et al. 2013) which aids in the cellular regulation of ROS and RNS (Lisjak et al. 2013).

Other than NO and H_2S, carbon monoxide (CO) has also been reported to be accumulated in a significant amount under UV-B stress by the increased expression of HO-1 as it has been found in soybean plants. This response was claimed to be associated with increased ROS accumulation within the cell and serves as a protective mechanism against oxidative damage due to high UV-B exposure (Yannarelli et al. 2006). CO and NO can co-modulate one to another (Dulak and Józkowicz 2003). According to Song et al. (2008) NO was reported to be involved in CO-induced stomatal closure mediated by the NO/NOS-like pathway (Fig. 2.1). NO is thought to act as a downstream signaling component of CO action (Santa-Cruz et al. 2010; Bai et al. 2012). Upon abiotic stress, HO-1/CO system has been reported to be induced by sodium nitroprusside (SNP), a NO-releasing compound, which is further modulated by ROS (Noriega et al. 2007). Evidence has shown that

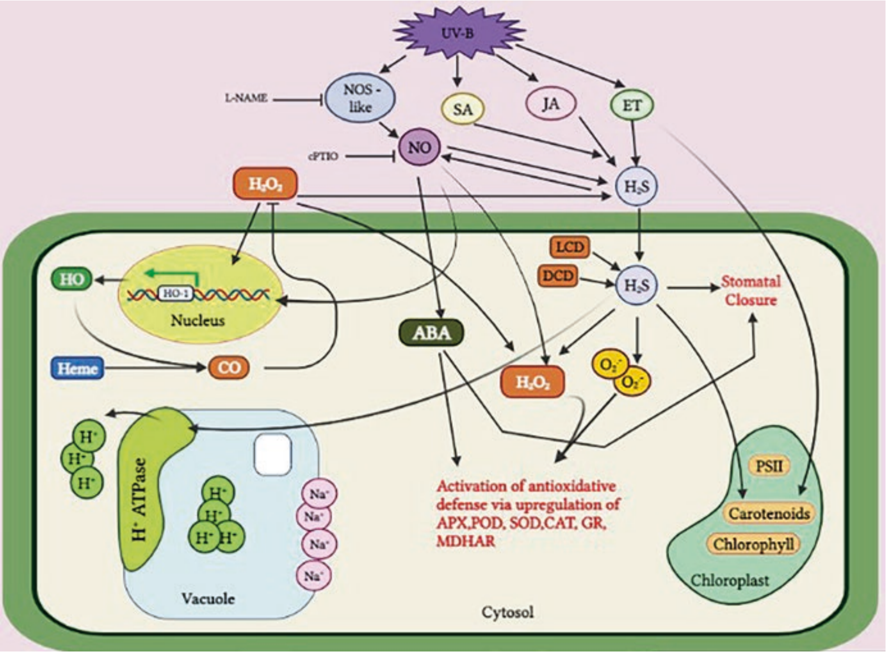

Fig. 2.1 Interaction between gasotransmitter mediated signaling pathways under UV-B stress. UV-B upregulates the production of NO, H_2S, and other gaseous signaling molecules like salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) which also induce H_2O_2. NO and H_2O_2 moves to nucleus and in turn upregulate the *HO-1* gene expression resulting in increased accumulation of CO via HO enzymatic pathway in cytosol. H_2S also acts in stomatal closure in association with ABA and NO, activation of the antioxidant defense system via upregulation of APX, POD, SOD, CAT, GR and MDHAR. It also aids in the upregulation of carotenoid biosynthesis taking place in the chloroplast. These complex interactions between gasotransmitters ultimately confer tolerance against UV-B mediated damages to plant cells. (Abbreviations: *NO* Nitric oxide, *H_2S* Hydrogen Sulfide, *SA* Salicylic Acid, *JA* Jasmonic Acid, *ET* Ethylene, *H_2O_2* Hydrogen Peroxide, *CO* Carbon monoxide, *HO* Heme Oxygenase, *ABA* Abscisic acid, *APX* Ascorbate peroxidase, *SOD* Superoxide dismutase, *CAT* Catalase, *GR* Glutathione reductase, *MDHAR* Mono-dehydroascorbate reductase, *PSII* Photosystem II, *LCD* L-cysteine desulfhydrase, *DCD* D-cysteine desulfhydrase, *NOS* Nitric-oxide synthase, *L-NAME* L-NG -Nitro arginine methyl ester, *cPTIO* 2-4-carboxyphenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide, $O_2^{\cdot-}$ Superoxide)

when HO-1 expression becomes upregulated, it eventually decreases H_2O_2 production and programmed cell death (PCD) is delayed as found in wheat aleurone layers (Wu et al. 2011). Yannarelli et al. (2006) have shown that ROS plays a key role in the UV-B-induced upregulation of HO-1 mRNA.

It has also been reported that the crosstalk between H_2O_2 and NO could be involved in the response against UV-B stress (Fig. 2.1). He et al. (2013) have established an interrelationship among Gα protein, H_2O_2, and NO during UV-B-induced stomatal closure in *Arabidopsis* leaves. Recently, Tossi et al. (2014) also showed that both H_2O_2 and NO generation play some important role in response to UV-B exposure via regulating the stomatal movement to reduce UV-B mediated damages.

2.4.2 *Interplay Between Gasotransmitters and Phytohormone Signaling under Adverse Conditions*

In plants, abiotic stress responses are regulated by crosstalk between multiple signaling molecules that mainly include the gasotransmitters like NO, H_2S, and CO as well as the phytohormones like ethylene, auxin, ABA, etc. As we know, exposure to excessive light intensity and UV-B radiation affects the photosynthetic efficiency of plants which may be due to the generation of excessive ROS (Takahashi and Badger 2011; Demarsy et al. 2018) and, as a response, it triggers the metabolism of NO, ethylene and other phytohormones as found in Arabidopsis (Magalhaes et al. 2000; Vanhaelewyn et al. 2016). Crosstalk between NO and ethylene was first evidenced by using chemical modulators like cPTIO which acts as NO scavenger and resulted in the repression of UV-B induced ethylene emission (Wang et al. 2006). (Fig. 2.1) Evidence has shown that in *Vicia faba* stomatal closure induced by UV-B radiation was promoted by NO accumulation in guard cells after the ethylene evolution was at its peak (He et al. 2011), and this event was inhibited by exogenous application of NO scavenger in guard cells. These observations have led to the conclusion that in the process of UV-B induced stomatal closure, ethylene acts as a signaling molecule upstream of NO.

2.5 Future Perspectives of Gasotransmitters in UV-B Stress

In recent years, considerable advances have been made in the research related to the biosynthesis of several gasotransmitters including NO, CO, H_2S, H_2, and CH_4, and their involvement in abiotic stress tolerance. These studies suggest that gasotransmitters play a key role as signaling molecules under adverse conditions by regulating the antioxidant defense system to maintain the redox homeostasis within the cell. Although these studies have reported the involvement of gasotransmitters in alleviating several abiotic stresses like salinity, drought, heat stress, heavy metal toxicity, etc., specific information regarding their role in adaption under UV-B stress is still lacking. Hence, the molecular aspect of the production pathways of these gasotransmitters and their association with abiotic stress responses, specifically under UV-B exposed conditions, remains of great interest to researchers. To achieve more in-depth mechanistic details on gasotransmitter action, some interesting area is claimed to be explored in near future like how these GTs modulate the stress responses, what are their targets in the cell under UV-B exposed conditions, how the metabolic pathways of these GTs interact with each other, etc. As we know UV-B is one of the major abiotic stresses in tropical countries from an agricultural perspective. A clear insight into the regulatory mechanism of GTs under UV-B mediated stress would facilitate safer breeding of tolerant crops to enhance yield and quality.

Acknowledgments The authors gratefully acknowledge Council of Scientific and Industrial Research, Govt. of India, (Ref. No. 38(1417)/16/EMR-II, dated: 17/05/2016 to SR), SERB, DST, Govt. of India (Ref. No. ECR/2016/000539) to SR for providing necessary financial supports. SD is the recipient of fellowship from the SERB, DST, Govt. of India (Ref. No. ECR/2016/000539) funded project. MM is the recipient of Inspire Fellowship from DST, Govt. of India (DST/INSPIRE Fellowship/2017/IF170001).

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Chapter 3
Signaling Pathways of Gasotransmitters in Heavy Metal Stress Mitigation

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Abstract Expanding population, industrialization and inadequate agricultural practices are complementing the ongoing environmental challenges and abiotic stressors. Heavy metals (HMs) are among the most common and hazardous pollutants and are posing a consistent threat to a plethora of crop plants as well as to the human population. However, plants produce certain endogenous molecules that vary from reactive oxygen species (ROS), phosphorylation cascades, phytohormones, and some of the gaseous signaling transmitters/gasotransmitters (GTs). GTs including methane (CH_4), nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfide (H_2S) have witnessed among the most potential gaseous signaling molecules which are well known to participate in the plant development as well as in the heavy metal stress amelioration. This chapter reviews the potential roles of GTs, their signaling cascades, and cross-talks between different GTs under heavy metal stress conditions.

Keywords Cross-talks · Heavy metal stress · Hazardous pollutants · Signalling cascade

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T. Aftab, F. J. Corpas (eds.), *Gasotransmitters Signaling in Plants under Challenging Environment*, Plant in Challenging Environments 5,
https://doi.org/10.1007/978-3-031-43029-9_3

3.1 Introduction

Heavy metals (HMs) are found to have hazardous impacts on the environment as well as on land plants, microbes, animals, and humans. Plants have the most prevalent encounters with these HMs i.e., copper (Cu), nickel (Ni), Cadmium (Cd), chromium (Cr), cobalt (Co), mercury (Hg), lead (Pb), etc. (Arao et al. 2010). Inadequate agricultural practices, use of chemical fertilizers, mining industry, poor solid waste management programs, and solid waste disposal methods has led to HMs contamination in the agricultural lands (Tóth et al. 2016). HMs stress leads to oxidative damage in the plant cells and further induces the functional disruption of multiple cellular enzymes, thus compromising growth and developmental parameters and yields losses. An excessive number of HMs inside the plant system destabilizes the physiological and metabolic machinery of plants (Shahzad et al. 2018). Also, there is an enhancement in the reactive oxygen species (ROS) generation, lipid peroxidation, and distorted cellular membranes. However, plants undergo a series of ameliorative networks to activate certain signaling cascades to cope with these HM stresses. In general, they involve phosphorylation cascades, ROS generation, calcium –calmodulin system, phytohormones, and the production of special gaseous molecules known as gasotransmitters (GTs) (Lamattina and García-Mata 2016). GTs include compounds with the following characteristic features such as (1) small gaseous molecules, (2) able to cross biological membranes without the help of some cognate membrane receptors, (3) endogenously produced or synthesized by certain enzymes, (4) target specific entities (Wang 2002). Recent literature shows that HMs stress generally stimulates the activities of four GTs in general, namely methane (CH_4), carbon monoxide (CO), nitric oxide (NO), and hydrogen sulfide (H_2S).

H_2S is among the gaseous signaling molecules after NO and CO with stinky egg odor and is found to be able to move freely across the membranes (Mathai et al. 2009; Wang 2012). Plants have strict enzyme-controlled systems to maintain the activation levels of H_2S. In plants, H_2S is known to regulate different processes of plant growth and development seed germination, and stomatal movements (Chen et al. 2020). Also, H_2S undergoes an interplay and coordinates with signaling molecules such as phytohormones and other stress-related molecules like CO, NO, and Ca^{2+}, etc. (Lin et al. 2012; Peng et al. 2016; Li et al. 2012).

Micro RNAs or (mi-RNAs) are also known to have specific roles in maintaining plant growth and developmental activities under harsh environmental conditions, thus helping in maintaining the regulatory activities of multiple physiological pathways (Sunkar et al. 2012). Shen et al. (2013) found that H_2S upregulates the transcriptional levels of miR 393a, miR 396a, miR 167a, miR 167c, and miR 167d in *Arabidopsis thaliana* to counter drought stress. HMs are capable of inducing endogenous H_2S levels in plants, e.g. under Cr stress in *Setaria italica*, Cd stress in Bermuda grass, Zn stress in *Solanum nigrum*, or Ni stress in rice (Fang et al. 2016; Shi et al. 2014; Liu et al. 2016; Rizwan et al. 2019).

Methane (CH_4) is another organic molecule that shows its functional activities as a gasotransmitter. Its biosynthesis inside the plants is enhanced under various abiotic stressors like high temperature, HMs, UV radiation, and salinity stress (Yao et al. 2019; Li et al. 2020). On the other hand, NO is also able to cross the membrane and interact with certain molecules inside the cells (Khan et al. 2021). Under abiotic stress conditions, NO is triggered and participates in different signaling cascades (Kolbert et al. 2019). NO initiates several physiological and biochemical activities in plants from root/shoot growth, photosynthesis, seed germination, floral regulation, and nutrient hemostasis (Buet et al. 2019; Khan et al. 2020a, b; Siddiqui et al. 2020). Also, NO shows its hormone-like and antioxidative properties in plants under stress. It helps the plants to encounter oxidative damage by neutralizing the ROS molecules by activating their antioxidant systems (Siddiqui et al. 2020; Singh et al. 2020). Exogenous NO application to seeds under Cr (VI) stress is found to have an improved germination potential with respect the untreated seeds. It might be the consequence of the applied NO in breaking the seed dormancy either by up-regulating gibberellins (GA) biosynthesis or by abscisic acid (ABA) catabolism (Signorelli and Considine 2018). CO is another odorless and colorless gaseous molecule with ubiquitous nature. Heme oxygenase (HO) is the endogenous source for CO production in plants which is well known for catalytic degradation of heme to generate free iron, CO, and biliverdin IXα (Fang et al. 2021). However, a crosstalk mechanism between these GTs is also observed during HMs stress amelioration and other physiological as well as molecular modulations in plants under multiple stress factors.

3.2 Gasotransmitters

The endogenously synthesized plant gaseous signaling molecules which play critical roles in plants developmental processes and plant stress protection are referred as to gasotransmitters (Fang et al. 2021; Kumar et al. 2021; Shivaraj et al. 2020; Yao et al. 2019). Of late several gases viz., CO, ethylene (C_2H_4), H_2S, NO, and CH_4 have been documented as gasotransmitters. However, among those, C_2H_4, H_2S and NO have been reported to be more responsive in affecting plant cellular processes (Shivaraj et al. 2020). The plant faces severe stresses and, to neutralize these factors, they have different strategies. Recently, GTs have been reported to ameliorate abiotic stresses and then, GTs stimulate the activities of antioxidant enzymes involved in the antioxidant defense system (Yao et al. 2019). This GT-mediated induced response leads to the alleviation of ROS imbalance. Thus, GTs protect plants from stresses and induce plant stress tolerance thereby restoring normal plant growth and development under adverse conditions (Fig. 3.1).

Both H_2S and NO have been reported to induce signaling response by stimulating protein post-translational modifications (PTMs), crosstalk with plant growth

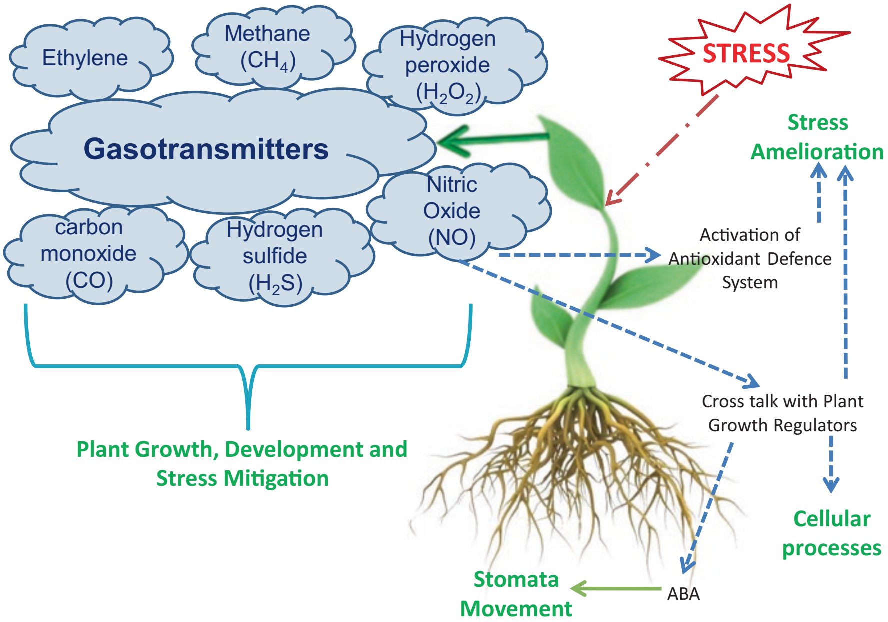

Fig. 3.1 Role of gasotransmitters in plant growth and development. Figure depicts, besides the production of hydrogen peroxide (H_2O_2), that of certain gasotransmitters (GTs) inside the plant system like carbon monoxide (CO), nitric oxide (NO), hydrogen sulfide (H_2S), methane (CH_4), and the gaseous phytohormone ethylene (ET), which undergo certain crosstalk mechanisms with other phytohormones to instigate diverse cellular processes, stomata movement with the help of ABA (abscisic acid), and the activation of antioxidant defence system in plants for stress amelioration under unfavourable environment

regulators (PGRs), calcium, etc. (Mishra et al. 2021). Although H_2S is a crucial signaling molecule found in animal cells, recent studies emphasized its significance in plants (Zhang 2016). It interacts with other gas signaling molecules such as NO. Also, H_2S plays a crucial role in normal plant growth and development processes, and plant stress protection from various abiotic stress factors ranging from salinity, drought, heavy metals etc. The biosynthetic pathways of NO, H_2S, and CO have been reported to interplay with calcium ions and some other important signaling molecules such as ROS through direct chemical interactions, competitive interactions for targets of bio-macromolecules, or via reciprocal influence on their synthesis (Kolupaev et al. 2019). These GTs have been further documented to affect the signaling through PTMs like GTs-mediated nitration, S-nitrosation, and persulfidation. Moreover, endogenous levels of GTs also affect the mechanisms of plant adaptations to various abiotic stresses viz., temperature (low and high), dehydration (water deficit conditions), and salinity (osmotic). In plants, NO, CO, H_2S, and CH_4 are involved significantly in the uptake and accumulations of heavy metals and their detoxification through crosstalk with different GTs which are associated with heavy metals (Fang et al. 2021).

Besides these GTs, ethylene is the only gaseous plant hormone that is referred to as a GT, because of its significance in plants developmental processes and plant stress protection (Karle et al. 2021). The expressions of genes involved in the ethylene signaling pathway are upregulated under salt stress. Various ethylene receptor genes (such as *ETR1*, *ETR2*, *EIN4*), ethylene signaling genes (namely *ERF1*, *ERF2*, *CTR1*, and *EIN3*), and MAPK cascade genes (such as *MKK2, MEKK1*, *MPK4*/6) have been reported to be upregulated in cotton plants under salinity stress (Peng et al. 2014). In cotton crops grown under salinity stress, when studied at proteomic and transcriptomic (mRNA sequencing analysis) levels, it has been found that a number of salt stress-responsive proteins can induce the alteration of miRNAs and further modulating the alternative splicing events. About 63 genes and their proteins products were identified after 4-hour exposure of cotton plants to salt stress, whereas 85 genes and their respective proteins have been identified after 24 hours of salt application in upland cotton plants. Furthermore, 158 genes/proteins were identified to interact/interplay during salt stress tolerance through the network of two specific clusters comprising cytochrome oxidase and ATP synthase in mitochondria. Thereby, a gene network is stimulated to confer ethylene-mediated salt stress tolerance in plants with the mitochondrion as a key site involved in providing salt stress resistance in plants.

Burgeoning evidence emphasized that ethylene with another plant growth regulators, i.e., polyamines, share a common precursor for its biosynthesis pathway (Kolbert et al. 2019). After the oxidation of polyamine, NO can be produced. Thus, there is an indirect metabolic connection established between the biosynthesis pathways of both ethylene and NO. Apart from ethylene, hydrogen gas (H_2) is also a potential candidate for mediating numerous physiological and stress-protective responses in plants (Karle et al. 2021). The synthesis of H_2 has been reported to be stimulated in response to various abiotic factors such as salt, heavy metals, UV radiation, drought, and temperature stress. Being a highly volatile gas, H_2 has been employed as an alternate donor supplement in plant stress investigations (Xu et al. 2013). However, the exact mechanism of action and mode of H_2-mediated stress signaling is not yet elucidated (Karle et al. 2021). Various reports suggest that abiotic stresses generally stimulate the generation of GTs in plants. As a response, different GTs further stimulate various stress-protective responses such as the accumulation of antioxidants, and the activation of antioxidant enzymes which further checks the levels of ROS in plants under abiotic stresses. This section highlights the significance of four major GTs namely, various NO, CO, H_2S, and CH_4 in plant developmental processes.

3.2.1 Nitric Oxide (NO)

Nitric oxide (NO) is among the oldest and most important gasotransmitters which is induced under herbicide, drought, and salinity stress in soybean, wheat, and tobacco, respectively (Yao et al. 2019). NO is also associated with the assimilation of nitrate in plants. The plants interact with microorganisms through either mutualistic or cooperative symbioses (Hichri et al. 2016). During initial symbiotic interactions, NO has been observed to favour plant-microbe association by repressing plant defense reactions. However, at a later stage of symbiotic interactions, NO has been reported to inhibit the nitrogen fixation. Thereby, NO is actively involved in the metabolism of both carbon and nitrogen. Increasing number of studies have revealed that NO is involved in maintaining energy status during a hypoxic environment, interacting with other plant growth regulators, ensuring the balance of ROS, and regulating the senescence processes.

NO is endogenously synthesized by plants at various plant developmental stages such as seed and fruit development as well as at stress alleviating responses (Kolbert et al. 2019). During the NO signaling pathway, the NO signal is generally perceived without the involvement of any NO-specific receptor and it is primarily perceived through S-nitrosation. NO has an antagonistic relationship with ethylene during various physiological processes viz., fruit ripening, de-etiolation, stomatal opening stimulated by darkness, and cadmium-ion mediated cell death in plants. Besides this, NO and ethylene also have synergistic effects during several abiotic stress responses such as UV-B stress-mediated stomatal closure, and molecular expression of iron acquisition genes in iron deficit plants.

Although being a reactive nitrogen species (RNS), NO is also referred to as a non-classical gaseous plant hormone that is involved in plant stress protection mediated through multiple enzymatic and non-enzymatic antioxidant defense pathways (Karle et al. 2021; Saddhe et al. 2019; Yao et al. 2019). During salinity stress, NO stimulates salt overly sensitive (SOS) and G-protein-linked signaling, Ca^{2+}-dependent pathways, the alternative oxidase (AOX) pathway, and mitogen-activated protein kinase (MAPK)-dependent pathways (Kaleem et al. 2018; Karle et al. 2021; Saddhe et al. 2019). In laboratory investigations, sodium nitroprusside (SNP) is used as a donor of NO and SNP has been reported to ameliorate abiotic stresses in plants. Escalating studies have emphasized that NO is an essential gas signal molecule that is crucial for maintaining cellular homeostasis in both uni- and multicellular organisms (Del Castello et al. 2019). Owing to its chemistry, NO is a versatile molecule. It is an unstable, redox, free radical, highly reactive, and short half-life gaseous molecule. Being lipophilic, NO easily crosses all the barriers imposed by the biological membranes. It is produced in living cells (both plants and animals) under normal and stressed conditions (Nabi et al. 2019). During stressed conditions, ROS concentrations increase and at the time NO may act as a stress/ROS-detoxifier and scavenges ROS thereby minimizing the detrimental effect of stress. It affects respiratory pathways such as mitochondrial electron transport pathways to induce antioxidant defense mechanisms and ROS mitigation under abiotic stresses in plants.

Both endogenous levels and exogenous applications of NO regulate abiotic stress tolerance in various plants subjected to abiotic stresses (Ahmad et al. 2018a, b; Nabi et al. 2019). In tomatoes, exogenously applied NO modulated the metabolism of osmolytes and antioxidants, stimulated the antioxidant enzymes of the ascorbate-glutathione pathway and promoted plant growth under cadmium metal stress

(Ahmad et al. 2018a). Recent studies revealed that NO interacts with other signaling molecules and, among them, NO works synergistically with H_2S during stress management. The endogenous concentration of NO and H_2S regulate specific defense-related entities and antioxidant defense mechanisms in plants (Bhuyan et al. 2020). Reports show that both H_2S and NO have well-established crosstalk mechanisms and induce abiotic stress tolerance against stresses like heavy metals, temperature, water, osmotic, and salinity (Singh et al. 2020). These GTs regulates the gene expression of certain genes to an extent to stimulate the levels of various antioxidative enzymes, and osmolytes mediated through signal transductions and cross adaptations.

The mode of action of NO involves the modification of various molecules of biological importance such as proteins, cGMP, fatty acids, and nucleotides. NO-produced RNS interact with the bio-macro/micro-molecules to amend their structure thereby modifying their function (Sánchez-Vicente et al. 2019). NO causes conformational changes in the protein structure that leads to altered stability and altered gene expression through two PTMs i.e., S-nitrosation (of Cys residues and metals) and the nitration of (Tyr residues). Further understanding of the underlying mechanism of action of NO with other GTs and plant hormones may help in elucidating the NO-mediated cell signaling under various environmental stresses. It would further help in developing specific biotechnological strategies to enhance crop productivity and ensure food security.

3.2.2 Carbon Monoxide (CO)

Carbon monoxide (CO) is another important player as gaseous signaling molecule endogenously produced in various plants cell and a variety of plant species and organs in response to stress, adaptive processes, and under distinct developmental plant stages (Fig. 3.2) (Jin et al. 2016). CO was just been recently identified as the second GT after NO in terms of discovery order (Sukmansky and Reutov 2016). Exogenously CO fumigations, its aqueous solution, and also artificial CO donors like hematin and hemin in combination with various genetic approaches like overexpressing heme oxygenase (HO) transgenic or knockdown mutants were till now used for illustrating the CO crucial role in regulating various plant's physiological functions (Lamattina and García-Mata 2016), such as plant growth and developmental processes, including germination, organ senescence and during alleviation of various biotic and abiotic processes. In addition to this, CO cross-talk with other signaling pathways was also confirmed in a variety of plant species. CO role in plants is intimately tied to its major endogenous enzymatic source, the heme oxygenase (HO) (Xuan et al. 2008). In plant cells, HO is normally found in the subcellular organelles mitochondria and chloroplasts (Dixit et al. 2014).

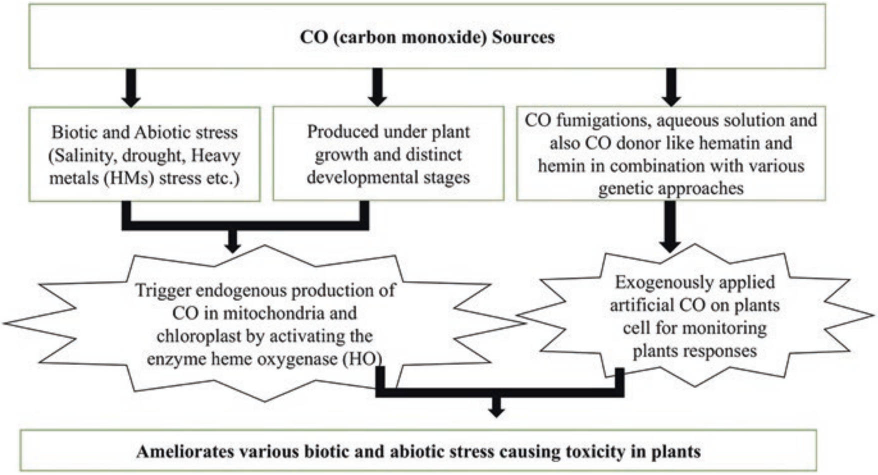

Fig. 3.2 Flow chart showing CO sources and its role in stress response in plants. The figure signifies the production of CO under harsh environmental conditions (abiotic and biotic stress) as well as under normal growth and developmental conditions in plants which further trigger the endogenous CO levels inside the cell organelles and activate the defense related signaling cascades. However, it also depicts the exogenous applications of CO to plants under stressful environmental conditions to achieve the normal functioning in plants

3.2.3 Hydrogen Sulfide (H_2S)

H_2S is a colorless, lipophilic and flammable molecule with a foul smell (rotten eggs) that impedes respiratory mechanisms in mitochondrial by damaging cytochrome *c* oxidase (Fotopoulos et al. 2015). At present, H_2S is reported as an important gasotransmitter or a secondary messenger due to its endogenous role in plants. H_2S plays its central roles in plants and further takes part in various physiological and metabolic processes such as formation of adventitious roots, seed germination, stomatal movement and tissue senescence, and increasing tolerance to various environmental stresses (Jin et al. 2013; Xuan et al. 2020; Aroca et al. 2018; Corpas et al. 2019). H_2S shows its signaling mechanism through gene expression modulation, interaction with the thiol (–SH) group of protein cysteine residues by persulfidation (PTM), and interconnection with other plant growth regulators (Freschi 2013; Asgher et al. 2017; Prakash et al. 2019). It has been extensively studied that plants which are grown under heavy metal (cadmium, nickel, chromium, copper, and lead) polluted soils can survive due to the positive effects of the H_2S gaseous molecule (Kushwaha and Singh 2020; Rizwan et al. 2019).

Sulfur metabolism is critical to the plants developmental process, and its deficiency leads to significant alterations in the normal growth and development in plants. Sulfur enters inside the plants system as sulfate (SO_4^{2-}) through specific transporters like SULTR (González-Gordo et al. 2020). Sulfur metabolism-related enzymes metabolize it into H_2S which is present in different subcellular compartments (chloroplast, cytoplasm, and mitochondria) of a plant cell. A chloroplast enzyme, sulfite reductase (SiR), in addition to ferredoxin reduces sulfite to sulfide during the sulfate assimilation pathway. The main sources of H_2S formation from cysteine through the action of two enzymes i.e., D-cysteine desulfhydrase (DCD) and L-cysteine desulfhydrase (LCD) in the cytosol. In mitochondria, H_2S is produced from the catalytic conversion of cyanide to β-cyanoalanine by β-cyanoalanine synthase (CAS), protecting the plant cell from CN^- toxicity that acts on the inner mitochondrial membrane (Gotor et al. 2019). In the persulfidation process thiol groups of cysteine is changed from –SH to –SSH and H_2S have been proposed to send a signal in this form which influences the protein structure and its function (Aroca et al. 2018).

Hydrogen sulfide is also interlinked with other plant hormones and signaling molecules like Ca^{2+}, ethylene, ABA, NO, and H_2O_2 (Shivaraj et al. 2020).

3.2.4 Methane (CH_4)

Methane (CH_4) is a volatile, odorless, gaseous molecule, slightly soluble in water with the potential to contribute to global warming and, accordingly, the planet's climate change (Boros et al. 2015; Li et al. 2019). Its protective role in various diseases has completely changed the conventional perspective of the biologically inactive molecule concept. Some of the roles in plants are illustrated in Fig. 3.3. CH_4 readily passes through membranes and is synthesized endogenously, producing its biological effect through exogenous donors (Liu et al. 2012; Boros et al. 2015). Concomitantly, it has been found to possess the characteristics to be a potent gasotransmitter mentioned by Wang (2014).

CH_4 is produced through both biotic and abiotic means. The abiotic pathways contribute a almost negligibly, while as a major proportion i.e., approximately 99% is through biotic ways. Furthermore, biotic microbial production accounts for almost 70%, while non-microbial such as animals, plants, soils, fungi, and oceans contribute to the rest (Fig. 3.3) (Wang et al. 2013; Fang et al. 2021). Until 2006, the plant has been considered a medium for the emission of soil CH_4 into the atmosphere (Keppler et al. 2006). Subsequently, studies and literature have shown the production, regulatory and protective role of CH_4 in plants under various stress conditions like UV, temperature, drought, salinity, injury, heavy metal/metalloid such

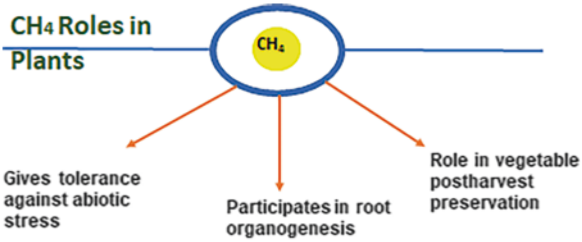

Fig. 3.3 Different roles of CH_4 in plants

as Cd and Cu, ROS, and pathogens (Hu et al. 2018; Mei et al. 2019). Although enzymes involved in the endogenous production of CH_4 are yet to be known (Fang et al. 2021: Li et al. 2019), reports have suggested that CH_4 generation in plants occur both under normal and stressed condition (Abdulmajeed et al. 2017; Martel and Qaderi 2017).

3.3 Heavy Metals as Abiotic Stressors

Due to industrial activities and sewage sludge, the concentration of heavy metals is increasing in nature at a rapid pace. Fe, Mn, Co, Cd, Ni, Hb, Zn, and As are some of the heavy metals that act as important nutrients in the soil but due to their increasing concentration, they are responsible for generating oxidative stress in plants. Due to their excessive concentration in soil, these metals are responsible for detrimental effects on plants by affecting the growth and development of plants (Ghori et al. 2019). Some heavy metals such as Zn, Mn, Cu, Ni, etc. are considered essential micronutrients in plants because of their role as co-factors for enzymes. Besides these metals, there are other heavy metals such as Cd and Pb, etc. which are not required in plants, and when their concentration increases to a certain limit, they become toxic (Burakova et al. 2018; Ali et al. 2017). Sources of these heavy metals are both natural and man-made. When plants encounter heavy metals, negative effects are clearly visible in the plants in the form of root browning, chlorosis, stunted growth, and plant death (Ozturk et al. 2015).

Heavy metals are responsible for causing the inactivation of various indispensable enzymes and proteins. These metals also interfere with the substitution reactions important for the metal ions from the biomolecules. Due to this interference, respiration rate, photosynthetic system, and homeostasis in plants are disturbed (Hossain et al. 2012). Heavy metals instigate and stimulate the generation of reactive oxygen species (ROS) as superoxide radical ($O_2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2). These ROS are responsible for oxidative stress which includes, among others, damage to the cellular membrane through lipid peroxidation. They are also known to cause damage to biomolecules and DNA strands (Barconi et al. 2011; Ahmad et al. 2012).

3.3.1 Heavy Metals and Their Toxic Effects

Copper plays a very necessary role in plants as it is important for ATP synthesis and assimilation of carbon. It is also an important part of cytochrome *c* oxidase and plastocyanin that are required in the respiratory system and the photosynthesis, respectively (Yadav 2010). But when the concentration of Cu increases in plants, it causes damage to macromolecules, biochemical pathways, and also DNA (Yadav 2010). It was reported by Bouazizi et al. 2010 that Cu stress causes inhibition in growth, and chlorosis and also causes retardation in the plant growth. Studies conducted by Neelima and Reddy (2002) in *Solanum melongena* showed that the growth parameters as root and shoot length of the plant were affected due to stress caused by Cu. Another heavy metal, Cr, causes contamination in the groundwater. Cr leads the retardation in growth, chlorosis, and damage to the roots (Shanker et al. 2003; Ozturk et al. 2015). Studies conducted by Yadav 2010 on the toxicity of Cr have found that it affects the chloroplast, carbon fixation, produces ROS, and also inhibits the electron transport chain. Studies conducted on the effect of excess Ni in the plant system have found that due to this metal, there is an arrest in the growth, and is also responsible for oxidative stress (Vatansever et al. 2017). The concentration of Ni in soil has increased mainly due to smelting, sewage mining, and excessive use of fertilizers (Aziz et al. 2015).

Among all heavy metals, lead (Pb) is considered one of the major contaminants that pollute the soil. Lead is mostly discharged from natural weathering processes and other anthropogenic activities like mining and smelting (Ashraf et al. 2015). In plants, Pb is known to cause chlorosis, stunted growth, and a decrease in the root length (Sharma and Dubey 2005). Studies conducted by Malar et al. 2014 on the Pb toxicity in water hyacinths indicated that at higher concentrations, Pb affects the plant growth. Due to the toxicity caused by Pb, antioxidative enzymes were also increased in plants to combat stress. Cd is toxic to plants and its toxic concentrations are a matter of concern. It has been observed that due to Cd, the photosynthetic system in plants is affected. Absorption and translocation of Ca, P, Mg K, and water are also reduced (Nagajyoti et al. 2010). Studies conducted on *Alternanthera bettzickiana* found that, at lower concentrations, Cd positively impacts the plants development processes whereas, at higher concentrations, the developmental activities in plants were affected (Tauqeer et al. 2016). Another heavy metal, Zn is considered an essential element for the normal growth of plants. But for the normal growth, Zn is required in very trace amounts as the higher concentrations of Zn are observed to cause toxicity in plants. When its concentration increases, Zn causes senescence, retards growth, induces chlorosis, and further impacts the overall developmental mechanism of plants (Nagajyoti et al. 2010).

3.4 Gasotransmitters Signaling Under HMs Stress Conditions

Plants are facing constant heavy metal constraints on their growth, metabolic activities, and yield. Plants adapted themselves by boosting substantial defensive mechanisms under the threat of uplifted levels of essential and non-essential metals. They regulate various defense strategies like metal sequestration, ions-trafficking phytochelatins (PCs), reduced glutathione (GSH), metallothioneins (MTs), and activation of antioxidant enzymes to competently counteract the heavy metal stress on the generation of ROS (Emamverdian et al. 2015; Choudhury et al. 2017). Nowadays, scientists are exploring a sustainable approach to attenuate hazardous toxicants which are posing a consistent threat to plants. In addition to synchronization of the antioxidative defense system and plant growth regulators, other biochemical approaches like biomolecule cascades, signal transducers viz. gasotransmitters (GTs) gaseous signaling molecules viz. NO, CO, H_2S and CH4 also contribute to the alleviation of consequences of toxic metal stress (Gu et al. 2018; Shivaraj et al. 2020; Alamri et al. 2020; Mukherjee and Corpas 2020). A GT type signal molecule endorses numerous physiological activities via enumerating biological processes such as activation of enzyme and metabolic activities, receptors at the target site of membrane germination, organogenesis, growth, development, etc. (Lamattina et al. 2013; Fang et al. 2021). In the following sections, we summarize the findings on regulatory functions and signaling response of GTs against toxic heavy metal stress.

3.4.1 NO Signaling Under Heavy Metal Stress

Heavy metals and metalloids induced phytotoxicity by hindering physiological, cellular, and metabolic functions. The toxicity of metals elevates the level of reactive oxygen species that causes an imbalance between antioxidant homeostasis (Sharma and Dietz 2009). Extensive research reveals the function of NO molecules in alleviating the toxic effect of heavy metals at both endogenous and exogenous capacity (Pető et al. 2013; Zheng et al. 2014). NO might act as a signaling molecule due to reductive/oxidative pathways like nitrite-NO-reductase (NiNOR) a membrane-bound enzyme, nitrate reductase (NR) in the cytosol, electron transport chain (ETC) cytochrome *c* oxidase in mitochondria, and a NO-like synthase in peroxisomes (Barroso et al. 1999; Rockel et al. 2002; Corpas et al. 2008; Neill et al. 2008; Farnese et al. 2016). These molecules act as stress markers to influence catalytic functions and improve the morphology of plants under various metal ions viz. lead, aluminium, cadmium, etc. owing to the overproduction of endogenous NO (Tain et al. 2007; Corpas et al. 2022).

Contradictory to these, some findings show both enhanced and reduced NO levels against different heavy metals in maize, rice, soybean, etc. Hence, these observations predict that the contradictory results might be a consequence of plant tissue, type, and duration of stress. Faria-Lopes et al. (2019) describe that NO in the form of S-nitrosoglutathione helped in boosting antioxidant enzyme activity by diminishing ROS levels against Al stress in wheat seedlings. Similarly, it was confirmed by Kováčik et al. (2019) that in the lichen, NO modulated Cd tolerance *via* escalating nutrients, minerals, and metabolites. Thus NO, a gaseous molecule, might regulate tolerance to toxic metals through the expression of associated genes.

Experimental observations revealed that NO stimulates seed germination, growth of different plant parts, and photosynthetic and enzymatic activity under both control and stressed condition (Gong et al. 2017a; Per et al. 2017; Nabaei and Amooaghaie 2019; Nagel et al. 2019). NO enhanced the rate of seed germination against Cu, As, Cd, and Cr metals in wheat, mung bean, tomato, *Catharanthus roseus*, and *Lupinus luteus* seed via initiation of α-amylase, β-amylase activity, and cGMP signaling pathway (Yang et al. 2010; Ismail 2012; Khan et al. 2020a, b). It was also found that NO exogenous treatment reduced the level of H_2O_2 and malondialdehyde (MDA) through the activity of protease and ATPase (Rather et al. 2020). Similarly, other reports show the involvement of NO in photosynthesis such as maintenance of light-harvesting complexes, chlorophyll molecules, and activity of enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) when exposed to different metals like Al, Cd, As, Ni, Pb, etc. (Gong et al. 2017a, b; Ahmad et al. 2018a, b; Rizwan et al. 2018; Bai et al. 2015). The upsurge in NO-stimulated photosynthesis might be due to mineral nutrients and the antioxidant defense system (Chen et al. 2018a). Several studies reveal the enhanced level of antioxidative enzymes in response to NO which significantly homeostasis the overproduction of ROS like H_2O_2, $O_2^{\bullet-}$, $^{\bullet}OH$ and expression of ascorbic acid (AsA), peroxidases (PODs), CAT, glutathione-S-transferase (GST), SOD, APX, and PCs (Sun et al. 2014; Souri et al. 2020; Singh et al. 2017; Kováčik et al. 2019). NO donor S-nitrosoglutathione mitigates the toxicity of metal ions by sequestering in vacuoles (Mostofa et al. 2015a; Tiwari et al. 2019).

Exogenous treatment of NO manifests a reduction in metalloid toxicity of As, Cd, and Pb in rice, wheat, mung bean, *Typha angustifolia* owing to the homeostasis of ROS, MDA, and augmentation in plant growth, biomass, and yield (Ismail 2012; Mostofa et al. 2015a; Zhao et al. 2016). The possible mechanism of NO for diminishing the toxicity of HMs comprises osmoregulation across the cell membrane and other related components (Ahmad et al. 2018a). The studies also indicate that NO ameliorates heavy metal stress via adaptive approaches like cell wall plasticity and expansion, signaling at phospholipid bilayer, and enhancing plant growth (Seabra and Oliveira 2016). Further, the recent reports reveal that NO alleviates heavy metal stress via induction of HM-associated metallo-chaperons (domain genes), specific proteins responsible for transporting the metal ions inside the cell under the catalysis of metallo-cofactor. Currently, with the help of transcriptomic studies, scientists can identify differentially expressed heavy metal-associated domain genes in relation to NO. The research work showing the ameliorative contribution of NO molecule against HMs toxicity in different plants is highlighted in Table 3.1. Nitric oxide attenuates varied activities in plants accounting for germination, photosynthetic molecules, stomatal and ion conductance, growth of different organs, and hormonal regulation (Munawar et al. 2019). From literature, it is concluded that NO regulates metal stress tolerance by strengthening the antioxidant defense system via equilibrating cell redox reactions (Sharma et al. 2020). In addition, on metal stress, plants normalize their tolerance by triggering the endogenous NO synthesis mechanism (Kaya et al. 2019).

Table 3.1 Ameliorative function of nitric oxide (NO) in metal stressed plants

Metal	Concentration	Plant	Regulatory function of exogenous NO	Reference
As	100–1000 µM	*Arabidopsis thaliana*	The activity of GSNOR and protein tyrosine upregulated cell viability	Leterrier et al. (2012); Pető et al. (2013)
	100 µM	*Brassica juncea*	Accumulation of As diminished, and expression of genes associated with N metabolism helped for enhanced ROS scavenging activity	Praveen et al. (2019)
	25–150 µM	*Oryza sativa*	Elevated activity of multiple antioxidants viz. CAT, POX, APX and SOD improved root growth via NiR activation	Singh et al. (2009); Praveen and Gupta (2018)
	250 and 500 µM	*Triticum aestivum* L.	The exogenous application of NO augmented biomass, water content, osmotic potential and level of antioxidants such as AsA and GSH	Hasanuzzaman et al. (2013)
	0.5–0.1 mg L^{-1}	*Pistia stratiotes*	ROS production diminished due to increased activity of different antioxidants which minimize the growth retardation factors	Farnese et al. (2013)
	100 and 2500 µM	*Vigna radiata*	NO minimize the inhibitory result of metal ions on seed germination, growth, photosynthetic efficiency and increased the antioxidant defensive system	Ismail (2012)
	50 and 100 µM	*Phaseolus vulgaris* L.	Enhanced plant morphology and decreased MDA content via reducing the effect of ROS at membrane. Elevated the antioxidant enzyme activity.	Talukdar (2013)
	100, 200, 400 µM	*Vicia faba* L.	Improved plant growth, yield, pigment molecules, upregulation of phytohormones and metabolites	Mohamed et al. (2016)
	100 µM	*Oryza sativa* L.	Inhibitory effect of As ameliorated via homeostasis of ROS generation and scavenged enzymes on roots formation and biomass	Kushwaha et al. (2019)

Cd	100 µM	*Cucumis sativus*	High concentration persuaded chlorosis and effectiveness of photosynthesis which became reduced by ROS scavenging enzymes.	Yu et al. (2014); Gong et al. (2017a)
	50–250 µM	*Arachis hypogaea* L.	Photosynthesis accelerated on NR and NOS induced activities. The level of ROS, MDA decreased by activation of antioxidative enzymes	Dong et al. (2020); Yuanjie et al. (2019)
	10–30 µM	*Oryza sativa ssp.*	Biomass, plant growth, antioxidant activity upsurges	Yang et al. (2016)
	50–100 µM	*Trifolium repens* L.	NO donor sodium nitroprusside (SNP) advances the Cd tolerance by abolishing oxidative damage, increasing ATPase activity, and homeostasis of hormones	Liu et al. (2015)
	100–400 µM	*Typha angustifolia*	NO minimized Cd ions toxicity via regulating antioxidant defence system, metabolism and accumulation of Cd in the cell wall of the root.	Zhao et al. (2016)
	150 µM	*S. lycopersicum* L	Limited Cd uptake, increased content of photosynthetic molecules, water potential and stomatal conductance. Boosted enzyme activities viz. AsA, GSH, phenolics and flavonoids	Ahmad et al. (2018a)
	5–200 µM	*Brassica juncea*	Roots adapted to Cd by enhancing the water and chlorophyll content and antioxidant enzymes activity	Verma et al. (2013); Per et al. (2017)
Pb	1500 µM	*Lupinus luteus*	Reduced the toxicity of Pb on morphology of roots by stimulating SOD activity	Kopyra and Gwóźdź (2003)
	100–200 µM	*Vigna unguiculata*	Enhanced photosynthetic machinery and defence system	Sadeghipour and Aghaei (2013)
	50–100 µM	*Triticum aestivum*	NO counters the effect of Pb by reducing the oxidative stress markers and enhanced antioxidant enzyme activity	Kaur et al. (2015)
Zn	10.8 and 21.6 µM	*Triticum aestivum, Phaseolus vulgaris*	The antagonistic effect of Zn^{2+} reduced by curbing the levels of sulfhydryl groups, GSH and SOD activity	Abdel-Kader (2007)
Al	30–250 µM	*Triticum aestivum*	NO donor molecules accelerate better plant growth via elevated antioxidative enzymes	Sun et al. (2014)
	1.2 mM	*Citrus grandis*	Improves the plant growth and stabilize the photosynthetic machinery by synthesizing malate and citrate that help to minimize the toxic effects on growth.	Yang et al. (2012)
Cu	100–400 mM	*Oryza sativa*	GSNO molecule expressed uptake of Cu and raised plant growth	Mostofa et al. (2015b)
Co		*Triticum aestivum*	Regulating the rate of redox reaction during photosynthesis process; SNP helped to moderate negative effects of stress	Ozfidan-Konakci et al. (2020)
Ni	200 µM	*Oryza sativa*	Raised endogenous NO constrained H_2O_2, MDA and augmented enzymatic and non-enzymatic activity of antioxidants	Rizwan et al. (2018)

3.4.2 H_2S Signaling Under Heavy Metal Stress

Plant growth and development are retarded by inhibiting photosynthesis, disrupting enzymatic activity and ROS production in heavy metal contaminated soil. H_2S reduces the uptake and translocation of these heavy metals through vacuolar compartmentalization, accumulation of osmoprotectants, antioxidant activities, etc. (Gong et al. 2020; Tian et al. 2016; Kushwaha and Singh 2020). Figure 3.4 shows the effect of H_2S under heavy metal toxicity. Under those conditions, the biosynthetic enzymes L-cysteine desulfhydrase (L-DES/LCD), D-cysteine desulfhydrase (D-DES/DCD), and CAS (cyanoalanine synthase) are activated and generate H_2S by using cysteine and cyanide as substrates (Alvarez et al. 2010; Gotor et al. 2010). H_2S production leads to the activation and maintenance of ROS detoxifying enzymes like SOD, POD, APX, CAT, etc. which then maintain the redox homeostasis. In rice plants, exogenous application of H_2S donor NaSH activates the antioxidantive enzymes and also enhances the expression of non-enzymatic antioxidants i.e. AsA

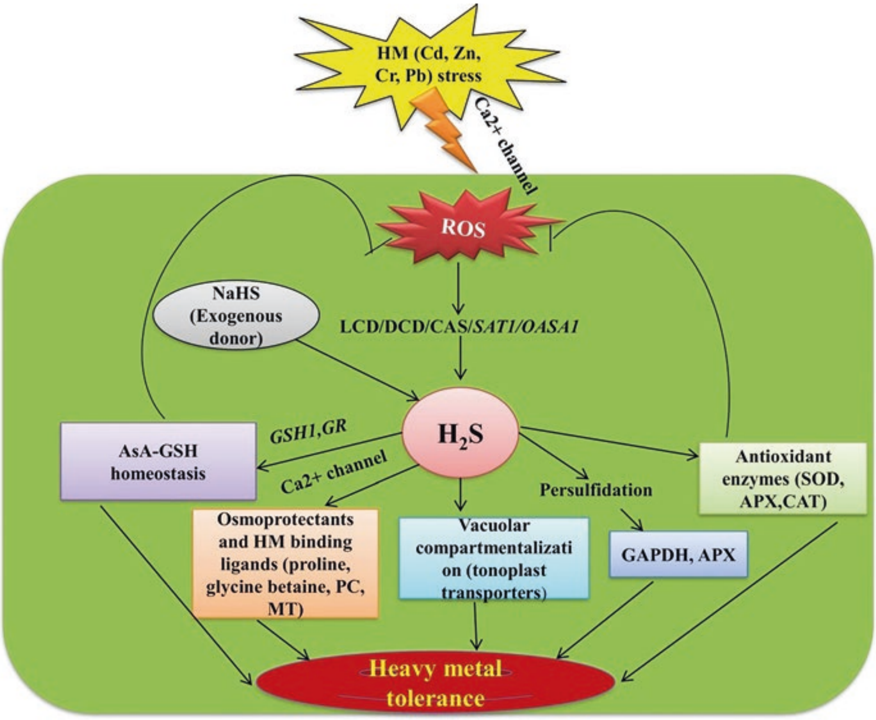

Fig. 3.4 H_2S signalling under heavy metal stress. HM- heavy metal, LCD- L-cysteine desulfhydrase; DCD- D-cysteine desulfhydrase, CAS- β-cyanoalanine synthase, SAT1- serine acetyltransferase 1, OASA1- cysteine synthase 1, GSH1-glutamylcysteine synthetase 1, GR- glutathione reductase, GAPDH-glyceraldehyde-3-phosphate dehydrogenase, APX- ascorbate peroxidase, SOD- superoxide dismutase, CAT- catalase, PC- phytochelatins, MT- Metallothionein

and GSH (Mostofa et al. 2015b). *Serine acetyltransferase 1 (SAT1)* and *cysteine synthase 1 (OASA1)* genes are upregulated by heavy metal triggered H_2S production which ultimately increases the phytochelatin and metallothionein content in plant cells and also the osmolytes (proline, glycine, betaine) maintain the ROS homeostasis by protecting protein molecules under osmotic stress (Fang et al. 2021; Jia et al. 2016).

Additionally, H_2S activates the tonoplast antiporters (Cd^{2+}/H^+) and therefore maintains the heavy metal homeostasis through vacuolar compartmentalization. In a protein, cysteine residues are targeted by persulfidation (-SH, S-S-, − S-OH to –SSH) which then enhances the expression of APX and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and subsequently help in metal tolerance (Aroca et al. 2015). H_2S elevates the level of GSH (reduced glutathione) by enhancing the expression of glutamylcysteine synthetase (GSH1) and glutathione reductase (GR) and also increasing the amount of reduced AsA, thereby neutralizing the HMs stress by the formation of these antioxidant molecules (Luo et al. 2020; Fang et al. 2014) (Fig. 3.4).

3.4.3 CO Signaling Under Heavy Metal Stress

Stressed plants can be protected from the harmful effects of HMs with the help application of CO (as shown in Table 3.2). Heavy metal-induced oxidative stress in plants can be relieved by CO (Zheng et al. 2011) or also by exogenous application of various substrates of CO fumes/its aqueous solution, hematin, hemin, and heme. CO treatments into *Brassica juncea* suppressed the production of ROS like $O_2^{\bullet-}$ and H_2O_2 (Meng et al. 2011). Oxidative damage associated with Cd toxicity was minimized by modulation of glutathione metabolism in *Medicago sativa* by CO application (Han et al. 2008). Hematin and CO when applied exogenously to *M. sativa* (alfalfa) root seedlings affected with $HgCl_2$ stress not only reduced lipid peroxidation but also caused root elongation mainly through the activation of antioxidants like monodehydroascorbate reductase (MDAR), SOD, GR, and decreasing lipoxygenase (LOX) activity (Han et al. 2007). In algae, CO lowers the HMs accumulation mainly by restricting the HMs uptake (Wei et al. 2011). CO treatment also eliminates oxidative stress in algae under Cu-toxicity through the activation of CAT enzymes (Zheng et al. 2011).

3.4.4 CH_4 Signalling Under Heavy Metal Stress Conditions

Metal contamination has become a widespread problem with the development of the industrial sector. It causes a serious disease effect on the health of plants, animals, and humans as well. In plants, metal exposure has been linked with inhibition of germination and plant growth, in many cases causing plant death. Studies revealed

Table 3.2 CO treatments and its role in ameliorating heavy metal stress in plants

Plant species	CO source	CO effect on heavy metal stress	References
Medicago sativa	Hematin	Ameliorates mercury stress	Han et al. (2007)
M. sativa	CO aqueous solution	Modulates glutathione metabolism in the roots to alleviate cadmium-induced oxidative damage	Han et al. (2008)
M. sativa	Hemin and hemin	Heme oxygenase-1 (HO-1) induction triggered by β-cyclodextrin-hemin (β-CD hemin, CDH) reduced the Cd-induced toxicity in *M. sativa*	Fu et al. (2011)
Brassica juncea	Aqueous solution of CO	Increases mercury tolerances	Meng et al. (2011)
Brassica napus	Hematin, hemin or HO	Enhances mercury tolerances	Shen et al. (2011)
M. sativa	Heme oxygenase-1 (HO-1)	HO-1-mediated CO production causes the downregulation of SA (salicylic acid) to reduce Cd-induced oxidative damage in the roots of alfalfa seedlings	Cui et al. (2012)
M. sativa	HO1	Ameliorates Al associated oxidative stress	Cui et al. (2013)
Chlamydomonas reinhardtii	HO1, CO	Reduced heavy metal stress	Wei et al. (2011)
C. reinhardtii	CO	Amliorates Cu induced oxidative stress	Zheng et al. (2011)
Oryza sativa	Hemin	Ameliorates zinc, Lead & chromium toxicity of rice seedling	Chen et al. (2017)
O. sativa	HO1/ferrous iron	Alleviates Zn tolerances	Chen et al. (2018a)
Chinese cabbage seedling	Heme	hemin reduced cadmium toxicity in Chinese cabbage seedlings through decreasing the Cd uptake	Zhu et al. (2019)

that plants develop oxidative stress with exposure to metals which thereby led to an ROS burst (Cui et al. 2017; Samma et al. 2017; Gu et al. 2018). Reduction in metal accumulation by inhibiting uptake and regenerating redox homeostasis are the two possible mechanisms through which CH_4 provides a shield against metal-related toxicity. For instance, a reduction in copper and Cu-induced proline content coupled with enhancement of total sugar content was detected in *Medicago sativa* seed which is thought to be inhibited from germination by copper accumulation (Samma et al. 2017). CH_4 was found to be the key player to alleviate the excess Cu and was successful in re-establishing the redox homeostasis. Gu et al. (2018) revealed that CH_4 induced alleviation of Cd and Al toxicity through regulation of their transporter and their associated genes. Samma et al. (2017) demonstrated the mitigative role of CH_4 in Cd-induced inhibition of seed germination, and seedling growth and alleviating oxidative stresses by applying exogenous CH_4 in alfalfa. The mechanism stated was through minimizing and controlling of lipid peroxidation and maintaining membrane integrity and emission of CH_4 has also been noticed in Cd stressed seedlings of alfalfa (Samma et al. 2017).

Inhibition of root elongation, nutrient disorder, and electrolyte leakage under Al stress has been found to mitigate through CH_4 application in alfalfa root tissue (Cui et al. 2017). Similarly, emission of CH_4 in root tissue of alfalfa plant under Cd stress and mitigation of Cd-induced inhibition of seedling growth with exogenous CH_4 application (Gu et al. 2018).

HM such as Cd, Al, and Cu cause oxidative stress and redox imbalance triggers the activity of antioxidative enzymes such SOD, CAT, POD, and APX activity. CH_4 pre-treatment further enhanced their activities via a cascade of signaling which targets the genes that synthesize those enzymes (Cui et al. 2017; Samma et al. 2017; Gu et al. 2018). ROS production under HM stress is considered to be the triggering signal for nonmicrobial CH_4 generation inside the plants. The ROS overproduction triggers the CH_4 generation which however leads in the upregulation in the activity of certain antioxidative enzymes. However after the ROS removal and their enhancement in ROS removal enzymes the levels of CH_4 gets reduced, thus gives enough evidence of CH_4 involvement in mitigation of ROS production under HM stress (Samma et al. 2017). Also, CH_4 maintains the GSH level under Cd stress by triggering the expression levels of specific genes such as γ-glutamylcysteinyl synthetase (ECS), glutathione reductase 1 and 2 (GR1/2), homoglutathione synthetase (hGS) and glutathione-S transferase which are involved in reduced glutathione (GSH) biosynthesis (Fig. 3.5) (Gu et al. 2018).

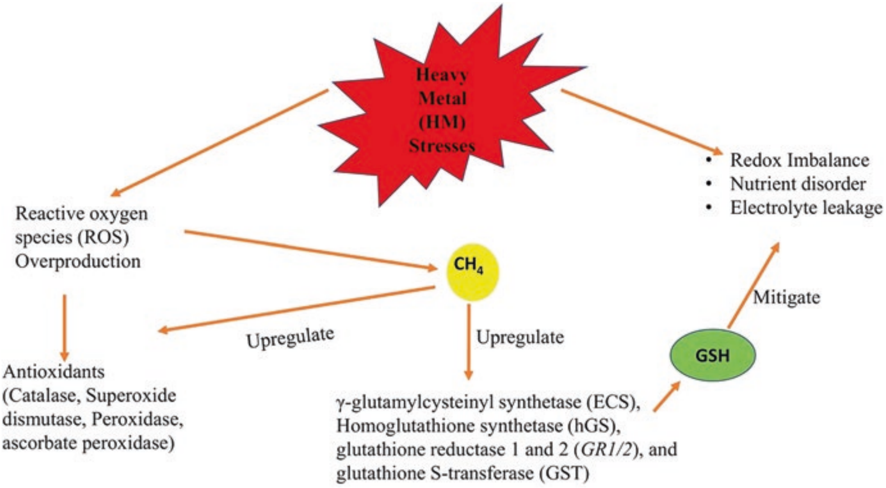

Fig. 3.5 Signaling mechanism of CH_4 in ameliorating the heavy metal (HM) stresses in plants

3.5 Crosstalk of Different Gasotransmitters Under Heavy Metal Stress

Gasotransmitters aid signaling processes in plants and are synthesized endogenously as well as exogenously. They are crucial for mediating signaling responses and function actively in regulating different mechanisms of plants under stressed conditions like metalloid toxicity and improved plant growth and metabolism (Alamri et al. 2020; Mukherjee and Corpas 2020; Yao et al. 2019). NO is considered one of the substantial gasotransmitters that is synthesized endogenously and also taken up by exogenous sources that help the plants to sustain, survive and counteract heavy metal toxicity conditions (Terrón-Camero et al. 2019). Endogenous NO levels become escalated in plants subjected to metal toxicity that is primarily due to higher NO-mediated transcripts (Besson-Bard et al. 2009). The exogenous levels of sodium nitroprusside (SNP) declined the ROS levels along with enhancing stress resistance and chlorophyll levels with improved nutrient absorption in *Lolium perenne* subjected to Cd stress (Chen et al. 2018a, b). The treatment of mustard plants with NO ameliorated Cu stress and associated adversities with higher germination index and antioxidant activities of APX, SOD, and GR respectively (Rather et al. 2020). They also reported reduced ROS and lipid peroxidation with enhanced photosynthetic rate and morphological attributes of the plants (Rather et al. 2020). Moreover, the treatment of SNP triggered the antioxidant levels and reduced superoxide and MDA contents in *Arachis hypogea*. Enhanced nutrient levels of Ca, Mg, Zn, and Fe have also been found (Dong et al. 2020). NO negatively affects the metal uptake except for Cd where positive regulation is observed (Terrón-Camero et al. 2019). The NO-mediated Cd mitigation in *Brassica sp.* depicts that stress alleviation is directly linked to S-assimilation and GSH synthesis (Per et al. 2017). To elucidate when plants were given SNP treatment along with GSH, the Cd toxicity was more efficiently ameliorated along with lowered ROS accumulation and stimulated antioxidant activities as well as photosynthetic and pigment levels (Per et al. 2017). NO-mediated amelioration of Cr toxicity in tomato plants was also observed along with inducing seed vigor index and germination rate respectively (Khan et al. 2020a, b). They also depicted the enhanced levels of proline, nitrogen, and metal ligands such as GSH and ascorbate with plummeting electron leakage as well as protein carbonylation (Khan et al. 2020a, b).

Another important gasotransmitter is H_2S which has been a well-known secondary messenger in plants under stressful conditions and induces other signaling messengers during the defense signaling cascade in plants (Luo et al. 2020). The exogenous application of H_2S mitigated Cr toxicity by limiting the electrolyte leakage, ROS, and MDA accrual along with enhancing the activities of physiological as well biochemical attributes in terms of chlorophyll levels, plant biomass, enzymatic activities such as SOD, APX, POD, GPX (glutathione peroxidase), CAT, etc. in cauliflower (Ahmad et al. 2020). Further, H_2S application also upgraded the photochemistry of photosystems through stimulating photochemical efficiencies, quenching as well as quantum efficiency. Consequently, an increase in the photosynthesis, antioxidants namely, SOD, POD, CAT, GPX, APX, GR, etc. was also reported that mitigated the Cu toxicity from wheat plants (Dai et al. 2016). Induced endogenous H_2S in rice is also known to upregulate ROS neutralizing enzymes and further regulates the redox homeostasis of plants. Higher chlorophyll synthesis through enhancing mineral acquisition of Mg, Zn, Mn, and Fe was also reported that maintained Cd homeostasis and toxicity in rice plants (Mostofa et al. 2015b). Moreover, H_2S synthesis is also stimulated during metal toxicity conditions in alfalfa plants grown under Cd toxicity through the biosynthesis of many enzymes such as LCD and DCD respectively that further regulated GSH metabolism as well as ROS homeostasis (Cui et al. 2014). Although, the exogenously applied H_2S further enhanced the endogenous levels to mitigate Cd-induced physiological damage to the plants by maintaining the levels of GSH pools and redox homeostasis (Cui et al. 2014). Moreover, the combinatorial treatment of H_2S and NO both mitigated CO toxicity in wheat by modulating their water levels as well as osmotic potential, stomatal conductance, transpiration rate, RuBisCO activity, intracellular CO_2 and rate of carbon assimilation (Ozfidan-Konakci et al. 2020).

CO is yet another molecule, kindred of NO and H_2S that induces the activation of antioxidants, CAT, APX, and POD to limit the ROS accrual during metal toxicity conditions along with promoting plant growth and development (Meng et al. 2011). Although CO application mitigated Cd-induced oxidative damage by regulating GSH pools and restoring the structural integrity in alfalfa plants (Han et al. 2008). In addition, hemin, a water-soluble CO donor led to activation of transcriptional expression as well as plummeted the Zn accumulation. This is directly co-linked to the down-regulation of Zn uptake along with reduced expression levels of genes encoding Zn homeostasis, *ZIP1*, *ZIP3*, *ZIP7*, and ZI*P*7 respectively, and enhanced Zn resistance in rice plants (Chen et al. 2018a, b). Additionally, another crucial player in maintaining redox homeostasis is CH_4 which dwindled metal toxicity in plants by blocking metal accumulation and improving plant growth and metabolism (Cui et al. 2017). CH_4 has been found to maintain nutrient balance and also regulates Al toxicity through modulating the expression levels of metal transporter genes namely, *ALMT1*, *MDH1/2*, *AACT*, and genes encoding organic acid synthesis (Cui et al. 2017). Alongside, Cd toxicity has also been observed to be regulated through GSH pools as well as expression of *miR159* and *miR167* along with targeted ABC transporters NRAMP6 respectively (Gu et al. 2018).

It is noteworthy that the most significant crosstalk is observed among different gasotransmitters during heavy metal toxicity that not only mitigated the negative effects but also promoted plant growth and functions in different aspects (Shivaraj et al. 2020). NO and H_2S in combination share their signaling mechanism where there are certain reports depicting H_2S upstream or downstream NO-signaling cascade (Corpas et al. 2019). Both NO and H_2S show an impact on each other, and overall expression is documented in the form of antioxidant responses of the plants

(Rather et al. 2020). There is a certain set of studies that suggest NO-H_2S crosstalk in plants under heavy metal toxicity. For instance, the exogenous H_2S donor enhanced the NO levels in alfalfa plants under Cd toxicity, whilst NO scavenger reverted their action (Li et al. 2012). Similar to this, NO and H_2S together mitigated Pb toxicity in *Sesamum indicum* by hindering their uptake and accrual along with improving antioxidant activities and nutrient uptake and assimilation of essential nutrients such as Mg, Zn, Mn, Fe, and P. All these processes regulated the mineral homeostasis as well as overall growth and metabolism of plants (Amooaghaie and Enteshari 2017). The interactive role of NO and H_2S in Cd stress mitigation in *Triticum sp.* plants was positively co-related to exogenously apply NO and H_2S donors. They reported stimulated plant dry matter, chlorophyll, antioxidant enzymes (CAT, POD, SOD, etc.), and mineral nutrients (Kaya et al. 2020). Moreover, NO and H_2S together can ameliorated Cd stress through modulation of ROS and osmoprotectants content in *Cynodon dactylon* respectively (Shi et al. 2014). Alongside, NO and H_2S inhibitors are found to restrict NO signals, whereas H_2S signals were mainly restricted by H_2S inhibitors only during Cd stress, thus can depict the active role of NO-signaling that mediates H_2S-induced Cd stress alleviation in *C. dactylon* (Shi et al. 2014). This is directly co-linked to the cryoprotective behavior of NO donors along with the combinatorial action of H_2S donors that caused Cd stress attenuation in alfalfa plants and depicts a classic example of crosstalk among different gasotransmitters during stressed conditions (Li et al. 2012).

Furthermore, NO also forms a signaling network along with phytohormones such as ethylene (ET), calcium, and Mitogen-activated protein kinases (MAPKs) and gives rise to a hormonal signaling cascade (Jalmi et al. 2018). NO-ET crosstalk has been observed to impact many plant responses subjected to toxicity of hazardous metals such as As, Cd, Cu, Ni, Pb, and Zn respectively (Sahay and Gupta 2017). NO-ET crosstalk is effective during Cd stress in pea plants that showed modulatory effects on both ROS as well as NO metabolism along with changing patterns of hormonal levels namely, jasmonic acid, salicylic acid, and ET respectively (Rodríguez-Serrano et al. 2009). In forging arguments, all these phytohormones escalated along with higher ROS levels together with limited NO production that was co-linked to Cd-mediated senescence. Therefore, the authors reported that ET and NO show antagonistic effects in plants under Cd stress and Cd-boosted nutritional disturbances, and led to NO reduction and subsequent alteration in protein nitrosation which favors ET biosynthesis (Rodríguez-Serrano et al. 2009). Strikingly, many other treatments with Cd enhanced the expression levels of genes encoding for proteins involved in ET as well as NO biosynthesis in *Glycine max* (Chmielowska-Bąk et al. 2013). The biochemical and molecular interactions among NO-ET signaling pathway and their crosstalk during metal toxicity in plants still requires to be explored. Although a complex network among different gasotransmitters during metal toxicity conditions is still being explored in detail to accomplish the main motive for completing this circuit and enable researchers working on gasotransmitters to understand their crosstalk elaborately during heavy metal toxicity conditions.

3.6 Conclusion

Studies on gasotransmitters regulation under the changing abiotic stress conditions is a topic of research hotspot for the scientific community. Among these abiotic stressors, HMs are evidenced to induce various GTs-related regulatory processes in the plant system. GTs are found to promote and up-regulate plant tolerance to diverse HMs constituents. These gaseous signaling molecules do not operate independently but undergo a series of cross-talks with other GTs and also coordinate with other molecules such as phosphorylation cascades, ROS molecules, calcium – calmodulin systems, phytohormones, etc. Thus, understanding and achieving more in-depth knowledge of these signaling cascades and the biological interplay between these GTs under HMs stress conditions requires more understanding and research applications.

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Chapter 4
Volatile Signaling Molecules in Plants and Their Interplay with the Redox Balance Under Challenging Environments: New Insights

Liliana Scelzo, Matías Alegre, Carlos Guillermo Bartoli, Andrea Galatro, Violeta Velikova, and Gustavo Esteban Gergoff Grozeff

Abstract Volatile molecules such as ethylene, nitric oxide (NO), and isoprene have important functions in plant growth and development. Under stress conditions, these compounds interact with each other and with the reactive oxygen species (ROS) processing systems, to undergo stressful situations. The coordination of several processes such as leaf senescence, fruit ripening, or growth under challenging scenarios requires a multilevel study, coupled with the study of the antioxidant systems that are able to maintain the redox balance within the plant cell. In this chapter, the authors emphasize the complex network that integrates volatile signaling molecules with ROS under hormonal control and the possible implications in the crop technology that will probably be an input in future genetic engineering programs.

Keywords Ascorbic acid · Glutathione · Tocopherols · Reactive nitrogen species · Reactive oxygen species · Isoprene · Abiotic stress · Hormones · Growth and development

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T. Aftab, F. J. Corpas (eds.), *Gasotransmitters Signaling in Plants under Challenging Environment*, Plant in Challenging Environments 5,
https://doi.org/10.1007/978-3-031-43029-9_4

4.1 Introduction

Since the first appearance of cyanobacteria, the Earth's atmosphere started to accumulate oxygen, converting the ambient from reducing to oxidant. The harvest of light and the synthesis of oxygen, as a sub-product of the photosynthetic process, also carried the production of certain forms of oxygen called reactive oxygen species (ROS) (Noctor et al. 2018). ROS can also act in two ways in the cellular metabolism: either producing harmful effects on molecules (e.g., proteins, DNA, lipids, etc.) or as signaling molecules (Mittler 2017).

Under stress conditions, plants have many systems to overcome these stressful situations and some volatile molecules contribute to coordinate the complex metabolic orchestration, including some gasotransmitters like ethylene, isoprene, and nitric oxide (NO), among others. This chapter aims to build a bridge between the stress conditions, the gasotransmitters, and the redox balance. First, we will briefly discuss the production of the ROS, and the ROS processing systems as the counterpart, and then we will introduce some volatile molecules that interact with the redox balance in plant metabolism.

4.2 Reactive Oxygen Species

ROS is a collective definition that includes oxygen radicals [*e.g.* superoxide radical ($O_2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$), peroxyl radical ($RO_2^{\bullet}$), hydroperoxyl radical ($HO_2^{\bullet}$)] and non-radical molecules, that are strong oxidizing agents [*e.g.* singlet oxygen ($^1O_2$), hydrogen peroxide H_2O_2, hypochlorous acid (HOCl), and ozone (O_3)] (Dvořák et al. 2021). One of the most stable ROS is hydrogen peroxide, which can also be actively transported through the cellular membranes by aquaporins (Bienert and Chaumont 2014).

Even though ROS can be generated in the symplast and the apoplast of the plant cell, the principal organelles that produce ROS are chloroplasts, mitochondria and peroxisomes. In chloroplasts, the production of ROS is located in the thylakoid membranes, where $O_2^{\bullet-}$ is produced at the level of Photosystem I, while $^1O_2$ is produced in Photosystem II basically (Asada 1999; Corpas et al. 2015). The $O_2^{\cdot-}$ anions can be dismutated spontaneously or generated by the action of the superoxide dismutase (SOD) enzyme in both the stroma and the thylakoid membrane. In mitochondria, the main ROS produced is $O_2^{\bullet-}$ and it is mainly associated with the activity of Complexes I and III in the inner mitochondrial membrane (Corpas et al. 2015; Gupta and Igamberdiev 2015). In peroxisomes, the $O_2^{\bullet-}$ is produced in the matrix and in their membranes (Corpas 2015; Corpas et al. 2017) and the H_2O_2 is the result of the activity of certain specific enzymes associated with photorespiration and other organelle pathways (Corpas 2015; Foyer 2018). The apoplastic ROS are produced by the NADPH oxidases, oxalate oxidases and through the spermidine degradation by the action of the polyamine oxidase (Wang et al. 2019), together with other isoforms of peroxidases (Dvořák et al. 2021).

4.3 ROS Processing Systems

4.3.1 Enzymatic ROS Processing Systems

Under physiological conditions, ROS are produced and then eliminated from the cells thanks to different ROS processing systems, which can be separated into enzymatic and non-enzymatic (Gupta et al. 2015, 2018). The enzymatic ROS processing system includes different enzymes like SOD, catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GPX), peroxiredoxins (Prx), and thioredoxins (Trx) (Table 4.1).

Besides their capacity to reduce $O_2^{\bullet -}$ to H_2O_2, ameliorating the ROS stress, SODs have a central role in the synthesis of H_2O_2 as a signaling transduction molecule and in the fine-tune responses of growth and development (Dietz et al. 2016).

The reduction of H_2O_2 can be catalyzed by three main enzymatic antioxidant systems: CAT, APX, and GPX. CAT transforms H_2O_2 to H_2O and O_2 in peroxisomes (Table 4.1). CAT activity can be also up-regulated under unfavorable conditions for plant growth (Mhamdi et al. 2012) and can control the redox homeostasis by reducing the levels of H_2O_2 (Sandalio and Romero-Puertas 2015). In *Arabidopsis thaliana*, there are three genes encoding different CAT isoforms: *CAT1* expression is related to the β-oxidation of fatty acids, *CAT2* is linked to the photorespiration pathway and *CAT3* is expressed under senescence-associated processes (Del Río and López-Huertas 2016). Under abiotic stress, CAT is up-regulated to decrease the impact of oxidative stress over different growth processes (Gupta et al. 2018).

The detoxification of H_2O_2 can also be catalyzed by the action of the APX, which uses L-ascorbic acid (AsA) as an electron donor and generates H_2O and monodehydroascorbate radical (MDHA) (Fig. 4.1). The different isoforms are located in the cytosol, chloroplasts (thylakoid and stroma), mitochondria, and peroxisomes, modulating the levels of H_2O_2 in these subcellular compartments (Pandey 2017; Pandey et al. 2017). In the chloroplasts, the APX is bounded to the thylakoid membrane and forms part of the water-water cycle (Asada 1999). The activity of these different isoforms enhances the role of AsA as an antioxidant and explains the abundance of this molecule in plant cells (Gest et al. 2013).

Another enzyme that catalyzes the same H_2O_2 scavenging reaction is glutathione peroxidase (GPX). It is part of the thiol peroxidases and uses glutathione (GSH) as an electron donor. Similar to APX, this ubiquitous enzyme is distributed in different subcellular compartments. In *A. thaliana*, there are eight isoforms that are induced by different signals (Bela et al. 2015). GPX and APX also regulate the ascorbate-glutathione cycle (Kumar et al. 2017) (Fig. 4.1).

Table 4.1 ROS processing systems in plants

	Subcellular compartments	Substrate	References
Enzymatic ROS processing systems			
SOD	Mitochondria, peroxisomes, nucleus, chloroplasts, cytosol, apoplast	$O_2^{\bullet-}$	Wang et al. (2016)
CAT	Peroxisomes	H_2O_2	Mhamdi et al. (2012)
GPX	Mitochondria, cytosol, endoplasmic reticulum, chloroplasts, plasma membrane, Golgi, endosome, nucleus.	H_2O_2	Bela et al. (2015)
APX	Cytosol, chloroplasts, mitochondria and peroxisomes.	H_2O_2	Maruta et al. (2016)
PRX	Cytosol, mitochondria, nucleus, chloroplasts, extracellular space and possibly in peroxisomes.	H_2O_2 ROOH ONOOH	Corpas et al. (2017) and Del Río (2020)
TRX	Chloroplasts, mitochondria, peroxisomes, cytosol	Cys-proteins	Sevilla et al. (2015)
Non-enzymatic ROS processing systems			
CAROTENOIDS	Plastids	$^1O_2$, triplet chlorophyll	Torres-Montilla and Rodríguez-Concepción (2021)
ASCORBIC ACID	Chloroplasts, mitochondria, peroxisomes, cytosol, apoplast	$O_2^{\bullet-}$, H_2O_2, $^\bullet OH$	Hassan et al. (2021)
TOCOPHEROLS	Membranes	$^1O_2$	Khalil et al. (2022) and Hameed et al. (2021)
GLUTATHIONE	Chloroplasts, mitochondria, peroxisomes, cytosol, apoplast	H_2O_2	Hussain et al. (2019)
PROLINE	Mitochondria	$^\bullet OH$, H_2O_2, $^1O_2$, $O_2^{\bullet-}$ and RNS	Alvarez et al. (2021)

SOD Superoxide dismutase, *CAT* catalase, *GPX* glutathione peroxidase, *APX* ascorbate peroxidase, *TRX* thioredoxins, *PRX* peroxiredoxins, $^1O_2$ singlet oxygen, $O_2^{\bullet-}$ superoxide, H_2O_2 hydrogen peroxide, $^\bullet OH$ hydroxyl radical, *ROOH* Alkyl-hydroperoxides, *ONOOH* peroxynitrite, *RNS* reactive nitrogen species

Apart from these enzymatic systems, other enzymes help to preserve the redox homeostasis, including dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), glutathione reductase (GR), glutathione transferases, peroxidases (PODs) and alternative oxidases. The redox hub, constituted by AsA and glutathione (GSH), is closely regulated by the activity of APX, MDHAR, DAR, and GR in the Foyer-Halliwell-Asada cycle (Foyer and Noctor 2011), with the participation of GPX and PRX (Fig. 4.1).

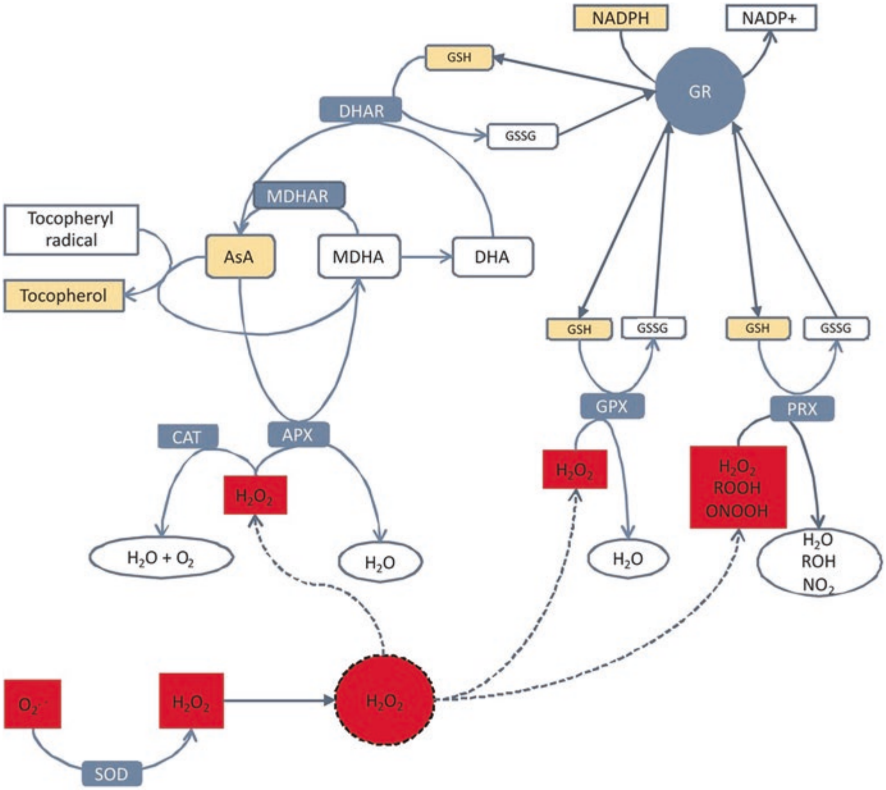

Fig. 4.1 ROS processing systems in plants. *SOD* Superoxide dismutase, *CAT* catalase, *GPX* glutathione peroxidase, *APX* ascorbate peroxidase, *PRX* peroxiredoxins, *DHAR* dehydroascorbate reductase, *MDHAR* monodehydroascorbate reductase, *GR* glutathione reductase, *GSH* glutathione, *GSSG* glutathione disulfide, *AsA* ascorbic acid, *MDHA* monodehydroascorbate, *DHA* dehydroascorbate, $O_2^{\cdot-}$ superoxide, H_2O_2 hydrogen peroxide, *ROOH* Alkyl-hydroperoxides, *ONOOH* peroxynitrite

4.3.2 Non-enzymatic ROS Processing Systems

Besides the enzymatic systems, there is a non-enzymatic redox system, constituted by different molecules that are capable (directly or via enzymatic reactions) of donating electrons to reduce ROS. The synthesis pathways of these molecules are very different, but they can roughly be classified into water-soluble antioxidants and liposoluble antioxidants. A brief description can be seen in Table 4.1.

4.3.2.1 Hydrosoluble Non-enzymatic Antioxidants

Ascorbic Acid

This multifunctional molecule is the most abundant and efficient hydrosoluble antioxidant that can neutralize the damage caused by ROS. It is found in the cytosol, mitochondria, peroxisomes, and apoplast at higher concentrations in meristems and photosynthetic tissues (Ishikawa et al. 2018). The concentrations range of AsA is wide and depends on the organs, the harvest, and postharvest time, but it can be between 0.45 μmol g^{-1} fresh weight (FW) in potatoes (*Solanum tuberosum* L.) to 73 μmol g^{-1} FW in “Acerola” (*Malpighia emarginata* D.C.) fruit (Davey et al. 2000); and even higher, rising to 128 μmol g^{-1} FW in “Peruvian camu camu” [*Myrciaria dubia* (H.B.K.) McVaugh] fruit (Chirinos et al. 2010).

Glutathione

Defined as a metabolic key, glutathione (GSH) is another important ubiquitous hydrosoluble antioxidant associated with the protection of the photosynthetic apparatus against ROS. GSH has other functions such as sulfur storage, signaling, and phytochelatins, apart from the neutralization of ROS. It acts in almost all the cellular compartments, including the endoplasmic reticulum, apoplast, peroxisomes, mitochondria, vacuole, chloroplasts, and cytosol (Gong et al. 2018).

4.3.2.2 Liposoluble Non-enzymatic Antioxidants

Within the group of liposoluble non-enzymatic antioxidants, the tocopherols represent the most important group protecting bio-membranes against ROS. There are four different isomers in plants, but α-tocopherol is the most abundant. They are synthesized in all the photosynthetic organisms, including higher plants, algae, and cyanobacteria. The α-tocopherol is more abundant in green tissues, while γ-tocopherol is abundant in seeds. They have an important role in stabilizing bio-membranes and removing ROS like $^1O_2$ (Muñoz and Munné-Bosch 2019) and they can be reduced again by the action of AsA (Munné-Bosch and Alegre 2002).

Carotenoids represent a group of liposoluble pigments and in photosynthetic organisms play several roles such as accessory light-harvesting pigments in the chloroplast, quenchers and scavengers of triplet state chlorophylls, $^1O_2$ and other ROS, dissipator of excess light energy as heat, and structural integrant of the thylakoid membrane, and precursor of hormones such as strigolactones and abscisic acid (Uarrota et al. 2018).

Summarizing, ROS processing systems in plant tissues have different regulating roles acting at different levels, protecting a diverse range of targets against oxidative stress conditions, and are strongly regulated by: plant hormones, such as ethylene; radicals, such as nitric oxide (NO) or other biogenic molecules like isoprene, all under challenging stress scenarios. The complex net of oxidants and antioxidants has important consequences in the growth and development of plants under optimal or stressful conditions. In the following sections, we will discuss some of the newest advances in the interaction of some gasotransmitters with the most important components of the redox balance.

4.4 Ethylene: The Gaseous Phytohormone

Ethylene is the smallest plant hormone that can be synthesized in plant tissues, and it is a gas, a hydrocarbon, and it is the simplest alkene that can trespass the lipid bilayer cell membranes. Its production is closely related to physiological stresses, such as wounding, chilling injury, flooding, high temperature, drought, and even nodulation and biotic infections, along with other physiological processes, such as growth, flower, and leaf senescence or fruit ripening (Khan et al. 2017).

The precursor of this hormone is an amino acid, methionine, which can be recycled in the Yang cycle to ensure great amounts of ethylene with low content of methionine (Adams and Yang 1979). In plants there are two synthesis systems: an autoinhibitory system, called System I, and an autocatalytic system, called System II. This distinction is very important to classify different fruit ripening physiologies: the fruit that raises the respiration rate together with the synthesis of ethylene when it ripens, has an autocatalytic system (System II), and they are called climacteric fruits; meanwhile, the fruit that lacks this increase in respiration rate and the ethylene rise, due to an autoinhibitory system of ethylene synthesis (System I), is negatively named non-climacteric fruits (Pattyn et al. 2020).

Similarly, to other senescence processes, fruit ripening is also characterized by changes in the metabolism, and increased ROS production (v.g. localized mainly in the chloroplast of tomatoes), by the end of this process (Steelheart et al. 2020). The deficiency of AsA synthesis can affect the fruit yield and the signaling of ethylene response, increasing the synthesis of H_2O_2 and the time for fruit ripening in tomatoes (Steelheart et al. 2020; Alegre et al. 2020).

4.4.1 Ethylene Role in Leaf Senescence

Senescence is considered the last stage of leaf growth and development that is associated with a specific gene expression (known as Senescence-associated genes or SAGs) (Buchanan-Wollaston et al. 2005). It is characterized by a drop in the photosynthesis, degradation of chlorophyll and pigments, protein, lipid, and nucleic acid, membrane disruption, and transport of different nutrients to different sinks, both in annual plants to the growing seeds, and in perennial plants to the branches, trunk and roots (Ferrante and Francini 2006). During the last stage of leaf development, the synthesis of ethylene triggers the senescence process, concluding with the leaf abscission, through the interaction with other hormones in different ways: strigolactones (Ueda and Kusaba 2015), cytokinins, auxin, and gibberellins delay leaf senescence (Zhang et al. 2017a, b); meanwhile, jasmonic acid (Hu et al. 2017), salicylic acid, brassinosteroids, abscisic acid, and polyamines accelerate this process (Saini et al. 2015; Pandey 2017).

In this case, the redox metabolism has a strong influence on the leaf senescence, since the increased ROS production causes damage in many organelles and finally triggers cell death (Mhamdi and Van Breusegem 2018). One of the final steps of this process was recently discovered, where WRKY42 could be the key transcription factor that stimulates the H_2O_2 production and other SAGs and salicylic acid regulated genes (Niu et al. 2020). The harmful effects of this ROS production can be controlled by different antioxidant systems. When leaves age, there is a strong decrease in the content of AsA (Hodges and Forney 2003), and this decrease can even be accelerated by the addition of exogenous ethylene (Gergoff Grozeff et al. 2010a). Furthermore, mature leaves of ethylene signaling *A. thaliana* mutants showed higher or lower content of AsA in insensitive or constitutive triple response plants, respectively (Gergoff Grozeff et al. 2010a). Similar effects were also demonstrated with the chemical inhibition of the signal receptor of ethylene, delaying the senescence process and maintaining higher contents of AsA (Gergoff Grozeff et al. 2010b). Years later, Zheng et al. (2020) found that in an AsA over-accumulating mutant ethylene-induced senescence can be also delayed. In the same way, reduced ascorbic acid also affects ethylene synthesis and signaling since AsA mutants are smaller and produce a higher amount of ethylene, and its inhibition increase photosynthesis and biomass (Caviglia et al. 2018).

Similarly, to AsA, GSH and GR are necessary to maintain the redox balance of the cell and contribute to maintaining the efficiency of the photosynthetic apparatus (Müller-Schüssele et al. 2020). Recent work also demonstrated that different leaves in the same plant have several mechanisms to control the synthesis of ethylene under drought stress, up-regulating the enzymes related to the synthesis pathway of GSH (Luo et al. 2021). These authors also found that the redox state of this antioxidant can affect the release of ethylene in drought-sensitive leaves.

At this point, ethylene has been the center of the research in leaf senescence. However, many other hormones accelerate or delay this process. Multiple and combined effects of different hormones, including pharmacological synthesis inhibitors or genetically engineered plants (knockdown or knock out), should be discussed in experimental layouts, to demonstrate the impact of each of them, including other hormones such as salicylic or jasmonic acids (Miao and Zentgraf 2007; Ji et al. 2016). It is fear to say that the technological effects of this combination have been recently discovered in a non-climacteric fruit (Serna-Escolano et al. 2021), but the role in this hormone-regulated process coupled with an exhaustive study of the enzymatic and non-enzymatic antioxidants systems in leaves remains uncompleted.

4.5 Nitric Oxide (NO): A Ubiquitous Signal in Plants

NO is another small gaseous free radical molecule, considered as a gasotransmitter in plants. NO involves a redox set of species with characteristic properties and reactivity such as nitrosonium (NO^+) and nitroxyl (NO^-) ions besides NO radical. Thus, reactions of NO in biological systems depend on the redox state of the cell and the interplay with other reactive molecules (Stamler et al. 1992; Lancaster 2015).

NO can react with molecular oxygen, $O_2^{\bullet -}$, and transition metals ($Me^{+/2+}$), to produce NOx, peroxynitrite ($ONOO^-$), and metal-NO adducts, respectively. These molecules have different biological implications in the regulation of the activity of enzymes and transition factors, peroxidation reactions, and changes in the tertiary structure and function of proteins (Lamattina et al. 2003).

NO has two phases in the redox metabolism of the cell: it can be either a pro-oxidant or an antioxidant. NO acts as a chain-breaking antioxidant, that can arrest lipid peroxidation reactions and photo-oxidative stress and it can also protect other macromolecules, such as proteins, DNA, and RNA (Beligni and Lamattina 2002, 1999a, b). NO can also interact with hormones like gibberellins, ethylene, auxin and abscisic acid (Simontacchi et al. 2013), or enhance the activity of antioxidant enzyme system, and alleviate metal-induced oxidative in plants (Sharma et al. 2019), among others.

When NO reacts with the $O_2^{\bullet -}$, it produces a strong oxidant, peroxynitrite ($ONOO^-$), which can nitrate tyrosine from peptides or proteins. NO can induce thiol-based modifications as well, leading to functional consequences in phosphorylating and non-phosphorylating proteins. Apart from these consequences, peroxynitrite can also modify lipids, inducing peroxidation or nitration in these molecules (Vandelle and Delledonne 2011).

During the last forty years, important advances have been made regarding NO synthesis and roles during the plant life involving several forms of NO donors and treatments, and an array of plant species (Kolbert et al. 2019).

Thus, NO has a broad range of functions in plants, including several growth and developmental processes like germination, flowering, and leaf senescence. It also participates in response to environmental stresses (Simontacchi et al. 2015; Mur et al. 2013) such as nutrient deficiency (Ramos-Artuso et al. 2018; Buet et al. 2019), or (coupled to ROS and hormone regulation) in fruit ripening (reviewed by Steelheart et al. 2019a), and in mechanisms involving herbicide toxicity (Chen et al. 2021).

4.5.1 Crosstalk Between Ethylene and NO in Fruit Ripening

Ethylene and NO display opposite effects in fruit ripening. As mentioned above, ethylene is a hormone mostly related to oxidative damage and senescence, while NO is present in growing organs and delays the senescence syndrome in plants (Simontacchi et al. 2013). Ripening can be assimilated as a senescence process in a terminal organ, the fruit that will irreversibly abscise from the mother plant. As in other senescing organs, in the early stages of fruit growth, higher levels of NO, together with other hormones, like cytokinins, auxins, and gibberellins can be found; but when fruit growth and ripening progress are in the later stages, the abundance of NO is lower and there is a rise in the ROS concentration (reviewed by Steelheart et al. 2019a), coupled to the rise in abscisic acid (non-climacteric fruit) and/or ethylene (climacteric fruit), depending on the fruit physiology (McAtee et al. 2013). Also, the difference between climacteric and non-climacteric fruit implies different modes of action of NO over the ethylene synthesis: in non-climacteric fruit, NO inhibits the 1-aminocyclopropane-1-carboxylate (ACC) synthase (Zhu and Zhou 2007); while in climacteric fruit, ethylene synthesis is inhibited at the level of the ACC oxidase (Zhu et al. 2006).

In climacteric fruit, NO and an ethylene signal inhibitor (1-methyl cyclopropane) have a strong effect on the maintenance of hydrosoluble antioxidants during postharvest. It was reported in tomatoes (Steelheart et al. 2019b) and in blueberries (Gergoff Grozeff et al. 2017) that the combination of 1-methylcyclopropene and a NO donor (S-nitrosoglutathione) maintained higher levels of AsA and GSH during postharvest. NO also inhibited the accumulation of lycopene (Eum et al. 2009), but increase the levels of AsA and flavonoids in tomatoes (Zuccarelli et al. 2021).

Other reports recently showed that NO stimulates the accumulation of AsA in fruits, such as sweet pepper (Rodríguez-Ruiz et al. 2017) or *Citrus* species (Zhou et al. 2016) and phenolics can also be accumulated in NO treated peach (Li et al. 2017) and *Citrus* (Zhou et al. 2016).

Nowadays, NO is a promising tool in fruit postharvest: it can act at the level of ethylene signaling and also maintaining the levels of certain antioxidants that are important for the human diet. The NO effects on ethylene receptors and downstream signaling events need more research. In addition, different NO sources with their advantages/disadvantages in postharvest technology use have been recently analyzed (Buet et al. 2021). Thus, NO donors may be incorporated into the packaging during postharvest treatments to delay the ethylene burst in climacteric fruit. However further research should be encouraged to gain future regulatory approval for postharvest employment (Buet et al. 2021).

4.6 Biogenic Isoprene: A Trait That Contribute to Plant Functioning in the Challenging Environments

Biogenic isoprene (C_5H_8, 2-methyl-1,3-butadiene) is the simplest isoprenoid emitted by a different kind of plant species. Plants do not possess structures where to store isoprene and, after its formation, it is released into the atmosphere. Isoprene is an extremely volatile and reactive molecule and, in the presence of sunlight and nitrogen oxides, hydroxyl radicals initiate reactions leading to the conversion of isoprene to toxic photochemical products (formaldehyde, methyl vinyl ketone, and methacrolein) (Apel et al. 2002; Seinfeld and Pandis 2006). Isoprene oxidation products are important components of secondary organic aerosol (SOA) particles (Matsunaga et al. 2005; Shallcross and Monks 2000) and they may have serious consequences on the radiation balance of Earth by scattering and absorbing light and participating in cloud formation. Thus, isoprene emissions have a high impact on air quality, in the global tropospheric chemistry, that can cause climate change. It is necessary to make more research plans that deals with the impacts of isoprene emissions over environmental factors, thus predicting global climate change and underline new policies of environmental management for future generations (Xie et al. 2013).

4.6.1 Biogenic Isoprene Is Involved in Complex Networks That Regulate Plant Response to Stress

The interest in the study of biogenic isoprene is associated not only because of its importance for atmospheric chemistry but also because of the suggested protective role in plants experiencing various stress stimuli. Under normal conditions, 0.5–2% of the assimilated carbon from photosynthesis is re-emitted as isoprene to the atmosphere (Guenther et al. 1995). Under dramatically stress conditions, this percentage even increases. Since isoprene biosynthesis requires a substantial amount of energy and carbon fixed through photosynthesis (Sharkey and Yeh 2001), it is assumed that it provides benefits to the emitting organisms (Sharkey and Yeh 2001; Velikova 2008; Vickers et al. 2009a; Loreto and Schnitzler 2010).

For nearly three decades of research, a substantial amount of evidence has been accumulated proving that isoprene can protect chloroplasts from different kind of abiotic stress. Isoprene was able to reduce the negative changes in photosynthesis (Sharkey and Singsaas 1995; Loreto and Velikova 2001), to reduce ROS, to limit lipid oxidation (Loreto et al. 2001; Loreto and Velikova 2001; Affek and Yakir 2002; Velikova et al. 2004, 2005; Vickers et al. 2009b; Behnke et al. 2010a) and induce cell death (Velikova et al. 2005, 2012).

4.6.2 Stress Responses of Plants with Chemically Manipulated Isoprene Emission

After discovering that isoprene is made from the products of the plastidic Methylerythritol Phosphate Pathway (MEP) pathway (Zeidler et al. 1997), experiments have relied on specific chemical inhibitors. The herbicide fosmidomycin, which inhibits 1-deoxy-D-xylulose-5-phosphate synthase of the MEP pathway (Zeidler et al. 1998), was used to study the physiological function of isoprene. Fosmidomycin feeding rapidly inhibit isoprene emission almost without altering photosynthesis (Sharkey et al. 2001; Loreto and Velikova 2001), making the use of fosmidomycin for helping to understand the role of isoprene emission in plant defense. A significant reduction of plant thermotolerance after fosmidomycin application was documented in several studies. The negative changes in photosynthesis were stronger in isoprene-inhibited leaves (Velikova et al. 2006, 2011) after exposure to heat. Ozone fumigation resulted in a higher accumulation of H_2O_2 and lipid peroxidation in isoprene-inhibited leaves (Loreto and Velikova 2001; Velikova et al. 2005), accumulating higher amounts of NO (Velikova et al. 2005). Higher NO emission, H_2O_2 production, and lipid peroxidation level were also detected in fosmidomycin-fed black poplar leaves exposed to singlet oxygen compared to isoprene-emitting leaves (Velikova et al. 2008). These results suggest that isoprene might be an effective mechanism to control ROS and RNS formed under abiotic stress conditions.

The development of transgenic plants has opened new perspectives in studying the role of biogenic isoprene in plant protection against stress. Both approaches were used, either by the insertion of the *isoprene synthase* (*ISPS*) gene in *A. thaliana* (Sharkey et al. 2005; Sasaki et al. 2007; Loivamäki et al. 2007) and in tobacco plants (Vickers et al. 2009b), or by knocking-down the natural isoprene emission in poplar plants (Behnke et al. 2007).

Since biogenic isoprene is a component of different biosynthetic pathways, it could be expected that genetically manipulated plants may have altered phenotypes and regulation of pathways competing with isoprene biosynthesis. *A. thaliana* plants overexpressing poplar *ISPS* gene were characterized by enhanced growth of the rosettes, higher dimethylallyl diphosphate level, and ISPS activity compared to wild type (Loivamäki et al. 2007). However, isoprene production did not have any gross effects on plant morphology in isoprene-emitting transgenic tobacco grown under control conditions (Vickers et al. 2009b). Moreover, no effect of isoprene was observed on physiological and biochemical traits in tobacco when plants were well-watered (Tattini et al. 2014). There were no significant differences in photosynthetic parameters, chlorophyll fluorescence, the concentration of chlorophylls, and total violaxanthin cycle pigments (Vickers et al. 2009b; Tattini et al. 2014), as well as in the level of ABA, starch or phenylpropanoids (Tattini et al. 2014). Genetically modified poplar trees that did not emit a significant amount of isoprene had similar biomass production and photosynthesis compared to the emitting trees when grown in plantations under field conditions (Monson et al. 2020). However, a dramatic reduction of carbon fluxes throughout the MEP has been demonstrated in isoprene suppressed poplar (Ghirardo et al. 2014). Under these conditions, a reallocation of carbon to another pathway, which induces profound metabolic changes was observed (Way et al. 2013; Kaling et al. 2015). Poplar lines with substantially reduced isoprene emission rates showed decreases in flavonol pigments but increases in the gene expression of carotenoids and terpenoids synthesis (Monson et al. 2020). The absence of isoprene emission in transgenic poplar trees generated a new transient chemo(pheno)type with suppressed production of phenolic compounds (Behnke et al. 2010b). Detailed analysis of transgenic poplar revealed wide metabolome and proteome rearrangements when comparing isoprene-emitting and isoprene-suppressed plants. Under optimal conditions, non-isoprene emitting poplars showed higher content of AsA (Behnke et al. 2009, 2010a), α-tocopherol, and de-epoxidation ratio of xanthophylls was enhanced (Behnke et al. 2009). Systemic changes in flavonoids, sterols, and metabolites of the carbon fixation were also recognized in those plants (Behnke et al. 2013). The absence of isoprene in poplar leaves triggers the rearrangement of the chloroplastic proteins (Velikova et al. 2014). The authors reported increased levels of histones and ribosomal proteins, and down-regulation of photosynthesis light reactions proteins, redox regulation, and defense against oxidative stress. Moreover, analysis of the whole proteome highlights some rearrangement of proteins and enzymes involved in photosynthesis, glycolysis, and the tricarboxylic acid cycle, as well as redox regulation, and protein translation (Vanzo et al. 2016). Analysis of lipid composition revealed differences in the double bound index between the isoprene-emitting and non-emitting poplar suggesting more pronounced changes in membrane structures when no isoprene was present (Behnke et al. 2013). Moreover, a lower level of unsaturated fatty acids, especially linolenic acids, in non-isoprene-emitting chloroplasts, was associated with fluidity reduction in thylakoid membranes, which negatively affects the photosystem II photochemistry efficiency (Velikova et al. 2015). Several modifications in the ultrastructure of chloroplasts were also related to the isoprene depletion (Velikova et al. 2015). The dissipation of energy excess is necessary. To evaluate this feature, non photochemical quenching (NPQ) was monitored in poplar leaves with genetically altered isoprene biosynthesis (Behnke et al. 2007; Velikova et al. 2015).

Important evidence for a wide cellular role of isoprene was obtained from the studies on transcriptional control of gene expression, using poplar, tobacco, and Arabidopsis plants with altered isoprene phenotypes. RNAi silencing of *ISPS* (Behnke et al. 2007) reduced the expression of different genes related to the shikimate and phenylpropanoid pathways, implying an important role for isoprene biosynthesis (Behnke et al. 2010a). Moreover, transgenic suppression of ISPS produced a reduction in several proteins that are associated with these two pathways (Monson et al. 2020). Transcriptomic analysis in *A. thaliana* and tobacco plants were also applied to explore the isoprene role as a standing component of the plant genotype and to understand how isoprene interacts with "*naïve*" metabolism, and what are the downstream changes in the higher terpenoids synthesis (carotenoids, monoterpenes, sesquiterpenes, tocopherols, cytokinins, gibberellic acid). Several genes that are crucial for photosynthesis, phenylpropanoid biosynthesis, and plant growth were up-regulated by isoprene in both *A. thaliana* and tobacco plants manipulated to emit isoprene as a natural metabolite (Zuo et al. 2019). Contrary to the observation for the shikimate and phenylpropanoid pathways, isoprene presence in poplar and transgenic tobacco reduced the expression of terpene biosynthesis-related genes and proteins (Zuo et al. 2019; Monson et al. 2020), indicating interspecific differences. Several proteins involved in carotenoid and ABA biosynthesis were at lower abundance in isoprene-emitting poplar lines, compared to non-emitting lines (Monson et al. 2020), and a similar pattern was observed in the tobacco system (Zuo et al. 2019). Reduced level of zeaxanthin, but a higher de-epoxidation ratio and lower AsA content in isoprene-emitting poplar lines compared to non-emitting ones were reported (Behnke et al. 2009). Potential interaction between isoprene and oxylipin pathway, a pathway which is initiated in the chloroplasts and produces C-6 aldehydes, alcohols, and esters that belong to wide group called green leaf volatiles (Hatanaka et al. 1987), has been also suggested. Indeed, the isoprene presence in poplar and tobacco plant systems increases the expression in several *LOX* (lipoxygenase) genes, increasing the production of green leaf volatiles (Behnke et al. 2010a; Zuo et al. 2019; Monson et al. 2020). It was also shown that isoprene affects the expression of genes that improve plant tolerance to a variety of environmental stress factors (Zuo et al. 2019). A strong relationship between gibberellic and jasmonic acid signaling pathways and isoprene presence has been revealed by gene expression analysis (Lanz et al. 2019).

Signaling advantages of the isoprene molecule might be conferred by its hydrophobic nature. Based on its solubility in lipids, it is assumed that isoprene can cross different cellular membranes and may influence different pathways in different cell organelles, and may interact with signaling components in the hydrophobic domain of the lipid bilayer membrane (Monson et al. 2021). Several studies suggested that isoprene can modulate ROS and RNS production through an unknown mechanism (Velikova and Loreto 2005; Velikova et al. 2008, 2012; Behnke et al. 2010a; Vanzo et al. 2016), and thus may affect cellular redox signaling. Recent multi-omic analyses indicated that isoprene exerts a broader role in initiating changes in gene expression, protein abundance, and plant defense compounds' production involved in stress tolerance. Genetic transformation to introduce or silence *ISPS* causes cellular modifications that affect several transcription factors that are important in signaling processes of the shikimate, phenylpropanoid, terpenoid, and oxylipin biosynthetic pathways (Monson et al. 2021).

4.6.3 Consequences of Genetically Engineered Isoprene Production for Plant Stress Responses

The general positive effect of isoprene in various stressful conditions was documented (Loreto and Schnitzler 2010). Heat tolerance of transgenic Arabidopsis overexpressing *ISPS* was enhanced (Sasaki et al. 2007) due to the thermostability of the light-harvesting complex of PSII in the stacked regions of grana thylakoids, and was able to increase the primary photochemistry efficiency of PSII at higher temperatures, suggesting some changes in the lipid bilayer membrane of the thylakoid (Velikova et al. 2011). On the other side, engineered poplar with negligible isoprene emission was also more sensitive to high temperature and light (Behnke et al. 2007, 2013; Way et al. 2011). Isoprene-emitting transgenic *A. thaliana* exposed to heat stress produced a lower pool of ROS and RNS, which was correlated with a reduction of H_2O_2 in isoprene-emitting plants (Velikova et al. 2012). Droughted transgenic tobacco genotypes with a constitutive promoter attached to the *ISPS* from *Populus alba*, showed no increase in lipid peroxidation and ROS content and were able to maintain a higher photosynthesis rate under mild to moderate drought better than the control non-emitting plants (Ryan et al. 2014). A comprehensive study clearly demonstrated that de-epoxidated xanthophylls, abscisic acid, soluble sugars, and phenylpropanoids were significantly higher in isoprene-emitting tobacco subjected to severe drought, and after re-watering, these plants maintained higher levels of metabolites than non-emitting genotypes (Tattini et al. 2014). These authors have suggested that isoprene-emitting plants can up-regulate the production of phenylpropanoids and non-volatile isoprenoids, which may protect leaves against different stress conditions. Isoprene-emitting tobacco plants were highly resistant to ozone oxidative damages compared to non-emitting controls (Vickers et al. 2009b). These plants accumulated less toxic ROS, their antioxidant level was higher, and showed a decrease in foliar damage and an increase in the photosynthesis rate. Isoprene presence in poplar leaves affected ozone-induced changes in NO emission (Vanzo et al. 2016). It was demonstrated that isoprene-emitting poplar ozone fumigation slightly stimulated NO production, while non-emitting poplar leaves released a significant amount of NO, thus triggering important changes in the pattern of the S-nitroso-proteome (Vanzo et al. 2016). Some proteins that are related to light and dark photosynthesis reactions, the Krebs cycle, protein metabolism, and redox regulation were the main targets of NO action in non-isoprene-emitting poplar plants. Moreover, some proteins involved in the detoxification of ROS showed increased S-nitrosation in non-emitting leaves. These results suggest that isoprene plays an indirect role in regulating the formation of ROS via the control of the S-nitrosation levels of the enzymatic ROS processing systems. Exposure to high doses of UV-B caused a similar decline in photosynthesis and PSII performance in both transgenic isoprene-emitting and non-emitting tobacco (Centritto et al. 2014). However, these authors reported that after the stress conditions, photosynthesis and PSII was recovered only in isoprene-emitting tobacco and this was associated with an increase of the antioxidant contents, suggesting that isoprene together with the antioxidants contributed to restore the photosynthetic apparatus upon exposure to UV-B radiation (Centritto et al. 2014). Remarkably, UV-induced metabolome adjustments were detected in transgenic poplar trees (Kaling et al. 2015). The development of phenotypic differences between isoprene-emitting and non-emitting poplar upon exposure to UV-B radiation was explained by the anthocyanin and proanthocyanidin pathways activation. Reduced accumulation in non-isoprene-emitting plants showed a decrease in phenolic compounds content, suggesting metabolic- or signaling-based interactions between isoprenoid and phenolic pathways (Kaling et al. 2015).

4.6.4 Contribution of Biogenic Isoprene to Plant Adaptation and Resilience in Current and Future Climate

Unambiguous experimental evidence has been provided that isoprene emission is a trait with a positive value, especially concerning protecting photosynthesis under stressful conditions. A logical question arises: Is there a broader adaptive scope of this trait? Many plants, approximately 20% of the perennial vegetation of tropical and temperate regions, constitutively emit isoprene (Loreto and Fineschi 2015). The capacity of plants to synthesize isoprene has been gained and lost many times during the course of evolution. Monson et al. (2013) believe that this trait has been conserved only in environmental conditions where isoprene benefits plant fitness. However, Dani et al. (2014) suggest that isoprene emission capacity arises under extensive speciation of genera. Sharkey et al. (2013) discuss ecological and biochemical reasons for the occurrence of isoprene emission. According to the "opportunistic hypothesis" (Owen and Peñuelas 2005) isoprene emission capacity is reduced when carbon shunted in the MEP pathway is needed for the production of more effective antioxidants and pigments, especially under severe stress conditions. Indeed, Beckett et al. (2012) demonstrated that isoprene protection occurs at the early stages of drought when the electron transport rate still drives the photosynthetic process and carbon fixation into volatile isoprenoids. Under severe drought, these authors demonstrated that when photosynthesis was ceased and isoprene emission was undetectable, zeaxanthin and lutein increased, suggesting a well-coordinated defense mechanism against drought, driven by volatile and non-volatile isoprenoids (Beckett et al. 2012).

4.7 Conclusions

A common "pathway" of three different gasotransmitters (ethylene, NO and isoprene) can be established. Isoprene is the most abundant biogenic volatile hydrocarbon compound that is naturally emitted by many plant species and it plays an important role in the chemistry of the atmosphere and, therefore, in air quality. NO is considered another air pollutant, but the most important source is diesel combustion (Hiroyasu and Kadota 1976).

The interplay between biogenic isoprene and NO with other molecules in plant metabolism is mainly related to stress conditions. Their interaction with enzymatic and non-enzymatic systems helps to alleviate ROS effects, improving physiological processes such as photosynthesis in plants suffering from stress conditions. The combination with other gasotransmitters that have hormonal action, such as ethylene, can even potentiate the beneficial effects on the plant metabolism. A new scheme for plant physiology research is needed to undergo the effects of the interactions of different molecules in a complex network, as can be seen in the metabolic pathways, including redundant or alternative routes under oxidative stress conditions (Fig. 4.2).

Although considerable advances in research on isoprene functioning in plants have been obtained, as far as the investigations regarding NO and ethylene effects, there is still a clear requirement for knowledge about precise signaling mechanisms to provide new insights into stress mitigation under scenarios of future challenging environments. This could be the base for future genetic engineering development.

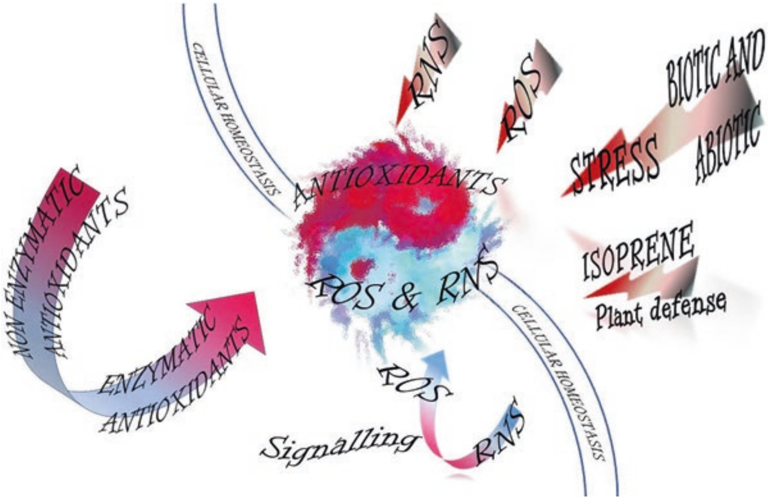

Fig. 4.2 Cellular Homeostasis in plants. The balance between ROS and RNS and the antioxidant systems as a counterpart. Stress conditions also can produce RNS and ROS, unbalancing the redox cellular equilibrium. Enzymatic and non-enzymatic antioxidants act together in the redox balance. RNS and ROS also act as signal molecules in many physiological processes

Acknowledgments Our work is supported by the National Scientific and Technical Research Council (grant PIP 2021–2023 11220200103019CO CONICET) and the National University of La Plata (grant 11/A337 and 11/A322) (Argentina). LS is a fellow student of the Faculty of Agricultural and Forestry Sciences UNLP, MA is a postdoctoral student of CONICET, AG, CGB and GEGG are carrier researchers of CONICET and VV is a researcher of the Bulgarian Academy of Sciences. The authors also would like to thank the editors for considering our chapter as part of this book.

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Chapter 5
Alleviation of Plant Stress by Molecular Hydrogen

John T. Hancock, Tyler W. LeBaron, Jennifer May, Adam Thomas, and Grace Russell

Abstract Gasotransmitters and gaseous-signaling molecules are hugely important for controlling cell function and especially so during stress challenges. Past research has concentrated on molecules such as nitric oxide (NO) and hydrogen sulfide (H_2S), although others such as ethylene and carbon monoxide (CO) are also important. Here, molecular hydrogen (H_2) is added to the mix. H_2 has been shown to ameliorate responses to a range of stressors in plants, including exposure to heavy metals, salinity, extreme temperatures, and UV radiation. Clearly, H_2 is an important gas, which may be useful for enhancing plant growth and food security in the future. Exogenous treatments with H_2 are easy in the form of hydrogen-rich water (HRW), but there are still issues with its wide-spread use. Furthermore, the molecular basis of the action of H_2 in cells is still not clear. Here, aspects of the use and the action of H_2 in plants are discussed, along with what might be learnt from other species.

Keywords Heavy metals · Hydrogen sulfide · Molecular hydrogen · Nitric oxide · Salinity · Reactive oxygen species · Redox

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T. Aftab, F. J. Corpas (eds.), *Gasotransmitters Signaling in Plants under Challenging Environment*, Plant in Challenging Environments 5,
https://doi.org/10.1007/978-3-031-43029-9_5

5.1 Introduction

In 1987, work in animals showed that endothelial-derived relaxing factor (EDRF) was in fact the gas nitric oxide (NO) (Palmer et al. 1987). This opened the door to studies not only on reactive nitrogen species (RNS) in biological systems, but also observations on other physiological gasotransmitters. Such analyses also sparked work on other small reactive compounds which could be involved in cell signaling, including nongaseous reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) (Veal et al. 2007), and reactive sulfur compounds such as hydrogen sulfide (H_2S) (Aroca et al. 2018). The year 2019 marked the fortieth anniversary of NO studies in plants (Klepper 1979; Kolbert et al. 2019), but more recently a new player has been added to the list, molecular hydrogen (H_2) (Wilson et al. 2017), which can alter plant cell activity (for example, Chen et al. 2017a), and may play a role in stress responses. As plants are sessile, they require convoluted strategies to overcome a range of stress challenges, which include exposure to UV light (Hideg et al. 2013), heavy metals (Morkunas et al. 2018), extreme temperature, both high (Niu and Xiang 2018) and low (Lyons 2012), drought (Farooq et al. 2009), flooding (Loreti et al. 2016), and salinity (Fahad et al. 2015).

The strategy for plants when under stress is to induce signal transduction pathways, which often lead to altered gene expression, and hence the complement of cellular proteins, enabling enhanced or new activities. Such actions are allowing the cells to manage the current stress, or even future stress challenges. The signaling invoked in plants involves a range of phytohormones (Khan et al. 2012), but it also involves numerous gasotransmitters, which are important mediators in other organisms as well. These include NO (Nabi et al. 2019), H_2S (Pandey and Gautam 2020), carbon monoxide (CO; Cui et al. 2012) and ethylene (Debbarma et al. 2019). Plant stress has a major impact on plant growth and productivity, and gasotransmitters are instrumental in the responses mounted by plants. Often there is an interaction and/or co-ordination of the signaling mediated by such molecules (Hancock and Whiteman 2016; Singh et al. 2020; Bhuyan et al. 2020). Here, the interactions of H_2 with other gaseous signaling molecules are discussed, with the focus on how H_2 alleviates plant stress.

5.2 H_2 Treatment of Plants

Hydrogen gas is hard to administer to plants. In mammals, hydrogen gas mixtures can be inhaled, and there are many examples of its use (Ge et al. 2017; Wu et al. 2019a, b), including in the treatment for COVID-19 (Chen et al. 2021a; Russell et al. 2021). However, the gas is highly flammable, raising safety issues, and is lighter than air, so H_2 will rapidly disperse into the upper atmosphere, making treatment of ground-level plants unpragmatic. Therefore, treatment of plants often involves the creation and diluting of a saturated solution of hydrogen in what is referred to as hydrogen-rich water (HRW). However, H_2 is not very soluble (Molecular Hydrogen Institute n.d.; Wilhelm et al. 1977) and will rapidly revert to the gaseous phase and be lost. This then may necessitate a frequent re-application of HRW to the plant tissues, either directly onto the leaves, or into the root feed water, to illicit an effect. However, as can be seen in Table 5.1, there are many examples of

Table 5.1 Examples of the use of hydrogen-rich water (HRW) in alleviating plant stress

Stress agent/ Conditions	Species used	Effects seen/comment	Reference
Aluminum	Maize seedlings	Alleviation of stress	Zhao et al. (2017)
	Alfalfa	Alleviated effects on root growth	Chen et al. (2014)
Cadmium	*Medicago sativa*	Alleviation of toxicity	Cui et al. (2013)
	Brassica chinensis L.	Reduced cadmium uptake	Wu et al. (2019b)
	Chinese cabbage	Reduced cadmium uptake	Wu et al. (2015)
	Brassica campestris ssp. chinensis	Reduced cadmium uptake	Wu et al. (2020a)
	Brassica chinensis and *Arabidopsis thaliana*	Mediated by iron-regulated transporter 1 (IRT1) and zinc-regulated transporter protein 2 (ZIP2)	Wu et al. (2021)
Mercury	Alfalfa	Tolerance to toxicity	Cui et al. (2014)
Salinity	Barley	Alleviation of stress	Wu et al. (2020b)
	Rice	Alleviates stress during germination	Xu et al. (2013)
UV-B	*Medicago sativa*	Alleviated stress	Xie et al. (2015)
UV-A	Radish sprouts	Anthocyanin biosynthesis	Zhang et al. (2018)
	Radish sprouts	Anthocyanin biosynthesis and ROS metabolism	Su et al. (2014)
Heat	Cucumber	Several parameters altered, including gas exchange, chlorophyll fluorescence, and antioxidant activities	Chen et al. (2017a)
Paraquat induced oxidative stress	*Medicago sativa*	Mediated by heme oxygenase (HO-1)	Jin et al. (2013)
Post-harvest	Lilly and rose	Increased vase life	Ren et al. (2017b)
	Kiwifruit	Delayed ripening	Hu et al. (2014)
	Mushroom *Hypsizygus marmoreus*	Enhanced antioxidant capacity and reduced postharvest senescence	Chen et al. (2017b)

the use of HRW in plants. With such a range of responses, including to heavy metals, temperature stress and light stress it is clear that plants can perceive and react to the presence of H_2 or HRW. Interestingly, one of the potentially significant uses of H_2 application is in post-harvest, where it may be useful to prolong storage of crops, particularly fruits (Hu et al. 2014) and flowers (Ren et al. 2017b). A new twist on the use of HRW is the formation of hydrogen nanobubble water (HNW) (Li et al. 2021b). This is suggested to increase the solubility of H_2 and prolong H_2 delivery.

For H_2 usage to be useful in practice, new and easier-to-use applications for the delivery of H_2 may need to be developed. These may come from disparate industries (Mayorga et al. 2020), for example, one potential donor is magnesium hydride (MgH_2) (Li et al. 2020b), a compound proposed for use in the solar-energy sector (Mathew et al. 2021). The kinetics of release of H_2 are slower and more sustained than just using HRW, but it was found to be more efficient when used in a citrate buffer. Another recently used compound for releasing H_2 in plants is AB@hMSN, an ammonia borane-loaded hollow mesoporous silica nanoparticle (Wang et al. 2021). However, there is a caveat here. If donor molecules are used, they are likely to leave behind by-products, and this could severely compromise the biologically safe use of H_2.

5.3 Molecular Targets of H_2

The hydrogen molecule is extremely small (relative to other signaling molecules) and relatively inert. Therefore, it is difficult to envisage how it is perceived by cells and acted on. Classical hormone-type signaling, for example with chemokines (D'Ambrosio et al. 2003), would use a protein receptor, but this is unlikely with a molecule such as H_2. Some signaling molecules, such as NO, will react with proteins, either through the prosthetic groups or via reacting with thiol groups (Feng et al. 2019). However, again, it is hard to see how this type of reaction would apply to H_2 because unlike NO, which is polar and a reactive free radical, H_2 is non-polar and not reactive. Therefore, other mechanisms must exist to account for the biological effects seen with H_2 administration.

One of the main thrusts of the argument regarding H_2 action is that it affects the antioxidant levels in cells. Many of these effects are indirect, with expression or accumulation of enzymes involved in the antioxidant capacity of the cell being altered (for example Zhao et al. 2017; Chen et al. 2017b). However, this can only happen if there is a direct perception of the H_2 molecule, and usually that is the aspect that is skirted in the literature.

It was reported that H_2 does have direct effects as an antioxidant by reacting with hydroxyl radicals ($\cdot OH$) but not with other ROS, such as the superoxide anion ($O_2\cdot^-$) or H_2O_2 (Ohsawa et al. 2007). $\cdot OH$ are known to be involved in plant stress responses, such as during heavy metal challenge (Cuypers et al. 2016), paraquat treatment (Babbs et al. 1989), and chilling and drought stresses (Shen et al. 1997). Therefore, the removal of $\cdot OH$ by a radical scavenger, suggested here to be H_2, could account for some of the effects seen. This being said, a later paper has suggested that a close investigation of the kinetics of this reaction does not support this notion (Penders et al. 2014), and in fact, it was suggested that the ·OH would react with other biomolecules before H_2, so that the effects of ·OH would not be mitigated against by H_2 addition. In a similar way, a second direct target was suggested to be the peroxynitrite molecule ($ONOO^-$). This would be produced by the reaction of superoxide ($O_2\cdot^-$) with NO, and as both are temporally and spatially produced together during stress responses, the presence of $ONOO^-$ is very likely. If H_2 removes $ONOO^-$, this could account for the effects seen. However, a close examination of the kinetics again, seems to rule out $ONOO^-$ as a direct H_2 target (Penders et al. 2014; LeBaron et al. 2019a).

With both ·OH and $ONOO^-$ being ruled out, it was suggested that a possible target could be the ferric (Fe^{3+}) ion (Penders et al. 2014). This would not be out of kilter with what has been reported for other gasotransmitters. One of the main actions of NO is the activation of soluble guanylyl cyclase (sGC) by a direct interaction of the NO with the heme prosthetic group of the enzyme (Xiao et al. 2019). With a foray into this area (Penders et al. 2014), the reduction of the iron by H_2 in myoglobin, cytochrome P450 and putidaredoxin was investigated, but it was concluded that there was no reduction of heme or iron-sulfur (Fe/S) clusters in these proteins. However, with a redox midpoint potential of −414 mV [relative to the Standard Hydrogen Electrode (SHE)], H_2 could thermodynamically reduce a range of heme groups in a variety of enzymes, and this is suggested as a focus of future investigation (Hancock et al. 2021). As discussed, enzymes such as the NAPDH oxidase homologues would be particularly interesting as they are known to be involved in a range of stress responses (for example, He et al. 2017). It is not inconceivable that sGC may be an H_2 target too. Clearly much more work is needed here, using a wide range of plant proteins which contain heme or Fe/S prosthetic groups, before such a mechanism can be ruled out. Nevertheless, there may need to be some caution here, as it cannot always be assumed that signaling pathways determined in animal systems are the same in plants. For example, the action of NO on a sCG has been thrown into doubt in plants (Astier et al. 2019). Therefore, the action of H_2 may be different too, although the use of other biological systems to advance plant science is a powerful tool, as discussed below.

Several effects of H_2 have been reported to be mediated by the enzyme heme-oxygenase (HO-1) (Jin et al. 2013; Lin et al. 2014). This enzyme catalyzes the breakdown of heme in a reaction which (1) involves oxygen, (2) uses NADPH as a cofactor and (3) produces biliverdin, CO and iron ions (Wilks 2002). However, the exact reaction with H_2 has yet to be reported, so it may be a consequence of downstream signaling which is yet to be determined. Another enzyme thought to mediate H_2 effects is glutathione peroxidase, an enzyme instrumental in the maintenance of intracellular redox. By the use of genetically deficient strains and inhibitors, it was shown that glutathione peroxidase was needed to mediate H_2 action in the *Ganoderma lucidum* fungus (Ren et al. 2017a). The enzyme is a selenium containing protein, making this an interesting potential H_2 target, unless the direct action of H_2 is upstream of the enzyme itself.

Table 5.2 Possible molecular targets and action of H_2

Molecular target proposed	Comment(s)	Reference(s)
Hydroxyl radical (·OH)	Kinetics do not support this mechanism	Ohsawa et al. (2007) and Penders et al. (2014)
Peroxynitrite ($ONOO^-$)	Kinetics do not support this mechanism	Ohsawa et al. (2007) and Penders et al. (2014)
Fe^{3+} ion	A range of heme groups could potentially be targets	Penders et al. (2014) and Hancock et al. (2021)
Heme oxygenase (HO-1)	No direct interaction reported	Jin et al. (2013) and Lin et al. (2014)
Glutathione peroxidase	Mediated effects in fungus	Ren et al. (2017a)
Spin states	Possible direct interaction, but not experimentally substantiated	Hancock and Hancock (2018)

Lastly, it has been suggested that because H_2 has two spin states that this could be a way for H_2 to influence other biomolecules (Hancock and Hancock 2018). However, to date, there is no experimental evidence of this.

As yet, no definitive mechanism of how H_2 interacts directly with biological systems has been identified, although several mechanisms have been suggested (Table 5.2). Therefore, much more work needs to be undertaken in this area. Despite this there clearly are effects in plants (Table 5.1), and this phenomenon can be exploited in the absence of a molecular mechanism, particularly as there appear to be no reports that H_2 application is harmful to neither plants nor animals. No H_2 mechanisms seem to leave by-products and so there seems to be no ramifications for food safety.

5.4 Signaling and Effects of H_2

Cell signaling events in plants, as with all species, is crucial for the organism to thrive and to survive stress challenges. The perception of an external signal, perhaps a biotic or abiotic stress, and the signal transduction pathway, leading to a response, involves a range of proteins and small molecules, and instrumental in many of these pathways are the small relatively reactive gasotransmitters, such as NO (Nabi et al. 2019) and H_2S (Pandey and Gautam 2020). Although, as discussed, it is hard to envisage how H_2 may have a direct interaction and effect on polypeptides, there is a body of evidence that shows that H_2 interacts, or has effects on, signaling events that involve other gasotransmitters and small redox compounds. Some of the evidence is discussed below.

5.4.1 Nitric Oxide, Stress and Hydrogen Gas Treatment

It has been known for several decades that NO is produced by plants and has a profound effect on controlling plant function (Kolbert et al. 2019). There is no doubt that NO has a central role in controlling cell function (Kumar and Pathak 2018), whilst more recently, it has been found that H_2 interacts in the NO pathways.

Decreased NO generation was reported when HRW was used to alleviate aluminum stress in alfafa (Chen et al. 2014). Fifty percent saturated HRW reduced the effects of a NO donor, suggesting that NO may mediate H_2 effects. In contrast, H_2 increased the NO production in tomato seedlings when root growth was being investigated. This was reduced by the NO scavenger 2-4-carboxyphenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO), which suggests that H_2 was not directly scavenging NO. The conclusion was that auxin-induced H_2 generation was then mediated by NO production from the enzyme nitrate reductase (NR) (Cao et al. 2017). Similar results were reported with cucumber, where HRW increased root growth and NO accumulated. Both HRW and NO increased the expression of cell cycle genes: *CycA* (A-type cyclin); *CycB* (B-type cyclin); *CDKA* (cyclin-dependent kinase A); and *CDKB* (cyclin-dependent kinase B). The effects were reduced by inhibitors of NR and nitric oxide synthase (NOS)-like enzymes, and NO scavengers (Zhu et al. 2016). NO also mediated root growth induced by H_2 in cucumber, where downstream proteins were identified as a plasma membrane H^+-ATPase and 14-3-3 proteins (Fu et al. 2000; Mhawech 2005; Li et al. 2020a, b). The latter being key regulatory proteins of such intracellular signaling cascades as mitogen activated protein kinase (MAPK) and p53. The enzyme NR was also found to be involved in NO generation when root formation was induced by a H_2 releasing donor AB@hMSN (Wang et al. 2021).

H_2 has the potential to be useful for postharvest storage of plant materials. One percent HRW [2.2 μM H_2] (calculated from the authors' information) and sodium nitroprusside (SNP: 150 μM) improved vase-life of cut lilies and these effects were reduced when NO was removed. It was also found, in a study of the genes expressed, that the chloroplast ATP synthase CF1 alpha subunit (AtpA) may be important in mediating these effects (Huo et al. 2018). Furthermore, nitrate accumulation was reduced in tomatoes by H_2, and this may have implications for the way fruits are stored (Zhang et al. 2019).

It is clear therefore, that H_2 has effects on, and is mediated, by NO metabolism, and it appears that this is not due to a direct scavenging of NO by H_2, which would be in line with what was previously reported (Ohsawa et al. 2007). However, H_2 may have effects through ROS too, which may also impinge on NO metabolism. To exemplify, abscisic acid (ABA) induced the accumulation of H_2 in *Arabidopsis thaliana*, which led to better drought tolerance. However, the effects also involved ROS and NO accumulation, with the enzymes NR and NADPH oxidase being used. In fact, it was found that the promotion of NO accumulation by H_2 was dependent on ROS production, showing what a complex and interdependent system H_2 is involved in (Xie et al. 2014).

5.4.2 *Reactive Oxygen Species, Antioxidants and Hydrogen Gas*

It is clear that ROS metabolism needs to be considered when the effects of H_2 are in question, especially as H_2 may have antioxidant and pro-oxidant effects (LeBaron et al. 2019b).

Even though the direct scavenging of $O_2{\cdot}^-$ and H_2O_2 were ruled out (Ohsawa et al. 2007), and the scavenging of ·OH was also cast into doubt (Penders et al. 2014), many reports suggest that H_2 has affects in plants through the modulation of the antioxidant capacity of cells. The postharvest treatment of Chinese chive with H_2 reduced oxidative damage and increased the activity of several antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POD) and ascorbate peroxidase (APX) (Jiang et al. 2021), resulting in an increased shelf life of the chives. Oxidative stress in *Medicago sativa* was also alleviated by HRW following UV-B exposure, and this too was mediated by changes in antioxidants, particularly flavonoids (Xie et al. 2015). HRW also allowed better tolerance to light stress in *Zea mays*, again mediated by antioxidant enzymes (Zhang et al. 2015). These included SOD, CAT and APX, which reduced the accumulation of $O_2{\cdot}^-$ and H_2O_2.

The tolerance bestowed on plants by H_2 administration to other stress challenges is also mediated by antioxidants. This includes aluminum tolerance in maize, where HRW altered the cellular levels of CAT, APX, SOD, and POD (Zhao et al. 2017). In rice seedlings exposed to cold stress, the SOD levels were altered, which appeared to be mediated by changes in the miRNA levels, in particular miR-398 transcripts. The authors suggested that this was imperative to maintaining the redox homeostasis of the cells (Xu et al. 2017b).

The changes in antioxidant activity observed in cells will not only relieve the tangible aspects of oxidative stress, as seen with less lipid peroxidation and protein oxidation, but it will also be part of the system which maintains the redox poise of the cell, which will be part of the complex interplay used in signaling (Shao et al. 2008). Lowering ROS will mean that reactions with NO will potentially be reduced, and so reducing the production of $ONOO^-$, which acts as a downstream signaling molecule of NO (Speckmann et al. 2016). It is known that ROS will also act on glutathione, a molecule instrumental in maintaining the cellular redox balance. It has been suggested that the redox of a cell is carefully kept in a “Goldilocks zone” (Alleman et al. 2014), and therefore any changes in intracellular redox molecules will feed into this. A good example of how such redox active molecules interact to give the effects in plants is seen with the legume–*Rhizobium* symbiosis system (Pauly et al. 2006), where GSH, NO and ROS were studied.

5.4.3 Hydrogen Gas and Ethylene Signaling

One of the most well-known gasotransmittters is ethylene (C_2H_4; $H_2C{=}CH_2$). It is involved in a range of physiological systems in plants, such as plant growth (Dubois et al. 2018), but is probably best known for its role in fruit ripening (Barry and Giovannoni 2007).

The interaction of ethylene with other gasotransmitters is not novel. For example, NO and ethylene has been reported to work together in the root development of cucumber (Xu et al. 2017a). Therefore, an interaction of H_2 and ethylene is no surprise. Postharvest senescence of rose flowers was reduced by H_2 application and this was mediated by changes in ethylene signaling. There was a reduction of substrates and biosynthetic enzymes: 1-aminocyclopropene-1-carboxylate (ACC); ACC synthase (ACS); and ACC oxidase (ACO). Gene expression of *Rh-ACS3* and *Rh-ACO1* transcripts, encoding biosynthesis enzymes, was also reduced. Interestingly, expression of the ethylene receptor, *Rh-ETR1*, was increased (Wang et al. 2020). These data clearly show that there is an influence of H_2 on ethylene metabolism and concomitant signaling.

A proteomic study also showed that H_2 and ethylene cooperated in signaling (Huang et al. 2020). Using cucumber roots as the model system, it was shown that inhibitors of ethylene signaling, $AgNO_3$ and aminoethoxyvinylglycine (AVG), reduced the adventitious root development induced by H_2 treatment. The proteomic analysis, using 2D-gel electrophoresis coupled with mass spectroscopic analysis, showed that HRW induced the up-regulation of nine proteins and the down-regulation of fifteen. The authors concluded that ethylene was downstream of H_2 and that six proteins were worthy of note and were probably mediating H_2 effects. These were RuBisCO, oxygen-evolving enhancer protein (OEE1), sedoheptulose-1,7-bisphosphatase (SBPase), threonine dehydratase (TDH), cytosolic ascorbate peroxidase (cAPX), and protein disulfide-isomerase (PDI).

5.4.4 Hydrogen Gas and Hydrogen Sulfide Signaling

H_2S is recognized as being toxic (Truong et al. 2006), but it is also now accepted as being a therapeutic gasotransmitter controlling key events in physiology and cell function (Wang 2003; Gadalla and Snyder 2010). However, as with the other small reactive compounds, H_2S does not act alone but is part of the complex interaction in which these molecules partake. It has been suggested that H_2S may act as a brake on some of the other signaling pathways (Hancock and Whiteman 2014). Alongside this, H_2S has also reported to be part of the H_2 signaling taking place in cells.

With the expression in Arabidopsis of a hydrogenase gene from *Chlamydomonas reinhardtii* (*CrHYD1*), which leads to H_2 biosynthesis, it was shown that endogenous H_2 was needed for osmotic stress tolerance in plants. Exposure to H_2 stimulated the production of H_2S and it was suggested that, to cause the modulation of the stomatal apertures, leading to the tolerance observed, H_2S was downstream of H_2 (Zhang et al. 2020a). A similar result was found with cut flowers. In a study of cut carnations, it was shown that a MgH_2 and citrate solution increased H_2S generation. The redox homeostasis was maintained whilst the expression of senescence genes was repressed (Li et al. 2020b). Hypotaurine, a H_2S scavenger, reversed the effects and it was suggested that the downstream effects of H_2 were mediated by H_2S, which is in line with the study on stomata (Zhang et al. 2020a).

It can be seen therefore, that H_2 is involved in the signaling pathways of a range of gasotransmitters, including NO, ethylene and H_2S.

5.5 What Might Be Learnt from Other Species

Working across the kingdoms of organisms can be rewarding, but it does come with some caveats. To exemplify, the characterization of the NAPDH oxidases from humans (Schröder 2020) has greatly helped advance the research on homologues of these enzymes in plants (Qu et al. 2017). Indeed, oxidase proteins from plants and animals could be combined to reconstitute activity *in vitro* (Desikan et al. 1996). On the other hand, the discovery of a NOS in animals (Bredt and Snyder 1990) has only led to controversy in plant science (Astier et al. 2018). Furthermore, the lack of a sGC signaling pathway in plants, so well characterized in animals, further emphasizes the caution that may need to be used (Astier et al. 2019). Having said that, deliberated below is how much can be learnt about the role of H_2 in biological systems by taking a broad approach.

If H_2 is able to enhance stress responses in plants, there needs to be an increase in the H_2 concentration in the relevant cells. This can be achieved via two mechanisms: either the endogenous production of H_2 can be increased, or the H_2 can be supplied exogenously.

Probably one of the most well-known endogenous biological systems for the production of H_2 is in the algae *Chlamydomonas* (Vargas et al. 2018). This organism is so good at generating H_2 that it has been suggested to be used as a biofuel (Scranton et al. 2015). Generation of H_2 is via a hydrogenase enzyme, and such mechanisms have been recently reviewed (Russell et al. 2020). If enzyme-based H_2 production can be increased in plants, either by the manipulation of the control of such enzymes, or by increasing the expression and relevant polypeptide accumulation, then targeted H_2 signaling can be used to enhance plant growth and survival. Model organisms such as *Chlamydomonas,* and then higher plant models such as *Arabidopsis*, will be instrumental in such work.

Alternatively, H_2 can be supplied exogenously. As discussed above, this might be from anthropogenic activity such as the application of HRW. However, plants, like many organisms, are likely to be in synergy or symbiotically with prokaryotes and fungi, which themselves can produce H_2. In humans, it has been suggested that increased H_2 production by gut microflora may enhance health (Ostojic 2020). Therefore, an increase in the prokaryotic production of H_2 around the root system of plants may have beneficial effects. On the other hand, H_2 oxidizing soil bacteria have also been shown to be beneficial (Zhang et al. 2020b). Manipulation of the soil bacterial flora therefore may be complicated but changing the H_2 metabolisms in the vicinity of the root system might have future benefits.

A study of bacteria may also help unravel how H_2 works. As the H_2 couple has a very reducing mid-point potential (−414 mV relative to SHE), then reduction of many protein prosthetic groups may be thermodynamically possible. This principle is exemplified by the reports on the reduction of cytochrome c_3 in *Desulfovibrio desulfuricans* (Peck 1959). Interestingly, following the redox reactions which may proceed downstream of this reduction, it was suggested that H_2S could be produced, which is known to be an important gasotransmitter in plants (Aroca et al. 2018), including under stress conditions (Singh et al. 2020), relevant to the discussion here. The study by Peck (1959) shows two important things. Firstly, the reduction of a heme group by H_2 is possible in biological systems. Secondly, once the heme is reduced there are possible downstream reactions which could potentially yield signaling molecules. As already mooted (Hancock et al. 2021), this needs to be explored further in plants and animals, not just in prokaryotes.

One of the biggest areas where other species can be useful to study is in the biomedical arena. Here, H_2 has been shown to have a benefit in a variety of diseases, including those listed in Table 5.3. H_2 has been found to relieve symptoms of COVID-19 and has been used for clinical trials (Guan et al. 2020). It has also been found to be of benefit in neurodegenerative disease (Chen et al. 2021b), rheumatoid arthritis (Yang et al. 2020) and diabetes (Yang et al. 2020). Therefore, it is clear that H_2 has a range of benefits for human health and for alleviating disease symptoms. Moreover, if mechanisms are known for H_2 action in the biomedical arena, can this be translated across and used in plant science?

It is not only the support that data such as that in Table 5.3 gives to the argument that H_2 has profound effects in biological systems, but it is the manner in which H_2 has its effects that is relevant here. Clearly, some of the effects and proposed mechanisms in animals are not directly relevant to plants. For example, a reduction in IL-6 levels or a dampening of a cytokine storm is not a mechanism which would be seen in plants. However, H_2 may have effects on analogous intercellular signaling molecules in plants, such as ethylene (Wang et al. 2020). Other effects may be much more relevant. As previously mentioned, H_2 may work through the action it has on antioxidants, an effect which has already been seen in plants. Accordingly, changes in antioxidants and a dampening of oxidative stress are a common feature in neurogenerative disease alleviation, the reductive effects on diabetes, cancer therapies, in mood alterations and in Hepatitis B (Ichihara et al. 2015). This is also a common feature of how H_2 alleviates plant stress (e.g., Zhang et al. 2015; Jiang et al. 2021). The biochemistry of animal and plant cells differs in detail but remains the same in principle. Therefore, a close study of the research on H_2 from the animal kingdom may be very beneficial to plant science in the future and vice versa. With the list of conditions for which H_2 may benefit human health, it is no surprise that H_2 has been mooted as a future therapy for humans (Ge et al. 2017; Wu et al. 2019a). With a focus on respiratory diseases at the present time because of the COVID-19

Table 5.3 Human diseases for which symptoms are alleviated by H_2 treatment

Disease/condition	Effect of H_2	Proposed molecular mechanism (if known)	Reference(s)
COVID-19	Severe symptoms alleviated	Dampens cytokine storm.	Russell et al. (2021), Hirano et al. (2021), Chen et al. (2021a), and Guan et al. (2020)
Neurodegenerative (e.g., Parkinson's disease, Alzheimer's disease)	Significantly improved scores assessed by the Unified Parkinson's Disease Rating Scale (UPDRS), or Alzheimer's Disease Assessment Scale-cognition cub-scale (ADAS-cog)	Reduces the loss of dopaminergic neurons and inhibits oxidative stress.	Chen et al. (2021b), Yang et al. (2020), Nishimaki et al. (2018), Ge et al. (2017), and Yoritaka et al. (2013)
Rheumatoid Arthritis	Reduced symptom severity	Relieves inflammation, possibly through reduction of IL-6-mediated responses.	Yang et al. (2020) and Ishibashi et al. (2014)
Ischaemia/reperfusion injury (e.g., stroke, brain trauma, cerebral infarction, cardiac arrest)	Significant increase in neurological improvement	Antioxidant, anti-inflammatory and anti-apoptotic effects. Inhibition of endoplasmic reticulum stress. Preservation of the blood-brain barrier and mitochondrial function.	Chen et al. (2021b) and Ono et al. (2017)
Metabolic syndrome and Type 2 Diabetes Melllitus	Improvement in urinary oxidative stress markers and cholesterol profile. Normalised oral glucose tolerance test	Decreases glucose and insulin levels. Stimulates energy metabolism. Increased urinary antioxidant superoxide dismutase enzyme. Reduced low-density-lipoprotein-mediated inflammation. Suppression of chemical modifications of serum lipoproteins in the plasma membrane	Yang et al. (2020), Ge et al. (2017), Song et al. (2013), Nakao et al. (2010), Suzuki et al. (2009), Kajiyama et al. (2008), and LeBaron et al. (2020)
Aiding anti-cancer therapy	Improving Quality-of-Life scores for radiotherapy patients	Radioprotection via antioxidant increase.	Ge et al. (2017) and Kang et al. (2011)
Mood disorders	Improved mood, anxiety and autonomic nerve function	Reduced accumulation of oxidative stress.	Chen et al. (2021b) and Mizuno et al. (2017)
Hepatitis B	May have potential to improve liver function and reduce viral DNA level	Reduction of oxidative stress.	Xia et al. (2013)

pandemic, the research and application of H_2 is likely to be of continued interest in the biomedical field (Russell et al. 2021). On the other hand, H_2 application has already been suggested to be hugely beneficial to agriculture (Zeng et al. 2014; Li et al. 2021a). The responses to H_2 are likely to be supported by common molecular mechanisms in plants and animals, be that through antioxidants or Fe^{3+} reduction, or other means. Plant science might have a lot to learn from the work being carried out on prokaryotes and higher animals, and vice versa.

5.6 Conclusions and Future Perspectives

It seems clear now that H_2 is a useful treatment for plants, alleviating a range of stress challenges (Table 5.1), as well as a potential regimen for the post-harvest storage of fruits and flowers, where it evidently delays senescence (Hu et al. 2014; Ren et al. 2017b). However, there are many aspects of the biochemistry of H_2 which are simply not clear. Firstly, it is not known what the direct targets of H_2 are in cells, even though several mechanisms have been suggested (Table 5.2), including scavenging radicals and other reactive signals, or acting through HO-1. Secondly, the full range of effects are not known, even though there are numerous reports of H_2 application being beneficial (Table 5.1).

The redox mid-point potential of H_2 is relatively low when compared to other biomolecules. Thermodynamically, it would be possible for H_2 to reduce Fe^{3+} to Fe^{2+} and this would have ramifications for many enzymes, suggesting the reduction of prosthetic groups, particularly many heme groups, is theoretically possible, although not widely reported. Additionally, selenium-containing enzymes may be targets. However, such reactions are likely not to be kinetically feasible without the certain environments that could lower the activation energy for such a reduction to take place. However, clearly, a comprehensive study of the proteins controlled by H_2 is required, even if it is simply to rule them out as being involved. Unlike the work with NO and H_2S (Baty et al. 2005; Hawkins and Davies 2019), a proteomic approach would seem to not be feasible with H_2 as no direct covalent post-translational modification of proteins have yet been reported for this molecule. On the other hand, downstream post-translational protein modification will occur, and a full compendium of such effects would be useful to know.

Although endogenous generation of H_2 in some plants is possible and may be able to be manipulated, manipulation of exogenous sources of H_2 would be a better approach as it would be easier. The presence of H_2 may be dictated by the surrounding microflora, but H_2 may be applied to plants as a treatment. Using H_2 as a gas is unlikely to be of use, but the generation of HRW or HNW may allow application to either foliage or roots, or both. Clearly, there are safety aspects from a physical point-of-view, as H_2 is extremely flammable, but from a biological viewpoint H_2 appears to be safe to use, both for plants and animals. As with other similar molecules, for example H_2S (Song et al. 2014), donor molecules may open up the better use of H_2 in the future, and the use of some are already being reported, such as MgH_2 (Li et al. 2020b) and AB@hMSN (Wang et al. 2021).

H_2 use in agriculture and horticulture has yet to be widely adopted, but there is a growing interest in this biologically safe treatment. As more is known about how it works, and the significance of any effects are more widely reported, H_2 may become an accepted way to enhance plant growth and crop storage in the future.

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Chapter 6

Understanding the Role of Nitric Oxide and Its Interactive Effects with Phytohormones in Mitigation of Salinity Stress

Mahima Misti Sarkar, Rewaj Subba, Swarnendu Roy, and Piyush Mathur

Abstract Nitric oxide (NO) is a highly reactive form of nitrogen species well discussed in varied literature and its significant role during physio-biochemical responses in plants has been well documented. Meanwhile, salinity is an important abiotic factor that limits the growth and production of almost all economically important plants worldwide. Due to salinity stress, there is substantial cellular damage, imbalance in ionic content, and osmotic strain in plants. Exogenous application of NO has shown positive results in alleviation of salinity stress by increasing photosynthetic activities, osmolytes content, stomatal conductance, etc. Exogenous application of NO in plant systems alleviates salt induced stress through maintaining ionic homeostasis by lowering the levels of cellular reactive oxygen species (ROS) content. However, the effect of NO is concentration dependent, as low concentrations initiate cell signaling while high concentrations induce nitrosative effects. Recent studies also revealed that NO is associated with numerous plant-signaling networks during salinity stress and interacts with the other plant growth regulators such as auxins, cytokinins, gibberellins, abscisic acid, ethylene, etc. Interactive roles of NO with phytohormones are known to cumulatively regulate responses even at molecular levels in plants during abiotic stressed condition such as salinity. With this background, the present chapter attempts to provide a holistic idea with special reference to mitigatory roles played by NO in plants under salinity stress including NO biosynthesis, NO-mediated physio-biochemical changes, and regulation at various molecular levels like transcriptome and proteome, along with

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T. Aftab, F. J. Corpas (eds.), *Gasotransmitters Signaling in Plants under Challenging Environment*, Plant in Challenging Environments 5,
https://doi.org/10.1007/978-3-031-43029-9_6

post-transcriptional and post-translational modifications. Furthermore, the chapter also deals with the interactive roles of NO with different phytohormones in plants under salinity stress.

Keywords Abiotic stress · Gasotransmitter · Jasmonic acid · Melatonin · Salicylic acid

6.1 Introduction

Nitric oxide (NO) is considered as an extremely reactive form of nitrogen which is formed inside the living cells causing oxidative damage in cells at high concentrations. However, at low concentrations, NO acts as an important gaseous signaling molecule that partakes in several plants physiological processes (Rather et al. 2020). NO can escape into the cellular compartments through the lipid bilayer of biological membranes (Del Castello et al. 2019). Talking about plant system, the idea about NO production is a topic of debate since several pathways has been reported to exist in plants that lead to NO production (Gupta et al. 2011). Several studies indicate the involvement of nitrate reductase (NR) pathway as the major contributing factor in NO generation in plants (Besson-Bard et al. 2008; Kolbert et al. 2019a). Involvement of NO in diverse plant responses towards important abiotic stresses such as drought (Gan et al. 2015; Jangid and Dwivedi 2017), salinity (Kaya et al. 2015; Klein et al. 2018) as well as heavy metals (Khairy et al. 2016; Terrón-Camero et al. 2019) has fuelled its research. NO assists plants to alleviate major abiotic stress like salinity by regulating stomatal movements, programmed cell death (PCD), modulation of proteins via post-transcriptional modifications (PTMs), including expression of stress responsive genes (Nabi et al. 2019).

Salinity in the soil can arise from either human interference such as frequent use of fertilizers, irrigation with salt-containing waters, or improper drainage system, or can occur by natural weathering and deposition (Hasanuzzaman et al. 2013). About 932.2 million ha are either saline or sodic areas and Asia alone comprises about 33.9% of the total land (Shahid et al. 2018). Higher accumulation of salt in the soils lowers seed germination, reduces plant growth and yield (Zhang and Dai 2019) which in turn highly impacts the economy (Srivastava et al. 2019).

In response to abiotic stress like salinity, plants undergo changes in terms of physiology, metabolism as well as regulation of genes (Arif et al. 2020). Lowering chlorophyll and carotenoid contents in plants under salinity stress greatly affects transpiration, gas exchange, and photosynthetic machinery (Pan et al. 2021). Increasing salinity levels causes hyperosmotic stress that results in membrane damage, nutrient imbalance, hampered photosynthesis, etc.

Reactive oxygen species (ROS) are normally produced during metabolism and play a vital role in the form of signalling molecules as to maintain cellular homeostasis (Hasanuzzaman et al. 2020). The involvement of ROS in growth, development, defense response, acclimation, and programmed cell death is well documented (Apel and Hirt 2004). However, different abiotic stresses like salinity triggers increased accumulation of ROS molecules leading to oxidative stress in plants that result in lipid peroxidation of plasmalemma, deteriorating membrane integrity, and damage towards proteins as well as DNA (EL Ghazali 2020). Furthermore, the higher levels of salinity have also seemed to affect the microtubule organization in the cortical cells of *Arabidopsis* and thus, interfere with the normal cell cycle (Shoji et al. 2006).

The concentration of cellular NO is very important for the manifestation of its effects. For example, at normal levels, NO produces beneficial effects through countering oxidative and nitrosative stresses, while on the other hand, at higher levels it generates oxidative as well as nitrosative effects that may even lead to cell death (Valderrama et al. 2007). Previous research has shown the positive effects of NO on alleviation of the salinity-induced toxic effects in plants (Shi et al. 2007; Fatma and Khan 2014). In this connection, the exogenous NO treatments in plants have shown beneficial effects in seed germination through improved activities of antioxidants during salinity stress (Fan et al. 2013). Reports for an increase in growth, relative water content (RWC), and photosynthetic pigments of plants have been obtained in salt-stressed plants subjected to NO application (Alnusairi et al. 2021; Sundararajan et al. 2022). Involvement of NO in maintaining photosynthesis, ROS through stimulation of various antioxidative enzymes (peroxidases, catalase, ascorbate peroxidase, etc) and production of osmolytes have been well documented by Sharma et al. (2020). H^+-ATPase in plasma membrane creates electrochemical gradients that direct the transport of Na^+ ions across the membrane (Serrano 1989). According to Zhao et al. (2004), NO plays an important role in regulating the H^+-ATPase activity thereby maintaining the K to Na ratio inside the cell. Additionally, interaction between NO and other phytohormones have been reported to mutually regulate plant responses during salinity stress (Grün et al. 2006; Campos et al. 2019)

Investigations have also revealed the existence of NO-mediated gene regulation and post-transcriptional modifications (PTMs) in plants exposed to salinity stress (Hasanuzzaman et al. 2018; Bhardwaj et al. 2021). NO in the modulation of gene expression in several plants like *Glycine max* (glutathione-s-transferases; *GST1* and *GST4*) (Dinler et al. 2014), *Triticum aestivum* (salt overly sensitive 1; *SOS1,* Na^+/H^+ exchanger; *NHX1,* aquaporin; *AQP* and osmotin; *OSM-34*) (Alnusairi et al. 2021), *Jatropha curcas* (*JcCAT1, JcCAT2, JcGR1* and *JcGR2*) (Gadelha et al. 2017), etc. has been previously reported. In addition, *S*-nitrosation (PTM promoted by NO) of transcription factors by NO is thought to play certain roles in transcriptional regulation (Mengel et al. 2013). PTMs of proteins are an important step for the proper functioning of proteins. Investigations show that NO aids in PTMs of proteins through metal nitrosylation, tyrosine nitration, and *S*-nitrosation (Astier and Lindermayr 2012) which can lead to protein methylation in plants as a response to stress conditions (Hu et al. 2017).

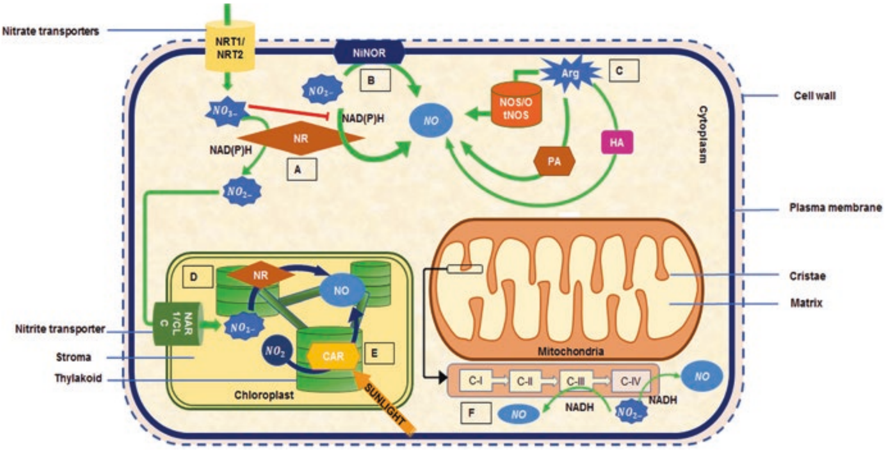

Fig. 6.1 Overview of pathways for NO synthesis in plants. (**a**, **b**, **d**, and **f**): Reductive pathways for NO synthesis in cytosol, plasma membrane, chloroplast, and mitochondria, respectively through the action of nitrate reductase (NR), nitrite-nitrate reductase (NiNOR), and complex III (C-III) and IV (C-IV) of electron transport chain. (**c**): Oxidative pathways for NO synthesis through nitric oxide-like synthase (NOS), polyamines (PA), and hydroxylamines (HA). (**e**): Non-enzymatic route for NO synthesis in chloroplast by carotenoids (CAR) in the presence of sunlight. (NOS/OtNOS indicate that in some plants enzyme NOS is similar to human NOS while in some enzyme NOS is similar to plant *Ostreococcus tauri*)

6.2 Biosynthesis of NO

In a plant system, NO can originate from different substrates following different routes including both enzymatic and non-enzymatic sources. These different enzymatic routes leading to the production of NO can be generally classified into reductive and oxidative pathways (Fig. 6.1) (Gupta et al. 2011; Corpas et al. 2022).

6.2.1 *Reductive Pathways*

Although the sources for the synthesis of NO have been a subject of much debate, the major contributor in the process of NO synthesis is thought to be the enzymatic reduction of nitrites by nitrate reductase (NR). NR is a principal enzyme (~200 kDa) that is involved in the assimilation of nitrogen through catalysing reduction reaction for the conversion of nitrate to nitrite in plants (Tejada-Jimenez et al. 2019). NR comprises two subunits with FAD, heme b$_{557}$ and molybdenum as prosthetic groups and, during the reduction process, NR utilizes NAD(P)H as their electron source (Hoff et al. 1992). In plants, nitrate transporters like NRT1 and NRT2 family transporters are involved in nitrate uptake from the soil (Crawford and Glass 1998). In the cytosol, nitrite is either generated by the action of NR on nitrate or through absorption from the soil. Nitrite is generally involved in the release of NO in plants upon the action of NR. However, nitrate (Ki = 50 μM) competitively binds with NR and acts as a competitive inhibitor of nitrite (Rockel et al. 2002). NR liberates NO when the concentration of nitrite levels is high in the plants, which is generally seen in the case of an anaerobic system (Yamasaki and Sakihama 2000). On the other hand, in an aerobic system, formation of nitrate is high which drives the reaction in plant tissues rather than the nitrite that results in the inhibition of NO production. Among the NR encoding genes of *Arabidopsis*, comparative analysis between *NIA_2* deleted plants and *nia_1* and *nia_2* double mutants, it was seen that majority of functional NR protein is encoded by *NIA_1* gene (Wilkinson and Crawford 1993).

Other than cytosol, studies have shown the presence of NO producing apparatus in plasma membrane and chloroplast. In the plasma membrane, nitrite NO-reductase enzyme (NiNOR), a membrane bound enzyme, regulates the nitrite:NO reductase (Chamizo-Ampudia et al. 2017). NiNOR in plasma membrane has been shown to generate NO parallelly with nitrate supply in mycorrhizal roots of *Nicotiana tabacum cv.* Samsun, but decreased under excess nitrate supply (Moche et al. 2010). For chloroplast-associated NR, NO production is well described in *Chlamydomonas*, where the cytosolic nitrite is transported to chloroplast via the nitrite transporter NAR1. However, higher plants lack NAR1, therefore, higher plants utilize ChLoride Channel (CLC) family transporters as an alternative for NAR1 to transport nitrite to the chloroplast (Monachello et al. 2009). Apart from these two cell organelles, production of NO is also seen in the inner mitochondrial membrane during the hypoxic condition where required electrons are assimilated from NADH through ubiquinone and electron transport chain during nitrite reduction (Gupta et al. 2018).

6.2.2 Oxidative Pathways

Apart from the reductive pathway which utilizes nitrite as substrates, there is evidence showing the presence of oxidative pathways for the generation of NO (Foresi et al. 2010; Gupta et al. 2011). In case of an animal cell, the enzyme nitric oxide synthase (NOS) majorly contributes during the synthesis of NO using L-arginine as substrate (Gupta et al. 2020a). Attempts have been made to identify the involvement of NOS in plants which remains elusive. The presence of plant NOS (OtNOS) has been reported in *Ostreococcus tauri*, where the enzyme resembled 45% human NOS and also utilized L-arginine as the substrate for NO biosynthesis (Foresi et al. 2010). Analysis of transcriptomes and genomes from several land and algal species was done by Jeandroz et al. (2016) using *OtNOS* and human *NOS1* sequence as templates. The search resulted in the majority of NOS-like sequences from algal species where binding sites for NOS cofactors were conserved. However, some studies also demonstrate the absence of NOS in some plants. For example, NO production in plants like *Helianthus annuus*, *Spinacia oleracea* and *Zea mays* does not occur through NOS activities as the NO liberation was found to be unaffected with the application of NOS inhibitors in these plants (Rockel et al. 2002). In addition to

NOS-like enzymes dependent pathways for NO biosynthesis, arginine-dependent polyamine (PA)-mediated NO production in plants has also been reported (Gupta et al. 2011). PAs are ubiquitous aliphatic amines that are present in all the cells of the plant system. According to Tun et al. (2006), PAs basically the spermidine and spermine were able to increase the production of NO in *Arabidopsis*. Another oxidative pathway for NO production is the hydroxylamine-mediated pathway that is generally seen in animals. However, this pathway was also reported in tobacco plants under anaerobic conditions (Rümer et al. 2009).

Apart from reductive and oxidative enzymatic pathways for NO production, there are reports of the presence of non-enzymatic production of NO in plants. For example, carotenoids exposed to nitrogen dioxide (NO_2) and light simultaneously generate NO in a non-enzymatic process (Cooney et al. 1994).

6.3 NO-Mediated Physiological and Biochemical Changes in Plants Exposed to Salinity

Primarily land degradation and environmental deterioration are brought about by excess salinization of soil which ultimately affects the total yield of agriculture (Farouk and Arafa 2018; Helaly et al. 2018). Plant growth and development become affected by the adverse effect of salinity through reduced physiological water status, and increasing ion and ROS accumulation. These negative impacts finally give rise to membrane damage, protein denaturation, DNA damage, and enzyme inhibition (Moradi and Ismail 2007; Sharma et al. 2012; Castillo et al. 2015). NO is a chief signaling biomolecule that is involved in various metabolic processes and plays a key role in the stress management strategies of plants (Xu et al. 2021). In this relation, NO was previously observed to palliate the negative impact of salinity in rapeseed (Zhao et al. 2018), wheat (Sun et al. 2019), and maize (Oliveira et al. 2016). Salinity imparts negative impacts on plants mainly by increasing ion toxicity and disrupting nutrient homeostasis through an increased Na^+ and decreased K^+ concentration, along with a reduction in the content of various micro- and macro-nutrients. NO was observed to help the plants by reducing the Na^+ accumulation and increasing the K^+ and nutrient content thus maintaining ion homeostasis and osmotic balance (Jamali et al. 2015; Hasanuzzaman et al. 2021). NO was also reported to accelerate some important seed germination parameters like germination potential, germination index, vigour index, germination velocity, vitality index, and embryonic growth under salinity stress (Zhang 2015; Ren et al. 2020). NO application can improve the tolerance of plants against salinity by growth promotion, maintaining ion homeostasis, and reversing oxidative damage (Sharma et al. 2020). Besides, increasing the biomass of plants under a saline environment, NO plays a vital role in the mitigation of salt-induced early senescence and leaf chlorosis (Adamu et al. 2018). This reduction in leaf senescence occurs due to the reduced ABA biosynthesis, Na^+ accumulation, and parallelly increased cytokinin biosynthesis, chlorophyll content, and photosynthetic rate (Kong et al. 2016). NO application also ameliorates the salinity stress of plants by improving the growth, photosynthesis, and osmolyte accumulation along with accelerated antioxidant enzyme activity and reduced electrolyte leakage, H_2O_2, and malondialdehyde (MDA) accumulation (Ahmad et al. 2016; Roychoudhury et al. 2021). Growth parameters like shoot and root length, shoot and root biomass, along with various beneficial physiological and biochemical attributes, were observed to be improved in plants exposed to salinity stress due to the application of NO. The exogenous application of NO was also observed to scavenge the oxidative molecules like superoxide anion, and H_2O_2 and thus reduced the electrolyte leakage and MDA accumulation simultaneously up regulating various enzymatic antioxidants *viz.* catalase, peroxidase, superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase, and polyphenol oxidase (Fan et al. 2013; Chen et al. 2014; Egbichi et al. 2014; Kaya et al. 2015; Klein et al. 2018; Khator and Shekhawat 2020; Ren et al. 2020; Sundararajan et al. 2022).

Non-enzymatic antioxidants also have great involvement in scavenging the ROS generated by stress. NO application was observed to increase the biosynthesis of non-enzymatic antioxidants (ascorbic acid, lycopene, β-carotene, total phenolics, flavonoids, and anthocyanin) in salt-stressed tomato plants, thereby, improving growth parameters and reducing Na^+ ion accumulation (Ali and Ismail 2014). NO was also observed to improve the tolerance against combined stress of salinity-alkalinity on muskmelon plants by acting in downstream of GABA (γ-aminobutyric acid) signaling, stimulating the antioxidant defense system which ultimately regulates ion homeostasis and membrane lipid peroxidation. In this connection, the GABA pretreatment increased the endogenous NO level but the external application of NO did not affect the endogenous levels of GABA (Xu et al. 2021). NO also showed its function in downstream signaling pathways of melatonin to increase the salinity tolerance of plants. For instance, in *Brassica napus*, the cordial action of melatonin and NO improved growth of the plant as well as reestablished redox and ion homeostasis by reducing the overproduction of ROS, thiobarbituric acid (a reactive substance), and Na^+/K^+ ratio with modulation in the transcripts of antioxidant defense-related genes (*sodium hydrogen exchanger 1, and salt overly sensitive 2*) (Zhao et al. 2018). External application of melatonin increased the NO synthase activity, polyamine content, and arginine utilization in plants. Moreover, NO helped to increase ATP content which ultimately was used to maintain K^+/Na^+ homeostasis by improving Na^+ efflux and K^+ influx (Yan et al. 2020). Increased (spermine + spermidine)/putrescine ratio indicated the benefit against salinity stress and NO was observed to reduce the negative impacts of salinity in cucumber seedlings by adjusting the proportions of these polyamines (Fan et al. 2013). In this connection, the external application of NO and spermidine on salt-stressed Bakraii seedlings (*Citrus reticulata* × *Citrus limetta*) resulted in an improved growth due to increasing leaf Ca^{2+}, Mg^{2+}, and K^+ concentrations, relative water content (RWC), photosynthetic rate, antioxidant enzyme activities, stomatal conductance, intercellular CO_2 concentration, and transpiration rate; and decreasing hydrogen peroxide, electrolyte leakage, MDA content, and leaf Na^+ and Cl^- concentration (Khoshbakht et al. 2018). NO was also observed to reduce glucose and ethylene sensitivity under salinity and thus improved salt tolerance by upregulating the antioxidant system, nitrogen assimilation, and proline accumulation (Sehar et al. 2019). Besides this, NO proved its capability towards mitigation of negative impacts of salinity stress by preserving photosynthesis, osmotic potential, and minimizing sodium ion toxicity and thus reducing the need of activating the ionic homeostasis (*SOS1/NHX1*) and osmotic (*AQP/OSM-34*)-related gene expression but enhanced D2-protein (photosystem II) activity (Alnusairi et al. 2021). Some of the recent studies that have role of nitric oxide in modulation of plant physiological and biochemical responses under salinity stress have been summarized in Table 6.1.

Table 6.1 Recent studies depicting the application of nitric oxide on plant physiological and biochemical responses subjected to salinity stress

Nitric oxide donor/ Stimulation of endogenous NO	Plant species	Alleviating effects in plants	Reference
S-nitrosoglutathione (GSNO)	*Pisum sativum* L. cv. Lincoln	Increased APX activity and *S*-nitrosated APX; increase in H_2O_2, NO and *S*-nitrosothiol (SNO) content	Begara-Morales et al. (2014)
Sodium nitroprusside (SNP)	*Hyoscyamus niger* L.	Stimulated germination; increased ROS-scavenging enzymes, DPPH activity, hydroxyl radical scavenging activity, ferrous ions chelation; reduced lipid peroxidation; increased callus fresh weight	Samsampour et al. (2018)
Sodium nitroprusside (SNP), 6-benzyl adenine, γ-aminobutyric acid	*Lolium perenne* (Bright Star SLT, Catalina, inspire, and SR4660ST cultivers)	Increased leaf fresh weight, dry weight, photochemical efficiency; reduced Na^+ accumulation, leaf chlorosis, necrosis	Ji et al. (2019)
Sodium nitroprusside (SNP)	*Capsicum annum* L.	Increased photosynthetic rate, Stomatal conductance, intercellular CO_2 concentration, transpiration rate, mineral uptake, plant growth, leaf RWC; decreased hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) accumulation	Shams et al. (2019)
Sodium nitroprusside (SNP)	*Brassica oleracea* L.	Increased chlorophyll a, total phenolics, glycine betaine contents, SOD, CAT, and POD enzymes activities; decreased H_2O_2 and MDA accumulation	Akram et al. (2020)
Sodium nitroprusside (SNP), Potassium chloride (KCl)	*Triticum aestivum* L. cv. Jimai 4	Increased root functionality, soluble protein content, SOD activity; maintained K^+/Na^+ homeostasis; decreased free proline content, superoxide anion radical generation rate, lipid peroxidation	Dong et al. (2020)

(continued)

Table 6.1 (continued)

Nitric oxide donor/ Stimulation of endogenous NO	Plant species	Alleviating effects in plants	Reference
Sodium nitroprusside (SNP), $CaCl_2$, H_2O_2	*Chenopodium quinoa* Willd.	Increased germination rate, relative germination rate, germination index; increased α-amylase and β-amylase activity and thus increased water-soluble sugars content; increased protein and amino acid contents	Hajihashemi et al. (2020)
Sodium nitroprusside (SNP)	*Oryza sativa* L. (Jinyuan85 and Liaojing763 varieties)	Increased the activities of glutamate dehydrogenase, sucrose synthase, sucrose phosphate synthase; increased plant height, biomass, nitrogen assimilation, proline, and sucrose content, antioxidant enzyme activities	Huang et al. (2020)
Sodium nitroprusside (SNP), Salicylic acid (SA)	*Crocus sativus* L.	Increased growth, compatible solutes accumulation, secondary metabolites biosynthesis; induced antioxidative enzyme activities	Babaei et al. (2021)
Sodium nitroprusside (SNP)	*Pisum sativum* L. cv. Jof and cv. Utrillo	Improved fresh-dry weight, RWC, chlorophyll a and b content; reduced tissue electrical conductance, H_2O_2, MDA content; increased antioxidant defense significantly; increased the ratio of K^+/Na^+ and Ca^{2+}/Na^+	Dadasoglu et al. (2021)
Sodium nitroprusside (SNP)	*Raphanus sativus* L.	Improved photosynthetic apparatus, sugar accumulation; enhanced FRAP; reduced H_2O_2 and lipid peroxidation; restored protein abundance; increased diameter of the central cylinder, the thickness of the casparian strip of hypocotyl	Hajihashemi et al. (2021)
Sodium nitroprusside (SNP)	*Glycine max* (L.) Merr.	Improved root and shoot length, fresh and dry weight, photosynthesis, chlorophyll contents, various antioxidant Enzyme activities (CAT, SOD, POD, APX); induced cell wall repair, sequestration of Na^+ In the vacuole, no swelling of thylakoids	Jabeen et al. (2021)
Sodium nitroprusside (SNP)	*Hordeum vulgare* L.	Enhanced phenolics accumulation, antioxidation enzymes activity, antioxidant capacity. Accelerated carbohydrate metabolism, amino acids biosynthesis	Ma et al. (2021)

(continued)

Table 6.1 (continued)

Nitric oxide donor/ Stimulation of endogenous NO	Plant species	Alleviating effects in plants	Reference
Hydrogen sulfide (H_2S), and melatonin (MT) induced endogenous NO	*Cucumis sativus* L.	H_2S acted downstream of MT, interacted with NO and MAPK cascades, and overall participated in the process of salt stress mitigation by regulating photosynthetic efficiency, antioxidant enzyme gene expression and activity	Sun et al. (2021)
Sodium nitroprusside (SNP)	*Lens culinaris* Medik.	Improved roots and shoots length, RWC, chlorophyll content, branch number, pods, seeds, seed yield, biomass per plant, enzyme activities (CAT, SOD, POD); reduced MDA, H_2O_2 content	Yasir et al. (2021)
Sodium nitroprusside (SNP)	*Nitraria tangutorum* Bobr.	Increased fresh weight, shoot and root elongation; increased ascorbate-glutathione cycle, antioxidant enzymes activities; decreased electrolyte leakage, malondialdehyde content, leaf senescence, root damage, Na^+/K^+ ratio	Gao et al. (2022)
Nitrosoglutathione (GSNO)	*Lycopersicum esculentum* L. 'Micro-Tom'	Increased plant height, root length, leaf area, soluble sugar, glycine betaine, proline, and chlorophyll contents, antioxidant enzyme activity; decreased $O_2^{\cdot-}$ production and H_2O_2 content	Wang et al. (2022)

APX ascorbate peroxidase, *$CaCl_2$* calcium chloride, *CAT* catalase, *FRAP* fluorescence recovery after photobleaching, *GSNO* nitrosoglutathione, *H_2O_2* hydrogen peroxide, *MDA* malondialdehyde, *MAPK* mitogen activated protein kinase, *MT* melatonin, *NO* nitric oxide, *$O_2^{\cdot-}$* superoxide anion, *POD* peroxidase, *SOD* superoxide dismutase, *SNP* sodium nitroprusside, *SNO* S-nitrosothiol content

6.4 Crosstalk Between NO and Phytohormones of Plants Exposed to Salinity

Phytohormones have their key regulatory role in plants to acclimatize under an abiotic stressed environment. Exogenous application of phytohormones or the use of any biotechnological tools to manipulate the endogenous phytohormones level can lead to balanced metabolism and healthier plants grown under different abiotic stress conditions (Wani et al. 2016). Auxin (AUX), gibberellin (GA), cytokinin (CK), ethylene (ET), brassinosteroid (BR), salicylic acid (SA), jasmonic acid (JA), and strigolactone are the well-known hormones of plants. Among all these phytohormones, ABA is known to have a key regulatory role in many abiotic stress-induced responses (Zhang et al. 2006). Under stressed conditions, ABA can interact with some other stress-responsive (ET, JA, SA) and growth-promoting (AUX, CK, GA, BR) hormones to combat the negative impacts of stresses (Verma et al. 2016).

NO is a free radical gasotransmitter signaling biomolecule that has a great role in various spectrums of signal transduction pathways and can lead to inducing cross adaptation against environmental stresses (Singhal et al. 2021). Recent studies have revealed the fact that NO is associated with numerous plant-signaling networks which can interplay with the other plant growth regulators (AUX, GA, ABA, ET, JA, BR, SA, H_2O_2, H_2S, melatonin) to combat various salinity stress and improve their growth and development (Singhal et al. 2021). For example, an integrated signaling network of NO, ABA, and AUX was observed to control the root morphogenesis of tomato plants under salinity stress. Their cordial signaling can improve the lateral root growth and root numbers of saline-stressed plants (Santos et al. 2020). The lateral root of sunflower seedlings was also observed to increase under salinity stress as a positive impact of the crosstalk between NO and the enzyme involved in ET biosynthesis 1-aminocyclopropane 1-carboxylic acid (ACC) oxidase. Here, the external application of NO reduced the biosynthesis of ET which facilitated the enhancement of lateral root formation (Singh and Bhatla 2018). Generally, the salinity stress reduced the AUX and cytokinin/ ethylene content but the externally applied NO has been observed to elevate their content in *Lactuca sativa* plant. On the other hand, ET content was decreased with the application of NO in comparison to the salinity-stressed plants. These results also support the interaction between NO and phytohormone signaling (Campos et al. 2019). In cucumber, NO was also found to have essential downstream signaling for AUX-induced tolerance against the alkaline-sodic stress that was evident from the abolishment of AUX function by NO scavenging (Gong et al. 2014). A previous study revealed that endogenous NO production is dependent upon the nitrate reductase enzyme activity, and externally applied 24-epibrassinolide (BR) in association with endogenous NO increased the antioxidant defense and decreased ABA content to counter salinity stress (Gupta and Seth 2020). Combinedly applied BR and NO was found to decrease the negative impacts of salinity by modulating the nitrogen, proline, and ABA metabolism (Gupta et al. 2017). These modifications in ABA content influenced by NO were a result of NO-associated post-translational modifications like tyrosine nitration and *S*-nitrosation of proteins. These modifications have their influence on the regulation of ABA signaling pathways (Prakash et al. 2018). NO and ET interplay also displayed their contribution towards the increase in total yield and stress tolerance level of crops under changing environments (Kolbert et al. 2019b). Similarly, crosstalk between NO and hydrogen sulfide (H_2S) has a potent role in abiotic stress alleviation and fruit ripening (da Silva and Modolo 2018; Mukherjee 2019). In this context, NO was reported to increase H_2S accumulation in salt-stressed barley plants in a dose-dependent manner and acts upstream of H_2S in order to mitigate the negative impacts of salinity (Chen et al. 2015; Singh et al. 2019). NO was also observed to interact with SA and H_2O_2 positively to initiate the amelioration of oxidative damage by inducing the methylglyoxal detoxification and antioxidant enzyme-mediated defense (Mostofa et al. 2015). Similarly, NO can contribute to SA-induced salinity tolerance in plants, SA increased the production of NO and that helped the *Capsicum annuum* plants combat salinity stress mainly by improving the ascorbate-glutathione cycle and antioxidant defense (Kaya et al. 2020). SA and NO together was also observed to improve plant health under salinity stress condition by increasing biomass, photosynthesis, osmolyte content, nutrient uptake, and antioxidant defense than untreated and saline stressed plants (Yadu et al. 2017; Ahanger et al. 2020). Combined application of NO and SA alleviated the negative effect of salinity stress by improving the morphological attributes, transpiration rate, photosynthesis, and PSII activity; and decreasing the H_2O_2 and ROS levels. Besides, the combined application also stabilized the cell membrane by lowering the electrolyte leakage and also induced the better activity of cell wall H^+-ATPase in *Gossypium hirsutum* (Liu et al. 2014). The combined application of JA and NO can also help the plants to reduce the detrimental impacts of salinity stress by antioxidant activity up regulations, metabolite, and osmolyte accumulation (Ahmad et al. 2018). Strigolactone is a phytohormone that acts as an essential signaling molecule in plants under salinity stress (Sarwar and Shahbaz 2019). External NO application also up regulated the strigolactone biosynthesis genes (*SlCCD7, SlCCD8, SlD2,* and *SlMAX1*) and its signal transduction genes (*SlD14* and *SlMAX2*) in salt-stressed tomato seedlings. These results indicated that NO is capable to combat salinity stress by improving endogenous strigolactone along with improvement in photosynthesis, and antioxidant activity (Liu et al. 2022). Exogenous NO substantiates plant growth and development under salinity stress by regulating levels of different phytohormones and their crosstalk that further modulates different physiological and biochemical (Fig. 6.2).

6.5 NO-Mediated Changes at the Molecular Level Influencing Plant Tolerance to Salinity

6.5.1 Transcriptomic Level

NO is responsible for the regulation of various stress-responsive transcription factors, phytohormones, and antioxidant defense-related genes in order to combat the negative impacts of environmental stresses (Huang et al. 2018). The antioxidant enzymes play a critical role in plants to elevate tolerance against abiotic stresses. In this connection, NO has been found to positively influence the over-activation of catalase (*CAT1*, *CAT2*) and glutathione reductase (*GR1*, *GR2*) genes in seedlings of *Jatropha curcas* grown under NaCl to check the negative effects of ROS (Gadelha et al. 2017). Biosynthesis genes of salt-induced enzyme glutathione-s-transferases or GST were observed to be significantly upregulated (*GST1* and *GST4*) under both salt stressed and NO pre-treated salt stressed *Glycine max* plants. These findings suggest that NO and salinity both have a regulatory role on GST gene and enzyme expression (Dinler et al. 2014). Root pre-treatment with combined application of

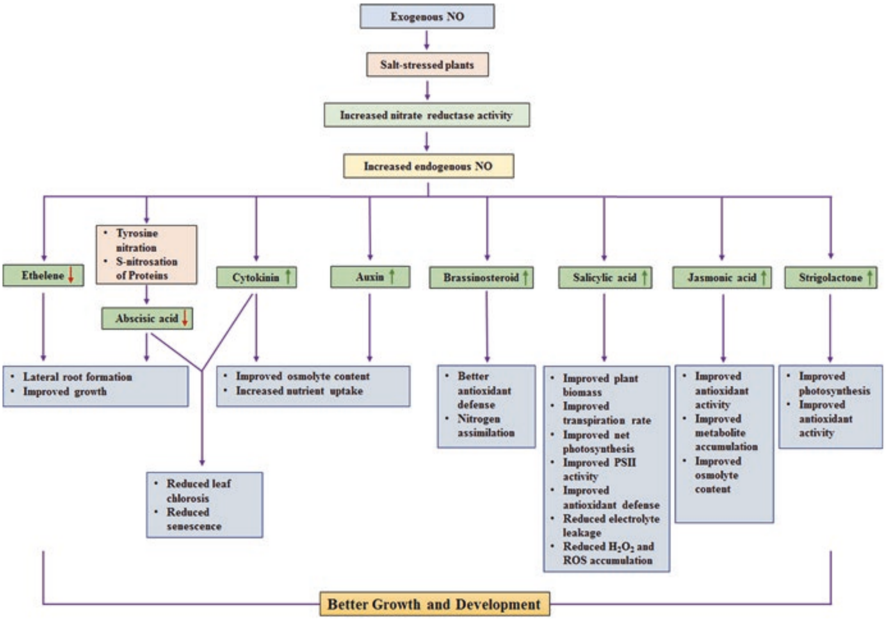

Fig. 6.2 Schematic representation describing the role of exogenously applied nitric oxide and its crosstalk with several endogenous phytohormones in order to alleviate the negative effects of salinity stress. NO application increased the endogenous NO through increased nitrate reductase activity due to which modulation in different phytohormone levels takes place. Further, the modulated phytohormone levels contribute to improving growth and development through different strategies

H_2O_2 and NO increased the transcript levels of some enzymatic antioxidants (glutathione reductase, cytosolic ascorbate peroxidase, catalase, manganese superoxide dismutase, dehydroascorbate reductase, and monodehydroascorbate reductase) and those involved in the biosynthesis of some non-enzymatic antioxidants like ascorbate (D- galacturonate reductase, L-galactose dehydrogenase, L-galactono-1,4-lactone dehydrogenase, myo-inositol oxygenase) and glutathione (gamma-glutamylcysteine synthase, glutathione synthetase) in leaves (Christou et al. 2014). Exogenous NO application in salinity stressed *Hylotelephium erythrostictum* plants significantly increased the Na^+ efflux, K^+ influx, and Ca^{2+} influx by modulating the genes associated with Na^+ and K^+ transport and Ca^{2+} channel respectively. This result identified the role of NO in the maintenance of K^+/Na^+ balance in plants through the Ca^{2+} signaling pathway (Chen et al. 2019). NO was also observed to have an inter-relation with *Aux/IAA17* (*AUXIN/INDOLE-3-ACETIC ACID 17*) and *RGL3* (*RGA-LIKE3*) genes which were overexpressed under NO influence to acquired resistance against salinity stress (Shi et al. 2017). A new compound Natolen128 was observed to modulate the NO accumulation level of *Arabidopsis thaliana* to improve the salinity stress by upregulating the expression of hypoxia-responsive genes including *PHYTOGLOBIN* and ethylene biosynthetic enzymes (Sako et al. 2021). Similarly, ABA was observed to increase H_2O_2 accumulation in plant cells, which can boost NO biosynthesis. The increased NO level resulted in MAPK activation and upregulation of the antioxidant enzyme biosynthesis-related genes to combat the negative impact of salt stress (Nawaz et al. 2017).

6.5.2 Proteome Level

Nitric oxide has its regulatory function on proteomic profiling of a plant that reflects the defensive responses of plants under salinity stress. NO is interactive with the metal centres of proteins like heme-iron, iron-sulfur clusters, zinc-sulfur clusters, and copper. These interactions ultimately form a stable metal-nitrosyl complex or produces various biochemical signals, which leads to structural or functional modifications of protein (Arora et al. 2016). In this relation, NO was observed to maintain iron homeostasis of sunflower seedlings under salinity stress by positively regulating the heme oxygenase (catalyst of ROS) activity, a catabolic enzyme of heme (a toxic iron sensor). NO binds/ interacts with the heme group of the heme oxygenase thereby reducing the rate of ROS production (Singh and Bhatla 2016). On the other hand, salinity has been studied to reduce the photosynthesis-related protein (Ribulose-phosphate 3-epimerase, large subunit of Rubisco, Rubisco activase A, and Quinine oxidoreductase-like protein isoform 1) abundance in *Avicennia marina*, a mangrove plant. Exogenous application of NO not only increased the abundance of those proteins but also the proteins related to primary metabolism, energy metabolism, RNA transcription, and stress response, and thus increased the plant salinity tolerance (Shen et al. 2018). Salinity also has a drastic impact on NO and redox homeostasis in the plant because salinity was observed to reduce the concentration of redox molecules (like nicotinamide adenine dinucleotide phosphate and reduced glutathione) and activities of some enzymes (like *S*-nitrosoglutathione reductase and catalase, and NADPH-generating dehydrogenases); and simultaneously increased the NO content along with glutathione reductase and glutathione peroxidase activity (Manai et al. 2014). Increased NO accumulation was observed to be positively correlated with increased G-protein-associated protein accumulation and antioxidative activities in salt-treated maize seedlings along with the activation of defense proteins, energy metabolism, and cell structure/division. G-protein signaling occurs upstream the NO biogenesis to increase the antioxidant defense of plants against salinity-induced H_2O_2 levels (Bai et al. 2011). Antioxidant defense through NO has a dual-action while involved in crosstalk with copper/zinc superoxide dismutase (Cu/ZnSOD) and FeSOD isoforms. Cu/ZnSOD activity in the roots of salinity stressed sunflower seedlings and FeSOD activity in the cotyledons increased with the increasing availability of NO, indicating its signaling role in separate intracellular pathways (Arora and Bhatla 2015). 5-aminolevulinic acid (ALA) is an important component that participates in induced plant tolerance against stress. NO has also been reported to be associated with the downstream signaling of ALA through the activation of nitric oxide synthase and thus completed the ALA-induced salt tolerance of maize plants (Kaya and

Ashraf 2021). Increased NO accumulation in seedlings under salinity stress also can enhance the tyrosine nitration of cytosolic proteins and proteins present in oil bodies to increase their longevity for better survival under salinity (David et al. 2015). Further, the external application of NO has been known to maintain polyamine homeostasis in salinity-stressed sunflower seedlings by upregulating the enzymes involved in PA biosynthesis (arginine decarboxylase and S-adenosylmethionine decarboxylase) and downregulating the polyamine oxidase activity that involves in PA catabolism (Tailor et al. 2019).

6.5.3 Post-transcriptional and Post-translational Modifications

Various biological functions are orchestrated by NO-mediated modulations through post-translational modifications (PTMs) like *S*-nitrosation of metals and cysteine residues and nitration of tyrosine residues. In plants, these NO-PTMs target hundreds of proteins under different environmental conditions stating the importance of NO in plant-signaling processes (Begara-Morales et al. 2016; Sánchez-Vicente et al. 2019). For example, the external application of H_2O_2 and NO and their interplay resulted in the improved survival rate of citrus plants under high saline stressed conditions. NO-mediated PTMs (carbonylation, nitration, and S-nitrosation) appear to be the key molecular strategy to conduct the signaling, transduction and stress mitigation under salt stressed conditions. Malate dehydrogenase and glutathione S-transferases were the most common proteins to undergo PTMs (Tanou et al. 2012). Salinity stress was observed to increase NO levels in plants depending upon the increased nitrated protein content but not *S*-nitrosated protein content. The absence or negligible amount of post-translational modification of proteins may help the respiratory and photorespiratory enzyme activities of plants to adapt in a better way against salinity stress (Camejo et al. 2012). *S*-nitrosation and denitrosation of proteins in NO signaling pathways also regulate the function of various enzymes (glyceraldehyde-3-phosphate dehydrogenase and monodehydroascorbate reductase) to increase the salinity tolerance of sunflower seedlings. In this connection, the proteins of the cotyledons were *S*-nitrosated whereas denitrosation occurred in the case of root proteins (Jain et al. 2017). Moreover, the carbohydrate metabolism-related proteins were the major proteins to undergo PTMs along with some newly reported proteins like pectinesterase, phospholipase D alpha, and calmodulin (Jain et al. 2017). NO signaling with *S*-nitrosoglutathione reductase activity together was involved in mitigating *Chlamydomonas reinhardtii* response to salinity (Chen et al. 2016).

NO bioactivity also showed its either direct or indirect involvement in a various number of PTMs like SUMOylation, phosphorylation, persulfidation, and acetylation (Gupta et al. 2020b). In higher plants, PRMT5 catalyzes Arg symmetric demethylation which is a key component of spliceosome. NO was observed to regulate the PRMT5 activity positively via *S*-nitrosation (Cys-125) during salt stress responses. These observations made evident a mechanism by which plants produce stress-induced NO signal to protein methylation mechanism via *S*-nitrosation of

PRMT5 as a response against salinity stress (Hu et al. 2017). Exogenously applied NO reduced the salinity-induced oxidative stress through upregulation of some SOD isoforms expressions (MnSOD1, Cu/ZnSOD1, and Cu/ZnSOD3). But some SOD isoforms get downregulated and some remain constant. This differential expression of the same enzyme isoforms that took place may be due to the NO-mediated post-transcriptional modification (e.g. *S*-nitrosation) (Klein et al. 2018).

6.6 Conclusion and Future Research

Salinity, being one of the adverse abiotic stress compromises both the health and yield of plants. It has negative effects on almost all the physiological and biochemical processes that occur in the plant. Thus, it becomes crucial to find out strategies to tackle such negative effects of salinity. In this background, studies have shown the importance of NO as an alleviating agent in plants during salinity stress. NO being a signaling molecule has a crucial involvement in the regulation of metabolic and physio-biochemical activities. NO assists plants to overcome salinity through the regulation of Na and K ions, photosynthesis, senescence, RWC, and antioxidant levels. Several investigations have shown the ability of NO in regulating defense-related genes in plants. Coordination of NO with other signaling pathways is another important factor for gene regulation during salt-stressed conditions. In addition, NO facilitates post-transcriptional and post-translational modification which aids in inducing salt tolerance in plants. However, several gaps persist in NO-mediated salt stress alleviation in plants. A deeper investigation is required to understand the NO-mediated mechanism for salinity tolerance. Furthermore, molecular studies for the involvement of gene(s) during NO biosynthesis and NO-mediated signaling pathways in plants can help in better understanding NO-mediated responses during salinity stress in a near future.

Acknowledgements All the authors are thankful to the University of North Bengal for providing the necessary facilities for writing this chapter. RS [File No: 09/0285(11430)/2021-EMR-I] acknowledges the Council of Scientific and Industrial Research (CSIR) for providing Junior Research Fellowship (JRF).

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Chapter 7
Nitric Oxide – A Small Molecule with Big Impacts on Plants Under Heavy Metal Stress

Kuntal Bera, Kakan Ball, Puspendu Dutta, and Sanjoy Sadhukhan

Abstract Plants produce signalling molecules as a stress-response mechanism, triggering a cascade of stress-adaptation reactions that result in either programmed cell death or plant acclimation. Nitric oxide (NO) is a small gaseous molecule which, with its bioactive nature, it is capable of regulating redox signalling in living cells. The importance of NO in abiotic stress response, particularly in heavy metal stress tolerance, is widely acknowledged by experts in the area. It is also worth noting that NO is involved in a variety of physiological processes, including seed germination, growth and development, flowering behaviour, senescence, and others. Because of its crucial role in regulating gene expression, post-translational modifications, and synergistic or antagonistic effects as a signalling molecule, several authors refer to NO as a gasotransmitter molecule. A relationship between NO accumulation and plant stress has been discovered in various studies. Exogenous NO enhances antioxidant activity in nearly all plant species and lessens the effects of stress in plants. However, the primary function of NO in the response to metal toxicity is to lessen oxidative stress by initiating antioxidant defence mechanisms. Although the pathways are largely species-specific, in this chapter we have attempted to provide an update on NO production, interactions, possible cross-talk with other chemicals and/or hormones, and several pathways involved in heavy metal stress.

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T. Aftab, F. J. Corpas (eds.), *Gasotransmitters Signaling in Plants under Challenging Environment*, Plant in Challenging Environments 5,
https://doi.org/10.1007/978-3-031-43029-9_7

Keywords Nitric oxide · Heavy metal stress · ROS · RNS · Metalloids · Oxidative stress · Redox homeostasis

7.1 Introduction

Nitric oxide (NO) is a diatomic, small, and impermanent molecule that plays a crucial role as a significant redox signalling agent in plants. It is considered one of the smallest, simple, and highly poisonous gas with complex chemistry including several interconnected redox forms with varying chemical reactivities. It plays important role in different physiological processes starting from the germination of the seed to flowering, and/or senescence (Sun et al. 2021). Plants respond to a lack of or excess supply of heavy metals and metalloids through complicated signalling pathways in many cases controlled by nitric oxide (NO) (Nabi et al. 2019). NO plays an important role as a primary messenger in various plant signalling (Domingos et al. 2015; Moreau et al. 2010). Exogenous application of sodium nitroprusside (SNP – a NO donor) is the most common source of NO and can activate a plant's biological signalling in a variety of plant processes and in different stages of plant growth (Buet et al. 2019; Zhao et al. 2007).

It was discovered that animals' endothelial cells produced nitric oxide as a signal in response to vasodilators like acetylcholine or bradykinin, and the concept of free radicals has undergone a paradigm shift. Free radicals and reactive oxygen species (ROS) were once thought to be harmful metabolic by-products that were reliant on oxygen for respiration (Crawford 2006). Whenever plants are frequently exposed to various stressful conditions in their very natural ecosystems that might interfere with proper growth, development, and production, hence jeopardising global food security. Soil contamination as induced through anthropological activities has become a major environmental issue in recent decades (Wuana and Okieimen 2011). Annually, the rising quantity of heavy metals in agricultural soils poses a serious health risk to people. In China, it is reported that approximately 19.4% of farmland and 10% of forestland are contaminated with heavy metals. The two most common hazardous heavy metals in the region are cadmium and lead. It is also reported that among different types of heavy metals, cadmium (Cd) is the most dangerous heavy metal to organisms and the environment, accounting for 7 percent of heavy metal toxicity in agricultural soil and urban areas in China (Qin et al. 2013). Excess accumulation of Cd in agricultural soils has several negative impacts on the environment and plant organs, including disturbing soil micronutrient and macronutrient balances, and negatively contributing to root elongation. Cd also induces oxidative stress by increasing H_2O_2, which results in a decrease in plant growth and photosynthetic pigments (Hawrylak-Nowak et al. 2015). Lead (Pb), on the other hand, causes a plethora of issues in plants, including affecting seed germination and cell division via altering important enzymes and lowering photosynthetic activities, as well as slowing plant growth during the divisional stage in seed germination (Malar et al. 2014). In general, plants experience several changes in their normal physiology, cellular metabolism, and regulation of their genetic expression under heavy metal stress. NO modulators, such as the application of various L-arginine analogues, including L-N$^{\omega}$-nitroarginine methyl ester [L-NAME - a nitric oxide synthase (NOS) inhibitor] and bovine haemoglobin (as a NO scavenger) (Souri et al. 2020; Tamás et al. 2018), change the activity of NO-like synthase, demonstrating the involvement of NO in the increase of plant tolerance to abiotic stress, specifically in plants that have been exposed to heavy metals (Ahmad et al. 2021; Bhat et al. 2021; Rezayian et al. 2020; Souri et al. 2020). NO reduces metalloid accumulation and can activate ROS-scavenging antioxidants in plants under metal stress, in addition to its signalling role in the reduction of heavy metal toxicity (Bhat et al. 2021). Plant cells are protected from oxidative stress by NO, which can stimulate H_2O_2-suppressing enzymes (Zheng et al. 2009). However, the knowledge of the molecular and physiological processes of NO in reducing the effects of heavy metal toxicity is extremely limited when compared to the recent understanding of the interactions of NO with other abiotic stressors (Corpas et al. 2006). Further, some studies on the relation between heavy metal toxicity and NO levels are ambiguous. As a result, it is worthy to examine and discuss recent achievements in this interesting field to better reach the roles of NO in heavy metal stress tolerance.

7.2 Synthesis of NO and Its Source in Plants

NO is produced endogenously in stressed organs or cells, specifically in cellular organelles such as mitochondria, chloroplasts and peroxisomes, and also in cytosol using nitrate reductase (NR) as a regulative enzyme (Planchet and Kaiser 2006a). This molecule diffuses both at intra- and intercellular ways (Fröhlich and Durner 2011). The discovery of NO in plants was first reported in legumes i.e., *Glycine max* while experimenting with synthetic inhibitor herbicides and under controlled dark anaerobic conditions and was first reported by (Klepper 1979, 1990; Nishimura et al. 1986). For the first time, (Ninnemann and Maier 1996) validated a NOS-like activity in plants. However, it has yet to be determined which specific enzymes are responsible for NO production. Only there have been regular inputs in plant sciences about NOS-like enzymes and their homologs have been suggested to contribute to NO production in various plant groups, such as in algae like *Ostreococcus lucimarinus* and *Ostreococcus tauri* (Shivaraj et al. 2020). Peroxisomal NO production appears to be mediated by L-arginine dependent NOS-like activity where NADPH acts as an active electron donor (Corpas and Barroso 2014; Corpas et al. 2004). The production of peroxynitrite (ONOO-) in peroxisomes, a NO derivative generated by a very quick chemical reaction between NO and $O_2^{\bullet -}$, serves as evidence that NO is present in peroxisomes (Corpas and Barroso 2014). Piacentini et al. (2020) elucidated the distribution of peroxisomes in the root meristem of primary and lateral roots, suggesting NO production and thus keeping a homeostatic control of NO in roots. A protein named AtNOA1 (Nitric Oxide Associated 1) found in *Arabidopsis thaliana* was reported to have possible involvement in NO synthesis by encoding a GTPase; however, later it was shown that this protein did not produce nitric oxide although it could be indirectly involved in its production (Fancy et al. 2017; Moreau et al. 2008). In the absence of expounded evidence on the direct source of NO and the undeviating role of NOS-like enzymes, the scientific and researching community worldwide accepted and validated the theory of oxidative and reductive pathways of NO production in plants (Nabi et al. 2019; Shivaraj et al. 2020). The oxidative pathway in plants includes (i) L-arginine dependent and (ii) polyamines mediated nitric oxide production, (iii) although the synthesis of nitric oxide via the hydroxylamine-mediated pathway is still undistinguishable in plants, it is considerably more widespread in bacteria and animals. Nonetheless, in tobacco cell culture under aerobic conditions, relatively little amount of NO was produced by this pathway compared to other pathways (Nabi et al. 2019; Shivaraj et al. 2020). The reductive pathway for NO production depends on the availability of nitrite as the primary source for NO production (Yamasaki and Cohen 2016), and it includes (i) nitrate reductase (NR), (ii) plasma membrane-bound nitrite: NO reductase (NiNOR), (iii) cytochrome-c oxidase and/or reductase, and (iv) non-enzymatically nitrite reduction in an acidic conditions (Nabi et al. 2019). It has been difficult to pinpoint the source of nitric oxide in plants, and so its synthesis in plant cells is still up for discussion (Hancock 2012; Salgado et al. 2013).

7.2.1 Oxidative Pathway

The oxidative pathways of NO production are still not well deciphered. However, some mechanisms have been validated by scientists. According to Tun et al. (2006), using arginine as a substrate, plants produce polyamines such as spermidine and spermine, these polyamines in turn oxidized to nitric oxide (NO). This report was validated by Arasimowicz-Jelonek et al. (2009) in *Cucumis sativus* during drought stress and by Groppa et al. (2008) in *Triticum aestivum* under cadmium stress. Under high salinity, L-arginine hydrolyzed to urea and L-ornithine by high arginase activity which also catalyses the metabolism of polyamine formation and can also be oxidised by homologous nitric oxide synthase (NOS) which leads to NO production in plants (da-Silva et al. 2018). An increase or decrease in NO production can be modulated by regulating the expression of *AtARGAH1* or *AtARGAH2* genes which encode arginine amidohydrolases. Flores et al. (2008) reported that there is a vice-versa relation between arginine amidohydrolase and NO production. The hydroxylamine-mediated pathway is mainly found in animals and bacteria, where NO production depends on the direct reaction of hydroxylamine with ROS (Shivaraj et al. 2020). For example, in *Nitrosomonas* sp., hydroxylamine is produced from ammonium oxidation and it, in turn, catalyzes the reaction for NO production (Martens-Habbena et al. 2015).

7.2.2 Reductive Pathways

Plants also produce NO using reductive processes, which are widely accepted. The well-studied nitrate reductase (NR) pathway for NO production is one of the several reductive processes. NR was found in the cytosol and the plasma membrane (Planchet and Kaiser 2006b). In general, NR converts nitrate to nitrite at the cost of NAD(P)H, and during this reaction, NR catalyzes the transfer of a single electron from NAD(P)H to nitrite, leading to the formation of NO (Planchet and Kaiser 2006b). In a low-oxygen environment, NR is more pronounced, and nitrite levels must be higher than the natural substrate level of nitrate (Prochazkova et al. 2014). In plants, nitrite: NO reductase (NiNOR) is another pathway for NO production. This pathway uses NO_2^- as substrate, which is produced in a coupled reaction with NR bound to the plasma membrane. Prochazkova et al. (2014) have shown that NiNOR has a maximum capacity when the apoplastic pH is 6.1 for converting nitrite into NO.

NO production in mitochondria is regulated by nitrate reductase (NiR). Mitochondrial NiR uses the enzyme complexes III-V to catalyze nitrite reduction in plants. In anoxia, NiR-dependent ATP generation can be reduced by respiratory inhibitors like potassium cyanide, which inhibits cytochrome c oxidase (Complex IV), and myxothiazol, which then hinders the functionality of complex III, by blocking electron transfer from ubiquinol to the central moiety of cytochrome c reductase that is a heme group protein. Myxothiazol inhibits all complex III-mediated redox reactions. Under anoxic conditions, the ubiquinone cycle is bypassed by a step in complex III that reduces nitrates. During respiration, mitochondrial NiR-mediated NO production inhibits oxygen consumption, preventing or delaying complete anoxia (Aguirre et al. 2010).

Non-enzymatic nitrite reduction under acidic conditions can be explained by a chain reaction carried out by two molecules of HNO_2 interacting with one another and producing NO and NO_2^-, which can then be converted to NO and oxygen as follows (Moreau et al. 2010; Stöhr and Stremlau 2006):

$$2NO_2^- + 2H^+ \leftrightarrow 2HNO_2 \leftrightarrow NO + NO_2^- + H_2O \leftrightarrow 2NO + \tfrac{1}{2}O_2 + H_2O$$

However, NO production appears to be a more complicated process in plants, and no plant-specific enzyme with a similar activity has been discovered thus far. Thus, it requires more intensive and illustrative research (Corpas et al. 2022).

7.3 Role and Production of Nitric Oxide in Response to Heavy Metal (HM) Stress

Root tissues are the first tissues to be exposed and impacted by HM and metalloids since plants use their roots to absorb minerals and nutrients from the soil. Oxidative stress damage due to heavy metals causes anomalous metabolic changes even in small quantities. In Arabidopsis, there is a notable alteration in the development and length of roots when exposed to heavy metals. Oxidative stress and glutathione depletion have been reported in alfalfa roots under high cadmium (Cd) and mercury (Hg) accumulation (Ortega-Villasante et al. 2005). Heavy metal toxicity in plants results in morphological, physiological, and biochemical alterations in plant organs, eventually plant growth, reproduction, photosynthesis, antioxidant content and activity, cell division, and cell differentiation get negatively affected (Sharma and Dietz 2009; Kolbert 2016) (Table 7.1). Being an interesting topic, numerous studies have been conducted to shed light on how heavy metal stress affects plants and the role of NO in the recovery mechanism (He and Chen 2014; Kolbert et al. 2017; Pető et al. 2013).

Exogenous administration of NO, as well as endogenous NO, have been shown to reduce HM toxicity. Exogenous NO, for example, lowers ROS and arsenic-induced malondialdehyde (MDA) levels in rice and mung beans to reduce arsenic (As^{3+}) toxicity (Ismail 2012; Singh et al. 2016; Singh et al. 2009). The application of NO to wheat and rice has been shown to alleviate HM stress (Mostofa et al. 2014). In *Typha angustifolia*, the role of NO in reducing the effects of cadmium stress and with improved growth and higher biomass yield has been documented (Zhao et al. 2016). Although the accumulation of NO in response to HM varies depending on the metal, condition and specificity are also regarded as important factors. Excess accumulation of NO in peroxisomes affects the catalytic reactions as a result enhancement of primary lateral roots which takes place in Arabidopsis

Table 7.1 Different observations based on the responses of NO metabolism to heavy metal stress

Heavy metal	Plant species and tissue exposed	Responses observed	References
Al	*Citrus grandis* (seedlings)	Reduced harmful effects on growth and changed root metabolism to protect against oxidative damage.	Yang et al. (2012)
Al	*Triticum aestivum (seedling)*	An increase in antioxidant enzyme activity.	Sun et al. (2014)
As	*Oryza sativa (root or coleoptile)*	Raised antioxidant enzyme level and modified the root and coleoptile architecture.	Singh et al. (2009); Singh et al. (2017b)
As	*Pistia stratiotes (plants)*	Elevated antioxidant enzyme levels and decreased negative impact on photosynthesis.	Farnese et al. (2013)
As	*Vigna radiate* (germinating seeds)	Decreased the amount of As that inhibits seed growth and boosted antioxidant and certain hydrolytic enzyme activity levels.	Ismail (2012)

(continued)

Table 7.1 (continued)

Heavy metal	Plant species and tissue exposed	Responses observed	References
As	*Phaseolus vulgaris* L (plants)	Increased cellular activity of antioxidant enzymes and decreased membrane damage.	Talukdar (2013)
As	*Triticum aestivum* L. (seedlings)	A rise in the contents of RWC, Chl, Pro, AsA, and GSH as well as the levels of antioxidant enzymes.	Hasanuzzaman and Fujita (2013)
As	*Vicia faba* L. (plants)	Boosting the amount of metabolites, photosynthetic pigments, phytohormones, and seed yield.	Mohamed et al. (2016)
As	*Oryza sativa* (seedlings)	Reduced the detrimental effects on growth and chlorophyll content; changed silicon transporter gene expression levels.	Singh et al. (2016)
Cd	*Brassica juncea* (plants)	Altered the root architecture, boosted the amount of photosynthetic pigments and water in the leaves, and raised the concentrations of antioxidant enzymes.	Verma et al. (2013)

(Kolbert et al. 2017). Elevated NO accumulation with high heavy metal toxicity is not noticeable in all plants. Endogenous NO, for example, dramatically increases in soybean after 72 hours of heavy metal exposure (Kopyra et al. 2006). Similarly, after 48 hours, the endogenous NO level was significantly lower in the root of Cd-treated *Medicago truncatula* (Xu et al. 2010). The conflicting results could be attributable to discrepancies in the type of HMs studied, plant tissues investigated, stress duration, and NO quantification method (Planchet and Kaiser 2006a, b).

To resist HM stress, NO is involved in numerous adaptation mechanisms including relaxation and expansion of the cell wall, providing safeguard to the integrity of the phospholipid bilayer, and enhancing overall plant growth (Seabra and Oliveira 2016). Other mechanisms regulated by NO are the preservation of osmotic pressure, which protects the membrane of chloroplasts, chlorophyll pigments, and other associated components from the harmful effects of HM by maintaining the viscosity of the cytoplasm (Ahmad et al. 2018). Another way of HM alleviation by NO could be the induction of heavy metal association domains containing certain genes that function as metallochaperons.

Metallochaperons are proteins that function as the safe and site-specific transporter of metallic ions within a cell (Robinson and Winge 2010). They may eventually become metallo-cofactors in certain enzymes that took part in cellular metabolism. In a recent RNA-seq-based transcriptomic analysis, 14 HM-related domain-containing genes were shown to have diversity in their expression in response to NO donor S-nitroso-cysteine (CySNO). Although nitric oxide-mediated HM-stress tolerance depends on various aspects such as concentration of NO applied, exposure time, plant species, type of tissue or organ exposed to stress, and NO donor (Chen et al. 2010; He and Chen 2014; Kováčik et al. 2014). The generation of ROS is another notable event under heavy metal stress that led to several

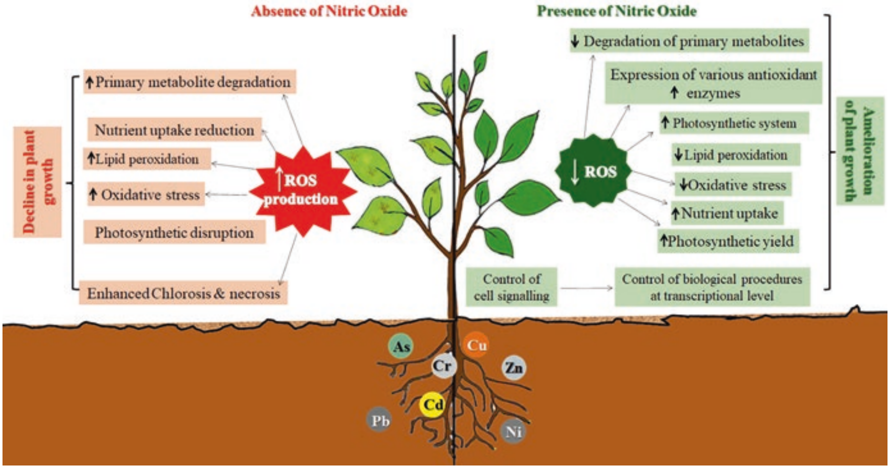

Fig. 7.1 Responses exhibited by plants in the presence and absence of NO under heavy metal stress. ROS production and oxidative stress are the most common responses exhibited by plants under different heavy metal-mediated stress environments, thus leading to various negative effects on plants. These are the degradation of various primary metabolites, reduction in nutrient uptake, photosynthetic imbalance, chlorosis and necrosis of various plant tissues that affect the normal growth and development of plants. Meanwhile, NO, especially under various heavy metal stress, play a crucial role in reducing ROS-mediated oxidative stress and associated negative effects of ROS by various means i.e., lipid peroxidation, enhancing photosynthetic system, inducing and participating in the expression of various antioxidant enzymes

negative effects in plants but the presence of NO plays a vital role in lowering the risks of ROS and also comes up with some beneficial responses by the plants (Fig. 7.1).

7.3.1 *Cadmium*

Cadmium (Cd), at very low concentrations, shows high toxicity and affects physiological activities in plants. Cd is easily absorbed by plant roots and stored in the top parts of plants, reducing agricultural productivity and posing a worldwide threat to food security. Cd can cause oxidative damage to various fundamental cellular components such as carbohydrates, proteins, DNA, and membrane lipids by increasing ROS generation via NADPH oxidase (Cuypers et al. 2010). In plants, Cd toxicity disrupts plant-water content (Perfus-Barbeoch et al. 2002), depletes nutritive components (Sandalio et al. 2001), and negatively influences photosynthetic pathways and components (Hsu and Kao 2004). Once plants are exposed to Cd, it has negative impacts on their roots and metabolites, resulting in yield loss.

The role of NO in reducing cadmium toxicity in a variety of plant species has also been well documented, with various findings indicating that NO is a vital compound in Cd detoxification for its anti-oxidative properties which lowers the Cd-mediated stress effects. For example, SNP lowers the Cd stress effects in rice leaves (Hsu and Kao 2004). Under Cd stress, a strong correlation has been found between NO and physiological responses by rice plants (Yang et al. 2022). The influx of Cd in the plant is initially repulsed by the root cell wall as it acts as a subcellular reservoir for cadmium and encumbers Cd diffusion to the cytoplasm (Richter et al. 2017). Cell wall-forming polysaccharides, mainly hemicellulose and pectin with many carboxylates (–COOH) and carboxyl (–OH) like functional groups attached in their branches, are involved in binding metal ions to the root cell wall (Wang et al. 2020). According to (Yang et al. 2022) endogenous and/or exogenous NO could improve the biosynthesis of hemicellulose and pectin, although the functional architecture of pectin is more complex than that of hemicellulose. Expression of pectin methylesterase genes *OsPME11* and *OsPME12* is upregulated by NO and results in higher PME activity which promotes pectin demethylesterification. This leads to the generation of a large quantity of low methyl-esterified pectins with numerous free carboxyl groups on pectin chains which enhance binding ability to metal ions in the root cell wall (Lionetti et al. 2017; Peng et al. 2017). According to (Corpas and Barroso 2014), peroxisomes are involved in the endogenous production of peroxynitrite ($ONOO^-$) leading to overproduction during cadmium stress in *Arabidopsis* which, in turn, could be involved in the Cd stress alleviating mechanisms. Application of SNP on the leaves and calluses of *Helianthus* under Cd stress was reduced (Gallego et al. 2005; Laspina et al. 2005). Hydroponically wheat cultivation with SNP supplementation demonstrated lower Cd toxicity as well as lower lipid peroxidation, H_2O_2 concentration, and less electrolyte leakage (Singh et al. 2008). NO has been shown to cause programmed cell death in response to Cd by activating MAPK and stress-activated protein kinase (Kulik et al. 2012; Ye et al. 2013). The reduction of Cd-induced oxidative damage and lipid peroxidation by foliar application of SNP was found to be beneficial, and it was hypothesised that this was due to either direct scavenging of ROS or induction of anti-oxidative enzyme activity (Wang et al. 2013).

7.3.2 Aluminium

Aluminium is ranked the third among the most prevalent metals in soil. However, it is absorbed by plants only if the soil pH falls below 5. Al is phytotoxic even when absorbed in a small amount. By enhancing free radical imbalance and antioxidant level, Al promotes oxidative damage in the cell (Yamamoto et al. 2002; Zhang et al. 2012), and also increase ROS production. Cellular redox equilibrium can be disturbed by this increased oxidative stress. Being a non-redox element, Al can induce oxidative stress by electrostatically interacting with the oxygen donor ligands (such as carboxylate or phosphate groups) that cause cytotoxicity (Jones and Kochian 1997). Cellular inflexibility caused by aluminium toxicity destroys cellular constituents like lipids, proteins, and nucleic acids, which results in cell death. The role of NO during Al stress has been studied, and found that NO reduced the Al^{3+} toxicity in rose mallow roots (Tain et al. 2007). In sour pummelo seedlings, it has been found that Al hinders development and slows down photosynthesis, but these effects are reversed by supplementing with SNP, which reduces Al accumulation in the shoots as well (Yang et al. 2012). NO protects plants from Al mediated oxidative damage through the scavenging of ROS and the production of antioxidant enzymes (Delledonne 2005; Laspina et al. 2005). Previous research has revealed that Al may disrupt NO homeostasis by decreasing the activity of NOS-like enzymes, resulting in root development suppression (He et al. 2012). Plants can avoid Al stress by maintaining hormonal balance and inducing anti-oxidative enzymes in various sections of the plant.

7.3.3 Arsenic

Arsenic (As) is harmful to a wide range of organisms, including plants. As has recently been shown to influence the natural level of NO in plants, allowing them to respond to As mediated toxicity. It is now accepted that under As stress, the synthesis of NO is increased in comparison to its natural endogenous level. As (over 500 μM) can cause oxidative damage (Leterrier et al. 2012). To confirm that NO production was generated as a response to As, seedlings exposed to As were pre-incubated with 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3--oxide potassium salt (cPTIO, a NO scavenger), and NO was completely eradicated. This indicates an increase in NO levels caused by As which promotes nitrosative stress *following* As exposure. This could be because excess As accumulation provokes NO to react with superoxide radicals ($O_2^{\bullet-}$) and, as a result, strong oxidant peroxynitrite ($ONOO^-$) has formed which can facilitate protein tyrosine nitration and thus cause nitrosative stress in plants. Exogenous application of NO might stimulate metal transporters and lowers the As uptake by roots Singh et al. (2017b), enhance the development of more adventitious roots Kushwaha et al. (2019), boost the antioxidant defences system Souri et al. (2020), and showing other favourable functions in As detoxification and responsive mechanisms. Further, it is obvious that endogenous and exogenous NO, through functioning as an antioxidant molecule, plays significant roles in reducing As-induced stress in *Spirodela intermedia* (da-Silva et al. 2018). According to Singh et al. (2016), in rice plants, the application of NO significantly lowered the accumulation of As in the root and shoot region. Singh et al. (2017a) found the involvement of NO and biosynthesis of jasmonic acid in the regulatory network of As detoxification in rice. It was also found that in reducing oxidative stress caused by As toxicity, exogenous application of SNP to the growth medium could be beneficial as SNP promotes various enzymatic and non-enzymatic antioxidants reactions to withstand As-mediated stress conditions (Bhat et al. 2021). However, until now, the distinction between the functioning architecture of NO and its incitation mechanisms by As has remained a mystery. Surprisingly, several additional investigations have found that too much As inhibits endogenous NO synthesis, impairing plant development and growth. Furthermore, seedlings growing with As reduced NO accumulation, photosynthetic activity, and nitrogen content in *Pisum sativum*, but the As toxicity could be reduced through the application of sodium hydrosulphide (NaHS) (Singh et al. 2015). The drop in NO content caused by As could be due to As indirectly inhibiting the NO productive pathways. In *Pisum sativum*, endogenous NO metabolism was found to be controlled by As stress, with a contrasting observation in root (1.9 fold decrease) and leaves (3.3 fold increase) growth (Rodríguez-Ruiz et al. 2019). In summary, the findings suggest that As mediated stress may regulate the level of endogenous NO in plants, reducing or exacerbating As-induced damage.

7.3.4 Copper

Like Cd, Al, and As, copper (Cu) may cause plants to produce endogenous NO. Cu-induced endogenous NO levels have been shown to alleviate Cu toxicity in various plants. Meanwhile, when plants are under Cu stress, NO treatments have been demonstrated to promote the growth and development of plants (Shams et al. 2018). Excess Cu has been reported to increase the accumulation of NO in the adventitious roots of *Panax ginseng*, while SNP supplementation decreases Cu-induced toxicity via increasing antioxidant enzyme activity. cPTIO, on the other hand, negates SNP's protective effect, implying that SNP's protective effects were related to NO release (Tewari et al. 2008). Cu also promoted NO production in *Chlamydomonas reinhardtii*, which was positively related to proline synthesis, reducing oxidative damage by high Cu content. Furthermore, it has been reported that pre-treatment with SNP promotes proline synthesis and increases antioxidant activity in Cu-treated cells, whereas such an effect is reversed by providing cPTIO (Zhang et al. 2008). Furthermore, NR-mediated NO generation in *H. vulgare* shoots relieves Cu toxicity, as validated by SNP and cPTIO analysis (Hu et al. 2015b). As a result, the earlier findings suggest that Cu stress causes plants to produce endogenous NO, which is important in reducing Cu toxicity. Nonetheless, additional research is needed to determine the potential processes through which Cu stress influences plant endogenous NO levels.

7.3.5 Lead and Zinc

According to several recent studies, lead (Pb) and zinc (Zn) have been found to modify the synthesis of NO in plants. Pb exposure was observed with a surge of NO accumulation in *Pogonatherum crinitum* root cells by increasing NR activity.

Importantly, the external application of NO enhances Pb uptake in a dose-dependent manner. Further, it implies that NO plays a significant role in Pb uptake management since Pb uptake can be inhibited by employing cPTIO (Yu et al. 2012). In *Arabidopsis*, the involvement of NO in peroxisomal metabolism has been observed and the NO generation was stimulated by Pb supplementation (Corpas and Barroso 2017). Pb toxicity is also significantly reduced by exogenous NO (Sadeghipour 2017). It has also been reported that Pb toxicity raises endogenous NO concentration in *Zea mays*, which enhances melatonin-induced stress tolerance. According to a recent study, the enhanced tolerance to Pb toxicity can be completely removed by cPTIO (Okant and Kaya 2019). This demonstrates a factual affirmative relationship between NO and phytohormones in response to Pb stress. NO generation is induced by Zn as observed in *T. aestivum* seedlings where NO suppresses Zn-mediated root development (Duan et al. 2015). Furthermore, Ni promotes NO generation and SNP significantly improves Ni tolerance by regulating Ni absorption and ROS detoxification (Rizwan et al. 2018).

7.4 Regulation of Metal-Induced Oxidative Stress by NO

The common effect of heavy metals and/or metalloids can be represented by the induction of oxidative stress in plants (Kohli et al. 2019; Soares et al. 2019). Under such stressful conditions various highly reactive derivatives of O_2 i.e., ROS such as $O_2^{\bullet -}$, H_2O_2, $^{\bullet}OH$ and $^1O_2$ are being produced and accumulated in large quantities (Soares et al. 2019; Sytar et al. 2013) and interrupt the usual equilibrium of the cells. There are several negative impacts of oxidative stress in plants including lipid peroxidation, leakage of ions, oxidation of proteins, disintegration and dysfunction of various organelles due to membrane damage, alteration in DNA and all those effects lead to plants death by PCD (Demidchik 2015; Handa et al. 2018; Kohli et al. 2018; Shahzad et al. 2018).

Plants usually develop various approaches to withstand the oxidative stress situation. Plant growth hormones play a crucial role in regulating oxidative stress induced by abiotic stresses (Sharma et al. 2018; Tanveer et al. 2019). NO plays a multifunctional role under oxidative stress conditions. According to Sami et al. (2018), NO regulates phytohormones and controls downstream signalling pathways. Under specific conditions and depending upon plant species, NO excites the activation of various enzymatic and non-enzymatic antioxidants such as catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GPX), superoxide dismutase (SOD), ascorbic acid (AsA), proline and glutathione, thus helping the plant to tolerate or escape oxidative stress (Nabi et al. 2019). Moreover, NO is also involved in scavenging ROS and free radicals (Table 7.2).

Table 7.2 The impact of exogenous NO on the content of heavy metals in different plants

Plant species	Organ/ tissue/ cells exposed	Types of heavy metals	Exogenous NO (SNP/ GSH)	Heavy metal content	Observed effect on plant	References
Solanum lycopersicum	Leaf, stem, and root	Cd	SNP (100 μM)	Decreased		Ahmad et al. (2018)
Trifolium repens	Roots and shoots	Cd	SNP (50 μM)	Decreased	Antioxidant enzyme activity has increased.	Liu et al. (2015)
Typha angustifolia	Roots	Cd	SNP (100 μM)	Increased	Minimized the toxicity and increase the antioxidant activity	Zhao et al. (2016)
Vigna radiata	Roots and shoots	Cd	SNP (1 mM)	Decreased		Nahar et al. (2016)
Triticum aestivum	Leaves	Cd	NO (0.5 mM)	Decreased		Basalah et al. (2013)
Oryza sativa	Root/ coleoptile	As	50 μM		Modified the root and coleoptile architecture and increased antioxidant enzyme levels	Singh et al. (2009); Singh et al. (2017b)
Vicia faba	Shoots	As	SNP (100 μM)	Not affected	Increase growth, seed yield, photosynthetic pigments, phytohormones, and metabolite	Mohamed et al. (2016)
Pistia stratiotes	Leaves	As	SNP (0.1 mg L – 1)	Not affected	Increased antioxidant enzyme levels and reduced the negative effect on photosynthesis	Farnese et al. (2013); Farnese et al. (2017)
Spirodela intermedia	Roots and shoots	As	SNP (50 μM)	Decreased		da-Silva et al. (2018)
Triticum aestivum	Roots	As	SNP (100 μM)	Not detected	Increased antioxidant enzyme activity	Kaur et al. (2015)
Lolium perenne	Roots	As	SNP (100 μM)	Increased	Raised photosynthetic and antioxidant activity	Bai et al. (2015)

(continued)

Table 7.2 (continued)

Plant species	Organ/ tissue/ cells exposed	Types of heavy metals	Exogenous NO (SNP/ GSH)	Heavy metal content	Observed effect on plant	References
Oryza sativa	Roots and shoots	Cu	SNP (200 μM)	Decreased	Increased antioxidant enzyme activity	Mostofa et al. (2014)
Lycopersicon esculentum	Roots and shoots	Cu	SNP (200 μM)	Decreased		Wang et al. (2016)
L. esculentum	Leaves and roots	Cu	SNP (100 μM)	Not affected		Cui et al. (2009)
L. sativa	Seeds	Cu	SNP (200 μM)	Not detected		Shams et al. (2018)
Oryza. sativa	Roots and shoot	Ni	SNP (100/200 μM)	Decreased		Rizwan et al. (2018)
Brassica napus	Roots	Ni	SNP (0.2 mM)	Increased		Kazemi et al. (2010)
Citrus grandis	Seedlings	Al	10 μM		Minimized the negative effect on growth and altered root metabolism to protect from	Yang et al. (2012)
Triticum aestivum	Seedlings	Al	250 μM		Increased the level of antioxidant enzyme activity	Sun et al. (2014); Sun et al. (2015b)
Triticum aestivum and Phaseolus vulgaris	Seedlings	Zn	100 μM		Toxicity was reduced, and antioxidant activity was increased.	Abdel-Kader (2007)

7.5 NO-Mediated Regulation of the Photosynthetic Mechanisms Under Metalloid Stress

Metal toxicity induces ROS generation in plants which affects the overall photosynthetic performance. According to various studies, NO appears to be able to counteract the damaging effects of ROS in plants. In tall fescue, under Cr stress, exogenous application of NO has been reported to improve photosynthetic attributes (Huang et al. 2018). Under Cd stress, the exogenous application of NO has been recorded to enhance PSII efficiency as NO can scavenge ROS and ameliorates oxidative stress (Per et al. 2017). Arsenic induced photosynthetic damage also becomes reduced on exogenous application of NO either by modulating non-photochemical quenching, respiration rate and/ or reducing chloroplast damage (Farnese et al. 2017). Under strong heavy metal stress, the reduction of RuBisCO and activity of RuBisCO activase has been frequent, but this reduction can be overcome by the application of SNP (Khairy et al. 2016; Per et al. 2017). Under Cd stress, structural proteins LHCII and LHCI of peripheral antennas of PSII and PSI are damaged and reduced. This finally results in low chlorophyll fluorescence and reduced quantum yield. Further other important PSII proteins such as PSBP, PSBR, PSB27, and PSB28 are also severely affected by Cd-induced metal toxicity (Gong et al. 2017). NO proved to be efficient in successfully reversing the changes in these photosynthetic proteins together with stable activity of the electron transport chain and reduced ROS generation (Gong et al. 2017). Moreover, NO plays important role in the quick recovery of damaged PSII by metal toxicity since it promotes the transcription of PSII- associated genes like *psbA*, *psbB* and *psbC* (Wodala et al. 2008).

7.6 Plant Stress Response and NO-Mediated Posttranslational Modifications

Plants are sophisticated creatures with broad genomic architecture that express a significant number of genes for essential functions in everyday life. To perform various activities and functions plants have evolved systems to change the existing structure of the protein in various forms. This phenomenon is known as posttranslational modifications (PTMs) of protein. PTMs of many types have been discovered in living organisms and investigated. Both plants and animals exhibit the PTMs i.e., tyrosine nitration and S-nitrosation, which are both mediated by NO (Corpas et al. 2009a, b; Wiseman and Thurmond 2012). S-nitrosothiols (SNOs) are formed in proteins through S-nitrostion when NO is covalently linked to a particular cysteine thiol residue, and are regarded as responsible for reversible posttranslational modification. In living organisms, the process is almost ubiquitous (Anand and Stamler 2012). S-nitrosation is now been recognized to modify a large number of proteins. Both plants and animals have been reported with tyrosine nitration Corpas et al. (2009a, b), which is the attachment of the nitro (NO_2-) group to a tyrosine residue (Greenacre and Ischiropoulos 2001). After production, NO must be transformed into a nontoxic, easily accessible, and transportable form. This is accomplished by covalently attaching NO to the highly abundant redox-sensitive tripeptide molecule glutathione to produce S-nitrosoglutathione (GSNO), which is one of the very important SNOs since it is a key NO reservoir and long-distance signalling molecule. Since GSNO can regulate protein activity and gene expression, it plays an important role in plant development and responds to a variety of abiotic stresses (Begara-Morales et al. 2018). By lowering the accessible SNOs in the cell, S-nitrosoglutathione reductase (GSNOR) regulates the levels of S-nitrosothiols, in addition to disturbing the normal development and growth pattern of the plants (Kwon et al. 2012). The absence of GSNOR in plants causes an uprising in the level of SNO and impairs various plant immunity mechanisms (Feechan et al. 2005). It has been reported after a detailed analysis of Arabidopsis *atgsnor1–3* knockout mutants that the loss of GSNOR activity results in a general increase in SNO levels, including the S-nitrosation of AtSABP3 at cysteine residue 280 that antagonises plant immunity, (Wang et al. 2009). S-nitrosation of the protein kinase OST1 (an important component of the ABA signalling network) and the major ROS scavenging enzyme APX1 has been found to affect plant responses to abiotic stress in several studies, underscoring the critical regulatory functions of NO-mediated S-nitrosation (Wang et al. 2015; Yang et al. 2015). Other proteins have also been demonstrated to be regulated by S-nitrosation. Arginine symmetric demethylation is catalysed by the enzyme protein-arginine-methyltransferase-5 (PRMT5) in eukaryotes. However, new research suggests that NO can initiate S-nitrosation of PRMT5 at cysteine 125, favourably controling PRMT5 function under stress (Hu et al. 2017). Other PTMs documented such as phosphorylation, ubiquitination, acetylation, palmitoylation, and sumoylation are regulated by the process of S-nitrosation, exhibiting the impact of S-nitrosation on other essential PTMs. It has been identified in *atgsnor1–3* knockout mutant plants, that 1195 endogenously S-nitrosated peptides from 926 proteins are involved in numerous essential physiological functions such as chlorophyll metabolism, carbohydrate metabolism, photosynthesis, and various stress responses with significantly higher level of total SNOs accumulation, implying an wide regulatory control by nitric oxide at cellular level (Hu et al. 2015a). All these findings emphasize the significance of NO as a critical signalling molecule, both at baseline and stimulated levels. Tyrosine nitration, like S-nitrosation, is a "nitrosative stress signal" (Mata-Pérez et al. 2016; Sehrawat and Deswal 2012). In plants, there are hundreds of tyrosine nitrated proteins. Other PTMs facilitated by NO include nitration, metal nitrosation, and glutathionylation, in addition to S-nitrosation and tyrosine nitration. It's worth mentioning that NO-mediated PTMs can have either positive or negative effects on target proteins.

7.7 Cross-Talk Between NO and Hydrogen Sulphide (H_2S) in Heavy Metal Stress

The possible relation between NO and H_2S under various abiotic stresses has been established by several studies. According to Wang et al. (2012), a nearly 30% increase in NO level has been noticed in alfalfa when the seed is treated with 100 mM NaCl and 100 μM NaHS. According to this finding, NaHS acts as an H_2S donor, increasing endogenous NO synthesis. High levels of NO in the cell then activate NO scavengers, helping to counteract NaHS's effects. The promoted activity of plasma membrane H^+-ATPase is amplified by NO, H_2S, and H_2O_2 in cucumber roots (Jiang et al. 2019; Zhou et al. 2018). With other abiotic stress, this type of signalling interplays has also been frequent in plants exposed to heavy metal stress. SNP, a well-known NO donor, enhances antioxidant capacity when pre-treated with

NaHS (H_2S donor), thus successfully ameliorating aluminium-induced stress in *T. aestivum*, barley, and oilseed rape (Dawood et al. 2012). PCD is an important physiological process in the plant system encountered by the involvement of various endogenous molecules such as ROS, different elicitors, NO, H_2S, and salicylic acid in the signalling cascade which takes place (Kroemer et al. 2005). The action of NO as a promoter or suppressor of PCD depends on various aspects such as type of cell, cellular redox status, and availability of NO in the cell. It has also been well documented that interactions between NO and H_2O_2 metabolism regarding the promotion of PCD in various plant species occur. In *Chlamydomonas reinhardtii,* combining the exogenous application of NO and H_2O_2 induces PCD higher than that of NO alone. As an application of NO scavenger diminishes the NO-H_2O_2 interaction and reduces the PCD level, thus NO exhibits dual nature concerning upregulating or downregulating the process of PCD, while H_2S is only involved in the mitigation of delaying of PCD via ROS inhibiting pathways (Luo et al. 2020; Zhang et al. 2015). Under heavy metal stress, the synergistic role of NO and H_2S have been documented in PCD mitigation (Li et al. 2016; Shivaraj et al. 2020). For example, this synergic role-play is involved in the reduction of Al^{3+}-induced PCD (He et al. 2019), and the repair of photosynthetic apparatus in *Vigna radiata* under cadmium stress (Khan et al. 2020). In wheat, c-PTIO (NO-scavenger) masks the activity of pectin methyl esterase (PME) which checks the affinity between Al and pectin by pectin methylation in the cell wall of root cells and thereby prevents Al accumulation in roots (Sun et al. 2015a). H_2S plays quite a similar function in rice, where H_2S decreases the PME activity and therefore, reduces the negative charges in root cell walls. Thus pectin and hemicellulose content decrease significantly. Further low NO content has been reported to enhance the H_2S-mediated Al toxicity alleviation in rice (Zhu et al. 2018). In some cases of Cd-induced stress, a synergistic relationship has been found between NO and H_2S. The endogenous level of NO and H_2S and/or exogenous application of SNP and NaHS performs significant alleviation of Cd stress in Bermuda grass (Shi et al. 2014). NO and H_2S inhibitors have also been discovered to interfere with NO signals. Blocking of polar transport of auxin in *Arabidopsis* roots by activation of MAPK6 cascade as a result of ROS metabolism is an example of NO-H_2S crosstalk (Banerjee et al. 2018). NO regulates the expression of genes involved in the production, removal, and inhibition of H_2S. Since H_2S causes the development of lateral roots in tomato plants, masking the genes that express H_2S production by exogenous injection of NO prevents the development of lateral roots in tomatoes (Li et al. 2014). Accumulation of Ca^{2+} and expression of calmodulin 1 (CAM 1) has stimulatory support from NO and induce the inhibition of H_2S synthesis, whereas Ca^{2+} chelators and other channel blockers reduce the H_2S-induced formation of lateral roots. It is also found that in root development, NO activity is regulated by several post-translational modifications some of which also include the involvement of auxin and H_2S. Transcriptional study of miRNA genes established link between H_2S and auxin pathway, which is supported by cyclin-dependent kinase A (CDKA)/cyclin D (CYCD) and the auxin response transcription factors (ARF) and also evidenced by NO and H_2S interactions during development of roots (Mishra et al. 2021). In pea seedlings, NO and H_2S level has been increased significantly by exogenous application of NaHS, helps in restoration of redox status between ascorbate and glutathione, and also reduces ROS induced oxidative damage under arsenic (AsV) stress (Singh et al. 2015). These are some of the possible crosstalk between two important signalling molecules under heavy metal stress.

Exogenous administration of NO or NO donor (SNP) has been reported to be efficient in lowering different heavy metal-induced damage in plants, similar to endogenous NO. According to Kolbert and Ördög (2021), exogenous NO prevents boron (B) uptake in plants and reduces B-induced ROS generation, thus improving plant growth and yield. Exogenous NO as a signalling molecule increases the activity of antioxidant enzymes, scavenges ROS, prevents cellular oxidation, reduces electrolyte leakage, and the amount of soluble proteins in bamboo plants in Pb and Cd stress (Hill et al. 2010). Application of SNP under As stress successfully recovers the number of adventitious roots, reduces ROS generation, and improves activities of the ascorbate-glutathione (AsA-GSH) cycle enzymes (Kushwaha et al. 2019). Application of 20 μM SNP in sunflower plants exposed under Zn stress has been reported to exhibit a high level of tolerance with the promoted activity of SOD, APX and GR, also enhancement in AsA and GSH metabolism (Akladious and Mohamed 2017). Similar results were found by Namdjoyan et al. (2017), where 100 μM SNP was applied to *Carthamus tinctorius* exposed under Zn stress. (Liu et al. 2020) have also recorded the combined stress-mitigating effect of exogenous SNP and silica (Si) in maize exposed to Cd stress. They have found that the combined application of Si and SNP improves several important aspects of plant growth such as photosynthesis rate, pigment concentration, biomass, and yield.

7.8 Conclusion

NO is a very important messenger in plants' tolerance to various abiotic stresses, especially in heavy metal-induced stress. Through antioxidative defence, modulation of numerous cellular pathways, cellular signalling, and cross-talking with other signalling molecules, it helps the plant to recover from multiple phytotoxicity-mediated damages. Although the knowledge behind the molecular and physiological mechanisms, crosstalk with other phytohormones, and their signalling network at the molecular level under heavy metal stress is still quite limited and needs exploration and extensive research. However, by various research on this particular subject, it is well established that NO plays an immersive role in recovering from heavy metal stress. Since heavy metal stress is a burning problem in various agroecological systems, the above-mentioned information may prove valuable for further research and establishing metal toxicity-tolerant crop traits in the future.

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Chapter 8
Nitric Oxide: A Key Modulator of Postharvest Fruit and Vegetable Physiology

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Abstract Nitric oxide (NO) is a gasotransmitter molecule involved in a variety of physiological functions related to acclimation responses in plants for biotic and abiotic stresses. Thus, NO holds great promise in extending the shelf life of freshly harvested fruits and vegetables. Various investigations have identified the signalling function of NO in respiration, ripening, senescence, chilling injury alleviation and membrane damage to biological tissues by maintaining a greater antioxidant system. This chapter describes the relevance and role of nitric oxide to improve postharvest storage life and the quality of freshly harvested fruits and vegetables.

Keywords Nitric oxide · Chilling injury · Storage life · Quality

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T. Aftab, F. J. Corpas (eds.), *Gasotransmitters Signaling in Plants under Challenging Environment*, Plant in Challenging Environments 5,
https://doi.org/10.1007/978-3-031-43029-9_8

8.1 Introduction

Fruits and vegetables are an important part of a balanced diet and are essential for food as well as nutritional security. Vitamins, vital fatty acids, dietary fibre, minerals, pigments, and a variety of important bioactive substances are found abundantly in them. They are classified as "protective foods" because they contain high quantities of secondary metabolites, made up of a variety of phenolic and antioxidant chemicals (Jacob et al. 2012; Natesh et al. 2017). Hence, World Health Organization recommends the inclusion of at least 400 g of fruit and non-starchy vegetables in the regular diet. The fruits and vegetables are highly susceptible to microbial attack and self-decay due to high moisture content and pre-programmed senescence induced by various active physiological processes (respiration, ripening and ethylene production) and biochemical changes. Therefore, the time interval between harvest and consumption is of major concern. In addition, lack of infrastructure during the supply chain shortens our handling, storage and transport potential (Hu et al. 2017). Ultimately, such produce seeks less demand and acceptance by the consumers and ends up in huge post-harvest and economic losses (Zhang et al. 2019; Aghdam et al. 2020). Currently, the post-harvest losses range from 6.7% to 15% and 4.6% to 12.4% in fruits and vegetables, respectively (Rao 2022).

Both controlled and modified atmosphere storage, low-pressure storage, ozonation treatments and edible film coatings are some of the preservation and storage techniques often employed to reduce post-harvest losses (Bose et al. 2021; Reddy et al. 2021). Furthermore, the employment of ultraviolet, low temperature and air storage techniques necessitates a large amount of equipment and is cost-intensive (Pang 2012; Bose et al. 2021). Finally, preserving the quality of the product with extended shelf life while complying with food safety regulations has always been a huge concern. As a result, naturally occurring chemicals such as salicylic acid, methyl jasmonate, hydrogen sulphide, and nitric oxide have sparked researchers' interest in postharvest management of fresh fruits and vegetables.

Respiration, ethylene generation, and senescence are the key elements affecting the quality and shelf life of fruits and vegetables during storage. These are some of the vital processes that are impacted by intrinsic and extrinsic circumstances, through various signalling and response metabolisms. Nitric oxide (NO) is one of the biological signalling compounds that has acquired a lot of interest in the field of post-harvest physiology. It was well known for elicitation of resistance mechanism against some post-harvest fungal diseases, in addition to different functions such as delayed ripening and senescence in fresh fruits and vegetables (Gong et al. 2018; Aghdam et al. 2020, 2021).

Nitric oxide, a gaseous molecule with redox activity, coordinates a variety of physiological and biochemical functions in biological systems (Yu et al. 2014). NO is thought to be a prospective target for promoting germination, root architecture, nutrient acquisition, floral transition, delaying ripening and postharvest senescence, and enhancing tolerance to biotic and abiotic stresses, among other biochemically mediated functions (Sun et al. 2021). A good number of studies have demonstrated the role of NO in maintaining the sensory and nutritional attributes of horticultural commodities, by extending their shelf-life *viz*. sweet cherry (Zhao et al. 2019); persimmon (Shahkoomahally et al. 2015); peach (Gao et al. 2016); strawberry (Aghdam and Fard 2017); peach (Wu et al. 2018); grape (Ghorbani et al. 2017); and banana (Wu et al. 2014). This chapter elaborates on the potential use of NO in the modulation of post-harvest physiology and extending the shelf life of various fruits and vegetables in detail.

8.2 Biosynthesis and Signalling

The earliest appearances of NO in biological systems were seen in plants (Klepper 1979), and endogenous NO in plants is thought to be produced through enzymatic and non-enzymatic processes in the oxidation and reduction pathways. The major enzymatic mechanism in the reduction pathway is the formation of NO from nitrite under the action of nitrate reductase (NR) (Tejada-Jimenez et al. 2019; Chamizo-Ampudia et al. 2017; Kaiser et al. 2018). Non-enzymatic nitric oxide generation, on the other hand, involves mitochondrial electron transport chains in low-oxygen conditions and deoxygenated heme-proteins in acidic environments (Sami et al. 2018), nitrous oxide breakdown, and chemical reactivity between NOs and plant metabolites. The most important sources of NO synthesis in plants are the NR and L-arginine dependent pathways through nitric oxide synthase (NOS)-like activity (Kolbert et al. 2019).

For the first time, the biological involvement of NO in potato immunological responses has been revealed. NO helps in the metabolic switch from growth and development to a stress reaction (Ageeva-Kieferle et al. 2021). In horticultural crops, NO has an important role in plant development (germination, seedling growth, biomass accumulation, and yield), defence, and post-harvest management (respiration, ethylene production, and senescence) (Sun et al. 2021).

NO interacts with other gaseous molecules and phytohormones in plants to serve as a signalling molecule (Buet et al. 2021). They are involved in immunity signalling pathways and disease resistance (Prakash et al. 2021), chilling injury (Ziogas et al. 2013; Sehrawat and Deswal 2014; Jiao 2021), postharvest disease alleviation (Yang and Liu 2019; Khaliq et al. 2021), ripening and senescence (Khaliq et al. 2021; Mansouri et al. 2021).

8.3 Role of NO in Postharvest Management of Fruits and Vegetables

The perishable nature of fruits and vegetables favours a large amount of postharvest losses for a variety of causes and is a major problem across the world. Numerous approaches and strategies have been implemented to decrease postharvest losses. NO, as an eco-friendly compound with low food safety concerns, can play an important role in regulating and signalling various biochemical pathways such as colour (Gheysarbigi et al. 2020), respiration rate (Steffens et al. 2021), ethylene production (Liu et al. 2019), ripening (Zuccarelli et al. 2021), senescence, alleviation of chilling injury, biotic and abiotic stresses (Siddiqui et al. 2021).

8.3.1 Effect on Nutritional Quality and Shelf-Life

In addition to the supply of major nutrients like carbohydrates, lipids, proteins, vitamins, and minerals, fruits and vegetables also provide a diverse spectrum of bioactive chemicals including phenolic compounds and carotenoids, which have a functional role in human health (Jideani et al. 2021; Arumugam et al. 2021). Physiological weight loss, which steadily increases during storage with respiration and transpiration, is the primary cause of quality deterioration in harvested horticultural commodities (Zhang et al. 2019). Organoleptic properties of horticultural crops, particularly fruits, are influenced by organic acids and sugars present in them (Baccichet et al. 2021). Sugars are an important source of energy for a variety of metabolic processes (Duran-Soria et al. 2020). NO treatment increased the expression level of *PpaSPS1/2* in peach, which increased the activity of sucrose phosphate synthase (SPS) (cv. Xiahui 6). The expression of *PpaAI1* gene encoding the sucrose-cleaving enzyme fell significantly, resulting in poorer sucrose-cleaving enzyme activity and high sugar levels (Han et al. 2018). By maintaining a greater sucrose content and retaining more metabolites, freshness and shelf life were preserved (Aghdam et al. 2018). For all sorts of fleshy fruits, firmness is a key quality indicator. The hydrolase enzymes polygalacturonase (PG) and pectin methylesterase (PME) is responsible for the loss of firmness (Zhang et al. 2019). The NO treatment reduced the activities of PG, PME and endo-1,4-glucanase while maintaining larger levels of acid-soluble pectin and starch, which could explain the delay in fruit softening (Cheng et al. 2009). Sugar metabolism involves sucrose phosphate synthase, and sucrose-cleaving enzymes (*PpaAI1* and *PpaNI1/2* genes) and their lower encoding levels resulted in decreased sucrose-cleaving enzyme activity and maintained increased firmness in peach fruit (Han et al. 2018). The expression of calcium ion (Ca^{2+}) signal-related genes (*CNGC1, CPK1, CIPK2, CML31, CML48, ZIFL1*) in kiwi fruit differed substantially, and they were implicated in the control of the NO softening response (Yang et al. 2021). Colour is one of the most visible indicators of ripeness and quality (Alos et al. 2019). The pigments (carotenoids: carotenes plus xanthophylls) in the plastids are responsible for the orange and red colour in the fruit, and the breakdown of chlorophyll operates while the pigments increase in the plastids during ripening. The activity of the enzymes chlorophyllase and Mg-dechelatase was regulated by NO treatment thus delaying the breakdown of chlorophyll (Wang et al. 2015). Nitric oxide slowed the enzymatic browning promoted by phenylalanine ammonia-lyase (PAL), peroxidase (POD), and polyphenol oxidase (PPO) (Gheysarbigi et al. 2020; Zhao et al. 2020). Because of the vast spectrum of health-promoting qualities of phytochemicals, their retention at greater levels in fruits and vegetables is of major interest (Thakur and Sharma 2018). NO reduced reactive oxygen species (ROS) damage to cells and extended the shelf life of fruits and vegetables by maintaining larger levels of phenols, anthocyanins, carotenoids, ascorbic acid, and flavonoids (Zhang et al. 2020; Huang et al. 2021). The role of NO in phytochemical retention in various crops has been summarized (Table 8.1).

8.3.2 Effect on Postharvest Ripening and Senescence

In fruit and vegetables, respiration is a fundamental and inevitable physiological activity (Irtiza et al. 2019). There is a surge in ethylene synthesis in climacteric fruits parallel to the respiration process during climacteric peak, and they are marked by quick senescence, loss of sensory qualities, and poor nutritional quality. This marks the beginning of ripening and the first stage of senescence. Several biochemical, physiological, and structural changes occur throughout the ripening process. The rate of senescence is largely determined by respiration rate and ethylene biosynthesis. Ethylene biosynthesis is a major contributor to accelerated senescence because it generates a large number of ROS, which obstruct normal cellular metabolism, resulting in a shorter shelf-life of fruits and vegetables (Liu et al. 2019).

Nitric oxide works antagonistic to ethylene, inhibiting its production and gene expression (Mukherjee 2019; Zhang et al. 2020; Palma et al. 2019; Qian et al. 2021). NO was shown to repress the expression of genes involved in ethylene biosynthesis enzymes, as well as post-translationally modifing methionine adenosyl transferase (MAT) activity through S-nitrosylation, reducing the availability of methyl groups required to produce ethylene (Manjunatha et al. 2012). NO suppresses ethylene-induced fruit ripening through interacting with other phytohormones such as ABA, auxin, jasmonic acid, salicylic acid, gibberellic acid, cytokinin, brassinosteroids, and polyamines, as well as reducing ethylene production (Palma et al. 2019; Steelheart et al. 2019). Climacteric fruits ripen faster and are characterized by rapid senescence, loss of sensory qualities, and reduction of nutritional quality. NO gas fumigation greatly slowed the ripening and softening of kiwifruit with a significant change in the expression of genes involved in ethylene production and signal transduction. 1-Aminocyclopropane carboxylic acid oxidase (*ACO*), the ethylene receptors (*ERS1, ETR2*), and the ethylene-responsive transcription factors (ERF016, ERF7, ERF010, ERF062, ERF110, ERF037, ERF008, ERF113, ERF12, ERF95) were lower in the NO-treated kiwifruit (Yang et al. 2021). Tomato fruits treated with NO delayed the expression of *LeACO1*, *LeACOH2*, and *LeACO4* genes related to 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) activity (Eum et al. 2009). NO significantly delayed ripening and senescence in various crops (Table 8.2).

Table 8.1 Effect of Nitric Oxide on nutritional quality and shelf-life extension

Crop	Source and Conditions	Effects	Reference
Banana	SNP 5 mM at RT	Suppression of ACO activity and transcription of gene *MA-ACO1* which resulted in lower ethylene production, suppressed the activity of PG, PME, and endo-β-1,4-glucanases and delayed ripening and increased shelf-life up to 8 days.	Cheng et al. (2009)
Pistachio hull	SNP 15 μM at 2 ± 1 °C with 93 ± 2% RH	Inhibited the activity of PPO, PAL, and POD, increased activity of SOD, preserved total phenolics, flavonoids, and antioxidant activity, higher lightness values (L*) and lower browning index and colour changes.	Gheysarbigi et al. (2020)
Guava	SNP 1 mM at RT (20 ± 3 °C)	Prevented weight loss and breakdown of chlorophyll, ascorbic acid, phenols, and flavonoids, higher antioxidant activity.	Sahu et al. (2020)
Winter jujube	NO 20 μL/L at 0 ± 1 °C and 90–95% RH	Maintained total soluble solids, titratable acidity and reduced browning of the flesh.	Zhao et al. (2020)
Persimmon	SNP 1.5 mM at 1 °C and 90% RH	Delayed weight loss and ripening, retained greater total antioxidant activity, total phenolic compounds and firmness.	Shahkoomahally et al. (2015)
Tainong mango fruit	SNP 0.25 mM at RT (23 °C)	Repressed respiration rate, reduced softening, rot index, peel colour changes, and weight loss, maintained higher TSS, titratable acidity, ascorbic acid, and phenolic compounds.	Ren et al. (2017)
Mango (*cv.* Kensington pride)	NO 20 $\mu L/L^{-1}$ at RT (21 °C)	Higher pulp firmness, lower ethylene production by inhibiting ACC synthase and ACC oxidase activities, maintained better pulp firmness by reducing endo-1,4-d-glucanase, exo-polygalacturonase, and endo-polygalacturonase activities.	Zaharah and Singh (2011)
Litchi	SNP 2.0 mM at RT	Reduced PAL activity, degradation of anthocyanin and other bioactive compounds (phenolics, ascorbic acid), minimal pericarp browning, weight loss, loss of TSS, titratable acidity, and antioxidant capacity and extended the shelf life up to 8 days.	Barman et al. (2014)
Pointed gourd	NO 1 and 2 mM at RT	Maintained higher chlorophyll, phenolics, antioxidant activity and membrane integrity, while reducing the weight loss, yellowness, lignification, and electrolytic leakage and extending the shelf life by extra 3 days at RT.	Siddiqui et al. (2021)
Kiwifrut	NO 1.5% (v/v???) at 1 °C and RH 95%.	Delayed the increase in soluble solids and weight loss, maintained a higher vitamin C, total phenol content, antioxidant capacity and TA during 70 days of storage.	Saadatian et al. (2012)

(continued)

Table 8.1 (continued)

Crop	Source and Conditions	Effects	Reference
Red raspberry	NO 15 μM at RT	Prevented oxidative damage, preserved higher flavonoids and anthocyanin, and maintained the quality by affecting the activities of sucrose phosphate synthase, neutral invertase, glucose-6-phosphate isomerase, and sucrose synthase in the soluble sugar metabolism.	Shi et al. (2019)
Chinese bayberry	NO 20 μL/L^{-1} stored at 0.5 °C and 90% RH	Slowed down the loss of firmness, total phenolic content, and DPPH radical-scavenging action, decreased membrane permeability and lipid peroxidation delayed $O_2^{\cdot-}$ and H_2O_2 content generation and boosted SOD, CAT, and APX activities.	Wu et al. (2012)

SNP sodium nitroprusside, *RT* room temperature, *ACO* ACC oxidase, *PG* polygalacturonase, *PME* pectin methyl esterase, *PPO* polyphenol oxidase, *PAL* phenyl alanine, *POD* peroxidase, *SOD* superoxide dismutase, *TSS* total soluble solids, *DPPH* 2,4-diphenyl picryl hydrazide, *CAT* catalase, *APX* ascorbate peroxidase

Table 8.2 Role of Nitric Oxide in postharvest senescence

Crop	Source	Effect	Reference
Peach (cv. Xiahui 6) and cv. 'Xiahui (NO.5)	NO 10 μL L^{-1} stored at 4 °C.	Delayed ripening, increased the expression of PpaSPS1/2 and activity of sucrose phosphate synthase, reduced gene expression encoding sucrose cleaving enzyme PpaAI1 which resulted in delayed senescence.	Kang et al. (2016) and Han et al. (2018)
Sweet pepper	NO 5 ppm at RT	Delayed fruit ripening, lipid peroxidation and accumulation of reactive oxygen/nitrogen species, altered the activity of ascorbate peroxidase and lipoxygenase.	Gonzalez-Gordo et al. (2019)
Papaya (Sui you 2)	NO 60 μL/L, at 20 °C and 75% RH	Suppressed ethylene production, respiration rate, reduced softening and ripening related changes in peel colour, delayed ripening and senescence.	Li et al. (2014)
Lettuce shreds	NO 100 ppm at 4 °C and 12 °C	Delayed senescence by reducing the H_2O_2 accumulation and extending the shelf life up to 21–22 days in mature butter head shreds and 25–26 days in young shreds.	Iakimova and Woltering (2015)
Nectarine (*var.* nucipersica)	SNP 0.5 mM	Reduced lipid peroxidation by slowing down the activity of lipoxygenase and PME activity which delayed the senescence and improved the quality.	Jayarajan and Sharma (2018)

SNP sodium nitroprusside, *RT* room temperature, *RH* relative humidity

8.3.3 Effect on Antioxidant Systems

The formation of a variety of ROS as a result of oxygen-mediated metabolism or environmental challenges experienced by the organism is well-known and acknowledged (Zhang et al. 2019). Singlet oxygen, superoxide radicals, hydrogen peroxide, and hydroxyl radicals are examples of these ROS (Meitha et al. 2020). Increased ROS generation in fruits and vegetables during postharvest storage should be countered by antioxidant systems to prevent their cells from ageing, allowing them to have a longer shelf-life. Both lipid-soluble antioxidants (α-tocopherol and carotenoids) and water-soluble reductants (glutathione and ascorbate), as well as enzymes such catalase glutathione reductase, ascorbate peroxidase, superoxide dismutase, and peroxidases, are involved (Ghorbani et al. 2017). Ascorbic acid, reduced glutathione, vitamin E (α-tocopherol), polyphenols, and carotenoids are examples of non-enzymatic antioxidants (Zhang et al. 2019). Through the ascorbate-glutathione cycle, NO may usually postpone senescence and sustain the antioxidant system (Ma et al. 2019). By reducing ROS generation and membrane lipid peroxidation in the peel and pulp, NO treatment boosted the activities of superoxide dismutase, peroxidase, ascorbic acid peroxidase, glutathione reductase, and catalase, which improved the quality of table grape during storage. NO treatment, the expression of genes VvSOD and VvCAT were down regulated in the peel and pulp of grapes (Zhang et al. 2019). NO gas fumigation of peach fruits reduced ROS levels while increased ascorbate (AsA) and reduced glutathione (GSH) levels. Through the ascorbate-glutathione cycle, NO may be able to postpone fruit senescence. The activities of dehydroascorbate reductase (DHAR), ascorbate peroxidase (APX), glutathione-S-transferase (GT), and glutathione reductase (GR) were all boosted by nitric oxide. NO treatment raised the ratios of reduced/oxidized glutathione (GSH:GSSG) and ascorbate/dehydroascorbate (AsA:DHA) while retaining greater 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) scavenging capacity. The findings showed that exogenous NO might boost the AsA-GSH cycle's lowering ability and keep peaches' antioxidant capacity high during storage (Ma et al. 2019). Roles of NO in maintaining antioxidant system are described in Table 8.3.

8.3.4 Effect of on Chilling Injury

When sub-tropical and tropical fruits are stored under a temperature below 10–15 °C for a certain period, chilling injury happens. Some of the consequences of chilling injury are surface pitting, discolouration, uneven ripening, reduced flavour and texture, internal breakdown and decay. Chilling injury can be alleviated using methods like temperature preconditioning, intermittent warming, controlled or modified storage, chemical treatments, hormonal regulations and genetic manipulations (Wang 1989).

Chilling damage in tissues is associated with the development of oxidative stress from excess ROS (Hodges et al. 2004). To protect these tissues from ROS damage,

Table 8.3 Effect of Nitric Oxide on antioxidant system

Crop	Source of Nitric Oxide	Effect of Nitric Oxide on antioxidant system	Reference
Banana	NO 60 μL L^{-1} at 22 °C	Boosted the enzyme activity of SOD, POD, APX, and CAT, increased *MaSOD*, *MaCAT*, *MaPOD*, and *MaAPX* gene expression, and reduced electrolyte leakage, and levels of MDA, $O_2^{\cdot -}$, and hydrogen peroxide (H_2O_2).	Wu et al. (2014)
Grapes *cv*. Rish Baba	NO 0.5 mM at −0.5 °C and 95% RH	Reduced ion leakage, lipid peroxidation, MDA and (H_2O_2) content, enhanced POD, APX, SOD, and CAT activity, and maintained a healthy endogenous antioxidant defence system.	Ghorbani et al. (2017)
Winter jujube	NO 20 μL/L at 0 ± 1 °C and 90–95% RH	Higher SOD, CAT, APX, and GR activities, increased ROS scavenging ability resulting in the reduction of oxidative damage and the preservation of cell membrane integrity.	Zhao et al. (2020)
Tainong mango	SNP 0.25 mmol/L at RT	Increased the activities of SOD, CAT, and POD while decreasing the activities of LOX and PPO which was linked to lower levels of malondialdehyde, superoxide anion radical ($O_2{\bullet}^{-}$), and H_2O_2, protected from oxidative damages caused by ROS.	Ren et al. (2017)
Peach	SNP 15 μmol L^{-1}	Reduced H_2O_2 content and $O_2^{\cdot -}$ generation rates, increased the expression of *PpG-6-PDH*, *Pp6PGDH*, and *PpAOX* while down-regulated the expression of *PpGPI* and *PpHK*, signifying that the pentose phosphate and cyanide-resistant respiration pathways were stimulated which improved antioxidant ability.	Song et al. (2021)
Sweet cherry	SNP 60 mmol/L at 0 °C	Reduced accumulation of ROS, higher levels of antioxidant enzyme activity.	Ma et al. (2019)

SNP sodium nitroprusside, *RT* room temperature, *ACO* ACC oxidase, *PG* polygalacturonase, *PME* pectin methyl esterase, *PPO* polyphenol oxidase, *PAL* phenyl alanine, *POD* peroxidase, *SOD* superoxide dismutase, *CAT* catalase, *APX* ascorbate peroxidase, *GR* glutathione reductase, *MDA* malondialdehyde, *LOX* lipoxygenase, *ROS* reactive oxygen species

plants have evolved a complex antioxidant system, which also balances the production and removal of ROS. Studies suggested that there is a positive relationship between antioxidant enzyme activity and tolerance to chilling injury in fruits after harvest

The development of chilling injury symptoms might be because of the oxidative stress from excess ROS which eventually induces peroxidation and breakdown of unsaturated fatty acids in membrane lipids (Lyons 1973). Nitric oxide is one of the significant signalling molecules that is involved in many plant physiological processes. It is also known to protect plant cells against oxidative stress by reducing ROS accumulation (Xu et al. 2017; Zhang et al. 2019). NO possibly acts as an antioxidant, which can scavenge the ROS, and hence protect the plant cells from oxidative damage. Many studies have revealed that, NO, when applied exogenously, has improved chilling injury tolerance as well as reduced chilling injury damage in more than a few fruits (Zhu et al. 2006; Singh et al. 2016; Rehman et al. 2019) (Table 8.4).

Table 8.4 Effect of nitric oxide on alleviation of chilling injury and fruit quality

Crop	Source of Nitric Oxide	Effect of nitric oxide on alleviation of chilling injury	Reference
Washington Navel Orange	SNP 0.5 mM	Reduced the incidence of chilling injury, lipid peroxidation and hydrogen peroxide content in peel and pulp during storage and also induced the activity of antioxidant enzymes and DPPH radical scavenging activity	Ghorbani et al. (2018)
Sweet Orange	NO gas 10 muL/L	Reduced chilling injury and improved antioxidant concentration in sweet oranges at low temperatures and also maintained the quality.	Rehman et al. (2019)
Mango (Kensington Pride)	NO gas	Alleviated the chilling injury index and increased the tartaric and shikimic acid content in fruits in cold storage during ripening	Zaharah and Singh (2011)
Mango (Chausa)	SNP	Reduced the chilling injury incidence and electrolyte leakage irrespective of concentration	Barman et al. (2014)
Peach	SNP	Alleviated chilling injury, reduced internal browning index, malondialdehyde content, electrolyte leakage and lipoxygenase activity while maintaining firmness	Zhao et al. (2021)
Japanese Plum	NO gas	Chilling injury symptoms like flesh browning and translucency were significantly lower in NO fumigated fruits than that non-fumigated ones	Singh et al. (2009)
Cucumber	NO gas	Enhanced chilling tolerance in cucumber by improving antioxidant defence system	Yang et al. (2011)
Hami melon	NO gas	Decreased the chilling injury index and incidence, reduced membrane permeability and malondialdehyde content, inhibited superoxide production rates and also sustained higher antioxidant enzyme activity of Hami melon fruit during storage at 1 ± 0.5 °C	Zhang et al. (2017)

8.3.5 Effect on Postharvest Diseases and Pest

Several studies in the past have demonstrated the role of NO as a potential fumigant in controlling the postharvest diseases of fruits and vegetables. Exogenous application of NO could reduce fruit ripening and consequently increases the quality of fruits and vegetables such as strawberry, apple, cucumber and lettuce. Increasing evidence as a fumigant against a wide range of diseases and pests in fruits and vegetables enables NO in controlling post-harvest diseases (Table 8.5).

8.4 Cross-Talk Among NO with Ethylene, H_2S and Melatonin During Ripening

The interaction between NO and ethylene during fruit ripening is antagonistic. Various studies have confirmed the role of NO against ethylene-mediated responses during fruit ripening (Guo et al. 2014; Palma et al. 2019; Corpas et al. 2020). In this

Table 8.5 Effect of NO in controlling various pests and diseases

Fruits or vegetables	NO concentration and outcomes	Disease or pest controlled	Reference
Strawberry and Sweet cherry	8 h fumigation with 3.0% (v/v) NO	Winged *Drosophila*	Yang and Liu (2018) and Walse et al. (2016)
Lettuce	16 h fumigation with 0.5% (v/v) NO at 2 °C	Thrips and Aphids	Liu (2016) and Yang and Liu (2019)
Apple	24 h fumigation with 3% NO (v/v) at 2 °C	Codling moth	Liu (2016)
Apple	Exogenous applications of NO reduce the *Penicillium* rot	*Penicillium* rot	Lai et al. (2014)
Papaya	0.1 mM sodium nitroprusside (a NO donor) for 8 min reduces the lesion expansion	*Colletotrichum* rot	Hu (2019)
Tomato	Arginine percussor of NO induces resistance in tomato	*Botrytis* rot	Zheng et al. (2011)

context, NO forms a ternary complex (ACC-ACC oxidase-NO) with ACC oxidase enzyme and reduced the gene expression (MA-ACO1) involved in ethylene biosynthesis in fruits (Rudell and Mattheis 2006; Zhu et al. 2006; Cheng et al. 2009). It also reduced the concentration of ACC by inhibiting the activity of ACC synthase which results in lower ethylene production.

NO and H_2S show both complementary and inhibitory interactions which depend on the doses (Li et al. 2012; Lisjak et al. 2013). The interaction of NO and H_2S exhibits a synergistic effect on delaying the ethylene-induced ripening of fruits. Postharvest application of H_2S and NO enhanced the anti-ripening effects and reduced respiratory burst in harvested fruits (Chang et al. 2014). The molecular mechanism might be due to the down-regulation of the expression of the ethylene biosynthesis gene and also chlorophyll degrading genes (Mukherjee 2019). Both signalling molecules showed a synergistic effect on delaying the ripening and senescence of fruit. Therefore, the role of NO and H_2S is found crucial in regulating the ethylene-mediated changes during fruit ripening.

Recently, phytomelatonin is recognized as a powerful signalling molecule which affects several physiological processes, especially antioxidant systems as free radical scavengers. Melatonin enhances antioxidant capacity during the ripening of fruits. It also promotes the biosynthesis of pigments and flavonoids and triggers transcriptomic changes in grapes (Xu et al. 2017). Postharvest application of melatonin triggers an antioxidant defence mechanism during ripening and increases the shelf life of fruits and vegetables. The exogenous application of melatonin alters the polyphenol and carbohydrates and partially regulates the ethylene biosynthesis. However, melatonin is not an inhibitor or inducer of fruit ripening unlike NO or H_2S, but it acts as a regulator in the ripening process and regulates redox homeostasis. The application of melatonin delayed senescence by activating the NO synthesis during storage, which antagonizes the effect of ethylene (Liu et al. 2019). Melatonin regulates redox homeostasis and metabolic processes during ripening by increasing the concentration of NO which produces reactive nitrogen species (RNS), and melatonin maintains this RNS homeostasis (Singh et al. 2016).

8.5 Conclusion and Future Aspects

In the postharvest management of horticultural crops, nitric oxide (NO) has a variety of physiological and biological effects. NO, as a signalling molecule, makes a significant contribution to maintaining the quality and shelf life of fruits and vegetables by delaying critical processes such as ethylene biosynthesis, ripening, and senescence. The focus of future research would be on overcoming obstacles utilising existing and innovative procedures and treatments to improve NO efficiency. The application of NO donors in postharvest is mostly limited to an experimental level. Thus, the potential of NO to elicit endogenous levels of NO by regulating the sources, mechanisms behind its synthesis and interaction with other phytohormones and signalling molecules can be a great area of research.

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Chapter 9
Interaction of Hydrogen Sulfide with Phytohormones During Plant Physiological and Stress Conditions

Nandni Sharma, Deepak Kumar, Kanika Khanna, Ripu Daman Parihar, Sandeep Kour, Renu Bhardwaj, and Puja Ohri

Abstract Hydrogen sulfide (H_2S) has been identified as the third endogenous gas transmitter after CO (carbon monoxide) and NO (nitric oxide). It is a small, reactive signaling component that is synthesized in chloroplasts with the assistance of the enzyme sulfite reductase, a major enzyme of the assimilatory sulfate reduction pathway. Additionally, the synthesis of H_2S is carried out with the help of L-cysteine desulfhydrase and β-cyano-alanine synthase C1(CAS-C1). These enzymes are involved in the desulfhydration of L-cysteine and β-cyano-alanine in cytosol and mitochondrion, respectively. Initially, H_2S was considered to be a toxic molecule, but now recent literature has illustrated that plants utilize H_2S in a variety of processes, including organogenesis, growth, photosynthesis, and stomatal conductance. Furthermore, the exogenous application of H_2S has an influence on the versatile physiological and antioxidant system of plants under both favorable and unfavorable circumstances. Under stressful conditions, H_2S interacts with other plant hormones and gasotransmitters, thus modulating the plant response against different types of abiotic stresses like thermal stress, salinity, heavy metal toxicity, drought, etc. So, keeping into consideration all these potencies of H_2S, the current book chapter focuses on the interaction of H_2S with different phytohormones toward the regulation of abiotic stress response in plants.

Keywords Phytohormones · Gasotransmitters · Abiotic stress · Metal toxicity

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T. Aftab, F. J. Corpas (eds.), *Gasotransmitters Signaling in Plants under Challenging Environment*, Plant in Challenging Environments 5,
https://doi.org/10.1007/978-3-031-43029-9_9

9.1 Introduction

Sulfur is among the key elements that is crucial for the metabolism of a number of components like Met (Methionine), Cys (Cysteine), thiamine, biotin, nitrogenase, Fe-S cluster and Coenzyme A. Inorganic sulfur that is naturally present in soil can be utilized only by plants, fungi, algae and few prokaryotes. They convert inorganic form to organic form (Takahashi et al. 2011). Specifically, in case of plants, the inorganic form (SO_4^{2-}), absorbed by roots first undergoes reduction to form H_2S, which is then used for the synthesis of various amino acids, thus becoming the major component in the thio-metabolism cascade (Xuan et al. 2020).

H_2S, a toxic gaseous component that exerts serious effects on both animals and plants (Hancock 2017), has been documented to have potential to act as gasotransmitter in plants and perform essential functions during different developmental stages ranging from germination, root development, stomatal movement to abscission (Banerjee et al. 2018; Corpas and Palma 2020). As per previous literature, H_2S performs its function in dose dependent manner, i.e., acts as a cytotoxin at high concentration and a signalling entity at relatively low concentrations (Li et al. 2016; Jin and Pei 2015). So, the rate of homeostasis of H_2S must be maintained in the cell by the regulation of H_2S biosynthesis and degradation (Corpas and Palma 2020; Xuan et al. 2020). The role and functioning of H_2S in animal cells have been extensively studied, but there are only few reports on its role in plant cells. Research is being carried out in plants and many botanists have reported that, like other gasotransmitters, i.e., CO and NO, H_2S also functions as a signalling entity that modulates normal physiological and stress responses in plants (Khanna et al. 2021; Ahmed et al. 2021). The most important endowments of H_2S to plant physiology encompasses seed germination, root organogenesis, development of lateral roots and regulation of photosynthesis (Zhang et al. 2009a, 2010a, b; Fang et al. 2014; Chen et al. 2011). Moreover, H_2S has also demonstrated to possess protective roles against various biotic (Shi et al. 2015) and abiotic stresses that include osmotic, chilling, salt, heat and drought (Wang et al. 2010; Zhang et al. 2010b; Jin et al. 2011, 2013; Shen et al. 2012, 2013; Xie et al. 2014).

However, the exact signalling cascade regulated by H_2S remains unknown, so now the main focus of researchers is to understand the mechanism adapted by H_2S under both favourable and unfavourable circumstances. Moreover, it has been well documented that H_2S is involved in the per-sulfidation of various secondary metabolites and proteins. Additionally, H_2S has the potential to interact with ionic signals, other gasotransmitters and different stimulatory as well as inhibitory plant hormones (Li et al. 2016). As the name depicts, inhibitory hormones suppress the plant growth during stressful conditions whereas, stimulatory hormones stimulate the growth and development processes in plants. Thus, suggesting that all the physiological processes including cell division, germination, senescence and response to stress or adverse conditions are under the influence of both inhibitory and stimulatory plant hormones (He et al. 2019). Also, these phytohormones regulate the signalling transduction pathways by interacting with one another and also with other gasotransmitters including H_2S (Jin and Pei 2015; Xuan et al. 2020). Keeping into consideration all these points, the current chapter summarizes the biosynthesis and biological role of H_2S and also its interaction with phytohormones for the modulation of numerous growth and developmental processes under favourable and unfavourable circumstances.

9.2 Biosynthesis of Hydrogen Sulfide in Plants

Biosynthesis of H_2S in plants is mainly under the influence of various enzymatic pathways that usually occur in three main compartments of plant cells viz., cytosol, chloroplast, and mitochondrion (Aroca et al. 2018). The major enzymes involved in the process of biosynthesis include cysteine synthase (CAS, EC4.4.1.9), assimilatory sulfite reductase (ferredoxin) (SiR, EC 1.8.7.1), L-3-cyanoalanine synthase (CS, EC 4.4.1.1), D-cysteine desulfhydrase (D-DES, EC 4.4.1.15), L-cysteine desulfhydrase (cystathionine gamma-lyase, L-DES, EC 4.4.1.1) (Yamasaki and Cohen 2016). H_2S is known to be synthesized endogenously in plant cells either by reduction of sulfite (SO_3^{2-}) or by the breakdown of cysteine. In chloroplast, ferredoxin and SiR are necessary for the reduction of SO_3^{2-}. The process of H_2S biosynthesis starts with the absorption of atmospheric sulfur, i.e., SO_2 or sulfate (SO_4^{2-}) by the plant through root tissue. Sulfate is further transferred to upper plant parts with the help of sulfate transporters (Sultrs). After entry into the plant cell, sulfate preferentially enters into chloroplast with the assistance of Sultr3s, where it undergoes transformed into adenosine-5′-phosphosulfate (APS), which is then converted into SO_3^{2-} with the help of a catalyst, ATP sulfurylase (EC 2.7.7.4) and APS reductase (EC 1.8.99.2) respectively (Li 2015). Sulfite thus formed, is reduced to sulphide (H_2S) in the presence of SiR. Further, H_2S undergoes a reaction with O-acetyl serine to generate cysteine, a basic amino acid involved in the formation of polypeptides. Another mechanism of biosynthesis of H_2S from cysteine occurs in cytosol. Basically, H_2S is generated as a by-product in biosynthesis of cysteine that is carried out in presence of OASTL (O-acetyl serine thiol lyase, EC 4.2.99.8) enzymes. Firstly, acetyl-CoA and serine undergo catalysis in presence of SAT (serine-acetyltransferase, EC 2.3.1.30), resulting in the formation of an intermediary product, OAS (O-acetyl-Ser), which is then converted into cysteine by the incorporation of sulfide. This step is followed by L-DES based catalysis of cysteine into H_2S, NH_4^+ and pyruvate in presence of a cofactor, pyridoxal phosphate that helps in accelerating the rate of reaction. In a similar manner, D-DES catalyses the conversion of D-cysteine into H_2S, NH_4^+ and pyruvate (Li 2015). Moreover, CAS (in mitochondrion) is also involved in cyanide detoxification and promotes the transformation of cyanide at the expense of cysteine to β-cyanoalanine, which is further involved in initiation of H_2S biosynthesis. Sulfide thus synthesised is utilized for the synthesis of cysteine in presence of OASTL, which is further used by CAS for cyanide detoxification, thus resulting in the generation of cyclic pathway in mitochondria (Fig. 9.1).

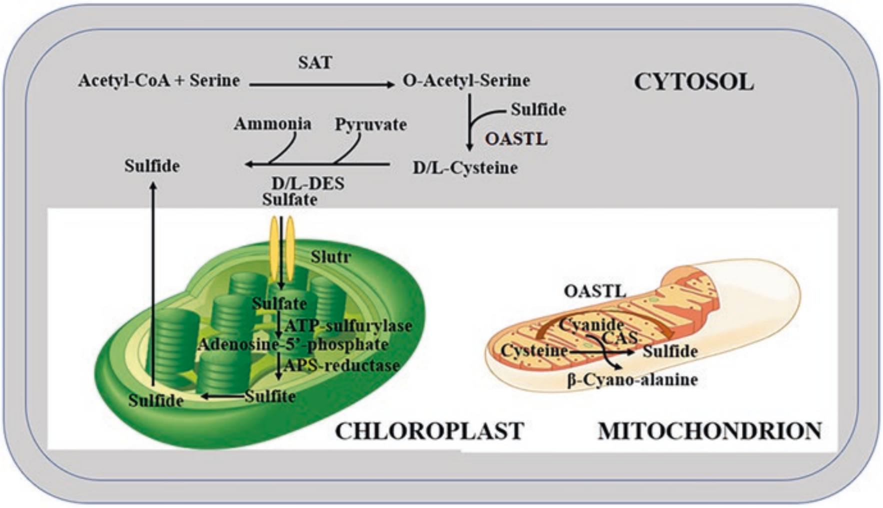

Fig. 9.1 Biosynthesis of hydrogen sulfide in plants. Hydrogen sulfide (H_2S) biosynthesis occurs in three subcellular compartments within plant cells, including the cytosol, chloroplasts and mitochondria. In the cytosol, the acetyl-CoA and serine undergoes a catalytic reaction facilitated by SAT (serine-acetyltransferase), resulting in the formation of OAS (O-acetyl-serine). Subsequently OASTL (O-acetyl serine thiol lyase) enzyme catalyzes the addition of sulfide to OAS (O-acetyl-ser) to form L/D cysteine. H_2S is then generated from L/D cysteine through a reaction catalyzed by L/D-CDES (L/D cysteine desulfhydrases), resulting in the release of ammonia and pyruvate. Chloroplasts generate H_2S during photosynthetic sulfate reduction. In mitochondria, H_2S is produced from cysteine via the release of cyanide and β-cyanoalanine, which is facilitated by the CAS (β-cyanoalanine synthase) enzyme

9.3 H_2S Mediated Post-translational Changes During Oxidative Stress in Plants

Since last one decade the knowledge of oxiPTMs (thiol-based oxidative post-translational modifications) has increased (Corpas et al. 2022). H_2S molecule acts as a signaling molecule in the process known as persulfidation. In this post-translational modification, H_2S mediates the conversion of the thiol present on cysteine residues (-SH group) into persulfide (-SSH) group of proteins. Post-translational modifications required for the function of many proteins are based on redox modifications which are cysteine-based (Buchanan and Balmer 2005; Chung et al. 2013). In protein, the thiol group reacts with hydrogen peroxide to form sulfenic acid (R-SOH) which thereafter undergoes reaction with H_2S to produce R-SSH which is a persulfide product. The product generated after reaction further reacts with ROS (reactive oxygen species) and generates R-SSOH (perthiosulfenic acid) which is a less stable product. In presence of higher concentration of oxidants, R-SSOH gets oxidized to form perthiosulfonic acid ($R\text{-}SSO_3H$)

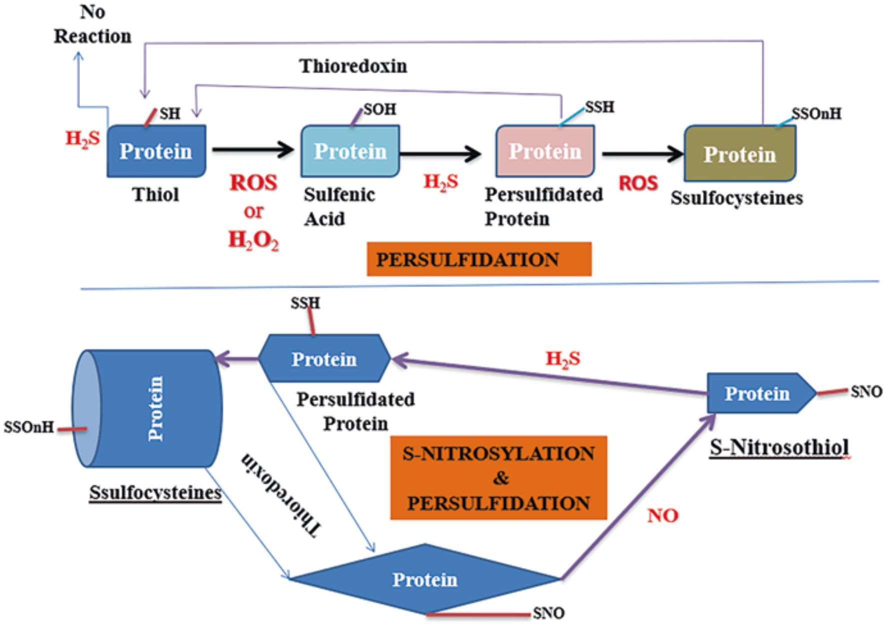

Fig. 9.2 Post-translational changes mediated by H_2S

and perthiosulfinic (R-SSO_2H) (Filipovic 2015). It has also been reported that thioredoxin regulates the persulfidation within the cell (Fig. 9.2) (Ren et al. 2017; Wedmann et al. 2016). NO (Nitric oxide), another signaling molecule also manipulates proteins through a process known as S-nitrosation (R-SNO). In this process, the thiol group of cysteine moiety of proteins gets attached to NO (Feng et al. 2019) and forms a product called S-nitrosothiols (Stamler et al. 1992; Hess et al. 2005). This product further reacts with H_2S and results in protein persulfidation (R-SSG). These persulfidated or modified proteins are more reactive due to the increased nucleophilicity of the -SSH group (Zhao et al. 2020). Studies have also reported that NO plays an important role in plant immunity and environmental interactions (Gupta et al. 2020).

9.4 Physiological Role of H_2S in Plants

H_2S has emerged as the major regulator of various physiological processes occurring in plants (Garcia-Mata and Lamattina 2010; Ali et al. 2014). These physiological processes include seed germination, root organogenesis, stomatal movement and photosynthesis (Dooley et al. 2013; Zhang et al. 2009b; Chen et al. 2011).

9.4.1 Effect of H_2S on Seed Germination

Seed germination is a stage through which the life cycle of plant begins. This is the phase when the plant is most susceptible to various unfavourable environmental conditions (Yuan and Wen 2018), so they must be protected during germination. As per reports, H_2S, when applied in proper concentrations, acts as main regulator of germination process under favourable and unfavourable conditions. For instance, as per the reports of Dooley and his co-workers (2013), the treatment of H_2S solution to seeds of corn, pea, wheat, and bean plants resulted in an enhancement in both germination rate as well as the seedlings size. Moreover, the germination time was also reported to be shortened (Dooley et al. 2013). Different stresses like heat, metal, and osmotic stress also induce oxidative damage in plants, especially during the germination phase. It has been reported that even under these stressful conditions, H_2S has the potential of promoting germination rate. For instance, wheat plants growing under aluminium, copper, or osmotic stress, increased H_2S content as well as seed germination rate after the application of the H_2S donor, NaHS (sodium hydrosulfide). In addition to this, uptake of copper by the plant was also restricted, thus ultimately lowering the contents of hydrogen peroxide and malondialdehyde in plants (Zhang et al. 2008, 2010a, b).

9.4.2 Role of H_2S in Lateral Root Formation

H_2S perform a remarkable role in developing lateral roots in plants by undergoing interactions with NO, H_2O_2, and indole acetic acid. Application of H_2S to *Ipomoea batatas* (sweet potato) seedlings resulted in enhanced number and size of adventitious roots (Zhang et al. 2009a; Mishra et al. 2021). Similarly, *Brassica napus* plants growing under selenium stress have been found to suffer from root growth inhibition, but the supplementation of NaHS has been documented to restore the normal root growth by modulating the antioxidative defense system of plants (Chen et al. 2014).

9.4.3 Role of H_2S in Photosynthesis

Stomata act as the apertures involved in the maintenance of gaseous exchange between the environment and plants. The stomatal movement influence various biochemical processes like transpiration and photosynthesis, thus, having a remarkable role in the normal growth and developmental processes in plants (Nunes et al. 2020). The stomatal movement has been reported to be regulated by various environmental factors and phytohormones. However, recent literature has reported H_2S as a potential agent that is involved in regulating stomatal movement (Jin and Pei 2016;

Garcia-Mata and Lamattina 2010). Even a minute dose (0.01 mM) of exogenously applied H_2S has enhanced the photosynthetic rate by increasing stomatal aperture and density in the case of *Oryza sativa* plants growing under normal conditions (Duan et al. 2015). Similarly, in *Arabidopsis* and fava bean, exogenous supplementation of AOA (H_2S synthesis suppressor), Na_2WO_4 (NO synthesis suppressor) or cPTIO (NO chelator) showed that H_2S acted as a downstream signalling molecule in NO-mediated signalling pathway that is involved in ethylene-induced stomatal closure (Hou et al. 2013; Liu et al. 2011). Certain reports also propose that under the elicitation of abscisic acid, H_2S becomes accumulated intracellularly in a short period of time and further results in persulfidation of Cys825 and Cys890 residues of NADPH oxidase RBOHD, thus resulting in the ROS overproduction. This induces closing of stomata and also have negative control on persulfidation of RBOHD thus ultimately inhibiting ABA signalling (Shen et al. 2020). Moreover, H_2S accumulated under the influence of ABA also regulates the persulfidation of SNRK2.6 (SNF1-RELATED PROTEIN KINASE 2.6), which is involved in regulating ABA signalling to promote stomatal closure (Chen et al. 2020a). Therefore, based on all these data, it can be deduced that H_2S, on one side acts as an activator of ABA signaling via persulfidation of SNRK2.6, but on other side acts as feedback regulator via persulfidation of RBOHD, thus having a control on stomatal movement.

9.4.4 Role of H_2S in Delaying Senescence

Senescence is a process that involves programmed cell death in plants during ripening of fruits, leaves fall, and also under challenging conditions (Gregersen et al. 2013; Aroca et al. 2021). This process is under the control of various phytohormones and signalling molecules (Woo et al. 2018). H_2S also regulates the process of senescence. For instance, senescent leaves of *Spinacia oleracea* were reported to contain a higher content of H_2S when compared to young leaves, thus depicting the role of H_2S in the senescence process (Chen et al. 2011). Furthermore, exogenous application of H_2S (0.5 mM) suppressed the destruction of photosynthetic pigments by regulating dark-dependent reactions in *Arabidopsis* leaves. Additionally, it was also involved in the regulation of SAG1 and SAG2 under prolonged dark periods (Wei et al. 2017). However, in case of DES1 mutant *Arabidopsis*, SAG1, SAG2, and other transcription factors were reported to be expressed, thus resulting in premature leaf senescence. According to another report, deficiency of DES1 induced the lipidation and accretion of ATG8 (autophagy-related protein 8) (Alvarez et al. 2012). But the application of donor has been found to have a negative impact on autophagy in *Arabidopsis* (Gotor et al. 2013; Laureano-Marin et al. 2016). Thus, it can be summarized from the reports available that H_2S is involved in modulating senescence by decreasing photosynthetic pigment degradation and ROS accumulation, negatively modulating autophagy, and positively inducing the expression of SAG genes.

9.4.5 H_2S-Mediated Suppression of Organ Abscission

Abscission involves the withering of mature plant organs naturally from the plant itself. This natural phenomenon is related to maturation and senescence (Gulfishan et al. 2019). But under unfavorable conditions, premature or abnormal abscission also occurs in plants (Yasong et al. 2018). And it has been well documented that the process of abscission is regulated by various phytohormones like ethylene, auxin, and salicylic acid (Taylor and Whitelaw 2001). Out of all, ethylene acts as an essential inducer of abscission (Botton and Ruperti 2019; Meir et al. 2019). It also causes IDA (INFLORESCENCE DEFICIENT IN ABSCISSION)-induced abscission by impairing the expression of ADPG2 (ARABIDOPSIS DEHISCENCE ZONE POLYGALACTURONASE 2), an abscission related gene in *Arabidopsis* (Wang et al. 2016). Various recent reports have revealed the participation of H_2S in delaying the ethylene-induced abscission in tomato plants (Liu et al. 2020a). Furthermore, H_2S has been reported to play a vital role in the regulation of indole-acetic acid-related genes thus, leading to enhanced aggregation of auxin in the abscission zone, which further results in delaying the abscission of the petiole (Liu et al. 2020a).

9.5 Role of Hydrogen Sulfide in Ameliorating Abiotic Stress in Plants

Since, the activity of various phytohormones and other signalling transmitters is induced in plants growing under stressed conditions. In such plants, H_2S become also triggered and forms a signalling cascade. The stress alleviating potential of H_2S in plants growing under adverse stress conditions is described in the following sections and in Table 9.1.

9.5.1 Salinity Stress

Salt stress causes a disastrous impact on growth and productivity of various crops. H_2S has been reported to play an essential role in modulating the cellular processes in plants growing under salinity stress. As per the study, melatonin helps in inducing salinity resistance in pepper plants by triggering the levels of various antioxidants and H_2S (Kaya et al. 2020a). In cucumber plants, supplementation of H_2S provided salinity resistance in the plants growing under salt stress by modulating antioxidant activities and also by regulating Na^+/K^+ homeostasis (Jiang et al. 2019). Another study reported by Christou et al. (2013), depicted that supplementation of H_2S

Table 9.1 Effect of H_2S on plant growing under different abiotic stress conditions

S. No.	Treatment	Plant species	Type of Stress	Effect of H_2S on plant	References
1.	0.02 mmol L^{-1} NaHS	*Salix matsudana*	Water	Stimulation of organogenesis of plant root	Zhang et al. (2009a)
2.	0.02 mmol L^{-1} NaHS	*Ipomea batatas*	Water	Initiation of root organogenesis	Zhang et al. (2009b)
3.	0.02 mmol L^{-1} NaHS	*Glycine max*	Water	Stimulation of organogenesis of plant root	Zhang et al. (2009b)
4.	100 µM NaHS	*Vicia faba*	Water	Closing of stomatal aperture	Garcia-Mata and Lamattina (2010)
5.	0.6 mM	*Triticum aestivum*	Water	Increased activities of catalase, ascorbate peroxidase and decreased content of oxidative stress markers like malondialdehyde and hydrogen peroxide.	Zhang et al. (2010a, b)
6.	100 µM NaHS	*Arabidopsis thaliana*	Water	Induction of nitric oxide production in plants.	Scuffi et al. (2014)
7.	500 µM NaHS	*T. aestivum*	Water	Modulation in the expression level of abscisic acid in leaves and root tissues.	Ma et al. (2016)
8.	0.4 mM NaHS	*T. aestivum*	Water	Enhanced activities of superoxide dismutase, dehydroascorbate reductase, mono-dehydroascorbate reductase.	Li et al. (2017)
9.	400 µM NaHS	*Cucumis sativus*	Salinity	Declined lipid peroxidation and modulation in the activities of antioxidants.	Yu et al. (2013)
10.	NaHS	*Cyanodon dactylon* L.	Salinity	Maintenance of ROS in plants by modulating antioxidant defense system.	Shi et al. (2013)
11.	100 µM NaHS	*Medicago sativa*	Salinity	Maintains potassium and sodium homeostasis in plants.	Lai et al. (2014)
12.	NaHS	*Oryza sativa*	Salinity	Decreased absorption of sodium and potassium.	Mostofa et al. (2015)
13.	NaHS	*C. sativus*	Salinity	Modulation in various physiological processes like photosynthesis, carbon metabolism	Jiang et al. (2020)
14.	NaHS	*Malus hupehensis*	Salinity	Maintenance of sodium/potassium homeostasis, decreased oxidative damage and enhanced activities of antioxidant defense enzymes.	Li et al. (2020)

(continued)

Table 9.1 (continued)

S. No.	Treatment	Plant species	Type of Stress	Effect of H_2S on plant	References
15.	NaHS	*Kandelia obovate*	Salinity	Improved photosynthesis, hormone biosynthesis and antioxidant defense mechanism in plants.	Liu et al. (2020b)
16.	NaHS	A. *Thaliana*	Cadmium	Enhanced activities of genes involved in biosynthesis of cysteine, increased activities of antioxidant defense enzymes.	Jia et al. (2016)
17.	NaHS	*Medicago sativa*	Cadmium	Ameliorates toxicity of Cd, whereas H_2S scavengers suppress NaHS based heavy metal tolerance in plants.	Shivaraj et al. (2019)
18.	NaHS	*O. sativa*	Aluminium	Enhanced activities of antioxidant enzymes and sequestration of heavy metals to vacuoles.	Zhu et al. (2018)
19.	Exogenous H_2S	*Brassica oleracea*	Chromium	Increased uptake of nutrients, photosynthetic pigments, gaseous exchange parameters and antioxidant system.	Ahmad et al. (2019)
20.	NaHS	*Zea mays*	Heat	Modulated activities of antioxidant defense system and accumulation of proline.	Zhou et al. (2018)
21.	NaHS	*Cucumis sativa*	Heat	Stimulated antioxidant defense mechanism.	Liu et al. (2019a)
22.	NaHS	*T. aestivum*	Heat	Enhanced levels of nitric oxide and hydrogen peroxide in roots.	Karpets et al. (2019)

alleviated salinity stress by modulating antioxidant and ascorbate/glutathione redox states in strawberries. Furthermore, H_2S also participates in modulation of antioxidative species along with certain transcription factors like dehydration responsive element binding factor, salty overly sensitive gene, and glutathione/ascorbate biosynthesis (Christou et al. 2013). Additionally, H_2S has also been implicated in the NO-induced signalling pathway that has a direct role in salt mitigation in plants (Wang et al. 2012).

9.5.2 Heavy Metal Stress

Various industrial and anthropogenic activities are responsible for the soil pollution. An excessive amount of heavy metal exposure of plants delays of seed germination and seedlings growth, modulation of the antioxidant system, induction of chromosomal aberrations, and in some cases plant death. Heavy metal toxicity also imposes secondary stress in plants like oxidative stress and nutrient imbalance. However, H_2S treatment plays an essential role in the amelioration of heavy metal toxicity in plants. For instance, H_2S alleviated aluminium stress in *Brassica napus* plants by enhancing biomass and levels of various nutrients (Ali et al. 2015). It has also been reported to safeguard *B. rapa* L. *pekinensis* plants growing under cadmium toxicity from oxidative stress and growth inhibition (Zhang et al. 2015). The toxicity of mercury is controlled in rice plants by H_2S which positively regulates the levels of heavy metal chelators like non-protein thiols and metallothioneins, thus, ultimately suppressing its transit to shoots and its accumulation in roots (He et al. 2018). Additionally, H_2S communicates with several other signalling molecules, to protect plants from heavy metal toxicity, e.g., H_2S interacts with NO and helps *Sesanum indicum* plants to overcome lead stress by restricting absorption and transportation of lead throughout the plant (Amooaghaie and Enteshari 2017). In addition to this, H_2S is also reported to inhibit zinc uptake by *Solanum nigrum* (Liu et al. 2016). Furthermore, the exogenous amendment of NaHS (H_2S donor) also enhanced the germination rate in cadmium stressed wheat plants by modulating the activity of the antioxidative defense system of the plant. NaHS was also reported to mitigate copper-induced oxidative damage in *Coriandrum sativum* seedlings by promoting ascorbate, reduced glutathione, and dehydroascorbate contents in seedlings grown under copper stress (Karam and Keramat 2017). As per the reports of Kaya and Aslam (2020), integrated treatment of thiamine and NaHS mitigated cadmium toxicity in strawberry (*Fragaria ananassa*) plants. Rizwan et al. (2019), found that NaHS treatment enabled rice plants to resist nickel toxicity by enhancing the rate of photosynthesis and also by regulating the metabolism of nitrogen, eventually enhancing the growth of rice plants.

9.5.3 Drought Stress

Drought conditions affect plant growth and development because plant roots cannot absorb enough water from the soil to meet their needs for transpiration. Turgor pressure in leaves lowers due to shortage of water, which limits cell expansion, increases leaf area and photosynthesis, and therefore, restricts biomass formation (Chaves et al. 2002). Under water shortage and other osmotic stresses, the accumulation of relevant osmoprotectants including sugar alcohols, soluble sugars, glycine-betaine, certain amino acids and proline may provide resistance to plants (Rivero et al. 2014). H_2S also help plants to withstand drought conditions. In *S. oleracea* seedlings, treatment with NaHS enhanced stomatal conductance, and transpiration rate, and also increased RWC (relative water content) in leaves (Chen et al. 2011). According to the reports of Jin et al. (2013), H_2S plays an essential role in controlling the closure of the stomatal aperture in *Arabidopsis* growing under water-scarce conditions. Under such conditions, H_2S modulates ion channel flow, which in turn impacts stomatal closure and turgor pressure. The K1 channel has been identified as the primary osmolyte implicated in the H_2S-induced modulation of stomatal movement in *Arabidopsis* (Jin et al. 2017). Additionally, it has been documented that H_2S regulates the energy-producing ability of mitochondria and safe-guard *A. thaliana* plants against cellular aging by delaying the process of leaf senescence under water scarcity (Jin et al. 2018). It has also been observed that H_2S supplementation increased the enzymatic activity of catalase (CAT), superoxide dismutase (SOD), and peroxidases (PODs), thus suggesting the role of H_2S in modulating antioxidative defense system in plants to reduce the level of oxidative stress markers induced by water deficiency. A similar increase in glutathione reductase (GR), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), and gamma-glutamyl cysteine synthase was reported in H_2S treated wheat seedlings growing under the influence of water starving conditions (Shan et al. 2011). By modifying the expression of numerous drought-associated miRNA genes as *miR398*, *miR396*, *miR393*, and *miR167*, H_2S increased *Arabidopsis* ability to withstand drought (Shen et al. 2013). In *Citrus aurantium* plants, exogenously applied NaHS increased the content of PTMs (post-translational modifications) and lowered leaf protein carbonylation, indicating the significance of NaHS in lowering protein oxidation under water scarce conditions (Ziogas et al. 2015).

9.5.4 Heat Stress

Temperature extremes are one of the main variables that restrict the growth and productivity of plants. High temperature increases the fluidity of lipids present in membranes and causes denaturation and aggregation of proteins that ultimately lead to deactivation of catalysts present in mitochondria and chloroplasts, thus resulting in disruption of protein synthesis, cellular damage, and perhaps cell death.

Heat tolerance metrics in plants include regrowth capacity, cell vitality, survival percentage, electrolyte leakage, and malondialdehyde concentration (Li et al. 2012). H_2S is known to have a role in ameliorating heat stress in plants. For instance, H_2S increased the activity of various enzymatic antioxidants like CAT, cytosolic ascorbate peroxidase (cAPX), and manganese superoxide dismutase (MnSOD), aquaporins, and heat shock proteins gene expression in *F. ananassa* plants growing under high-temperature conditions (Christou et al. 2014). In response to heat stress, plants promote the accumulation of betaine, which is generated with the help of BADH (betaine dehydrogenase) enzyme. According to Li et al. (2015a, b), the exogenous treatment of NaHS greatly increased the BADH activity, thus resulting in the accumulation of betaine, which further improved the survival rate of maize plants grown under high temperature. Furthermore, in *Populus trichocarpa*, the application of H_2S donors reduced hydrogen peroxide and superoxide anion content produced due to heat stress, by modulating various enzymatic antioxidants, such as DHAR, monodehydroascorbate reductase, GR, and APX (Cheng et al. 2018).

One of the most significant factors affecting agricultural output in cold climates is low temperature. Low-temperature signals are detected and transmitted by signal molecules, which are subsequently employed to mediate responses to cold stress by several physiological processes and transcription factors. In a study, it was discovered that H_2S increased the expression of mitogen-activated protein kinase, which in turn controlled the expression of genes that reduce cold stress in *A. thaliana*, including inducer of C-repeat binding factor (ICE1), C repeat-binding factors (CBF3), cold-responsive 15A (COR15A), and cold-responsive 15B (COR15B) (Du et al. 2017). It is also reported to have a role in the cold stress response in *V. vinifera*, in which it lowered superoxide anion radical concentration, malondialdehyde content, and cell membrane relative permeability. H_2S enhanced the expression of the gene that encodes cucurbitacin C (*CuC*) synthetase, thus raising the concentration of CuC (triterpenoid secondary metabolite) in *C. sativus*, which in turn aided in providing tolerance to plants against low-temperature circumstances (Liu et al. 2019a). Recently, it was seen that exogenously applied NaHS increased blueberry plant resistance to chilling stress by increasing chlorophyll and carotenoids synthesis, PSII and PSI activity, and improving various photosynthetic attributes including stomatal opening and photosynthetic carbon absorption capacity (Tang et al. 2020).

9.6 Interaction of H_2S with Other Plant Hormones for Ameliorating the Effects Caused by Abiotic Stress in Plants

Involvement of H_2S and plant hormones in plant growth and development as well as in the amelioration of various abiotic stresses has been widely studied (Corpas and Palma 2020; Khanna et al. 2021; Rhaman et al. 2021). Also, it is well known that H_2S substantially interacts with phytohormones to ameliorate the effects of abiotic stresses (Huang et al. 2021). This interaction of H_2S leads to a complex signaling network in plant biology. However, the mechanism of interaction of H_2S and plant hormones along with the detailed roles of H_2S in signal transduction is not well understood. Under normal and stress conditions, the interaction entails two aspects of H_2S-mediated plant hormone signaling, one in which H_2S locates downstream in plant hormone signaling and the second is the plant hormones-mediated H_2S signaling where hormones endeavor signalling role in the downstream of H_2S (Li et al. 2021b) (Figs. 9.3 and 9.4).

9.6.1 Interaction of Hydrogen Sulfide with Stimulatory Phytohormones

In different plant species, the interplay of H_2S with stimulatory phytohormones like gibberellic acid (GA) (Xie et al. 2013, 2014; Zhu et al. 2021), auxin (AUX) (Zhang et al. 2019; Xuan et al. 2020) and melatonin (MEL) (Gu et al. 2021; Rehaman et al. 2021; Sun et al. 2021) is involved in various physiological processes, such as germination, root growth, fruit ripening, and also abiotic stress resistance.

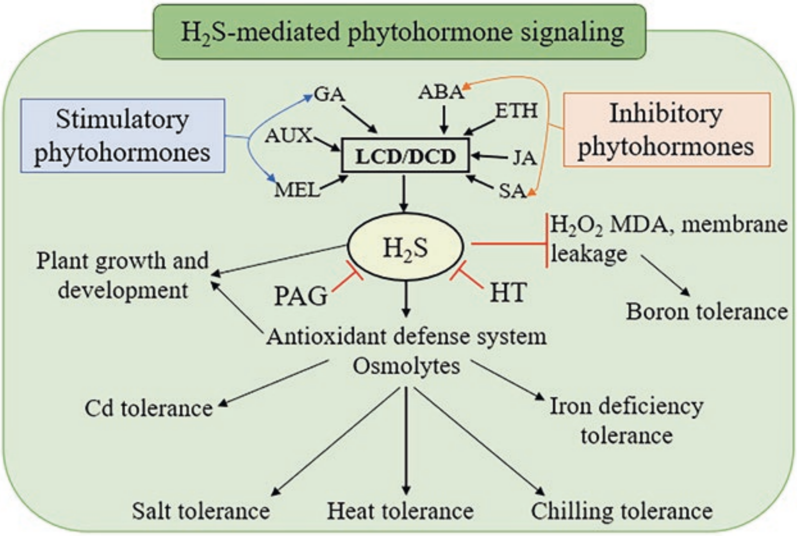

Fig. 9.3 H_2S (hydrogen sulfide)-conciliated phytohormone signaling. *GA* gibberellic acid, *AUX* auxin, *MEL* melatonin, *ABA* abscisic acid, *SA* salicylic acid, *ETH* ethylene, *JA* jasmonic acid, *LCD* L-cysteine desufhydrase, *DCD* D-cysteine desulfhydrase, H_2O_2 Hydrogen peroxide, *MDA* malondialdehyde, *Cd* Cadmium, *HT* H_2S scavenger hypotaurine, *PAG* DL-propargylglycine

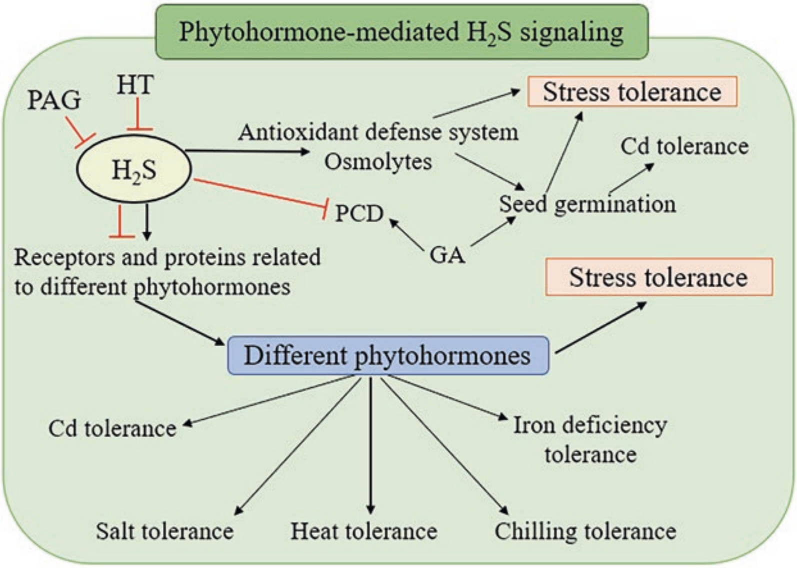

Fig. 9.4 Phytohormone-mediated H_2S signaling. *GA* gibberellic acid, *Cd* Cadmium, *HT* H_2S scavenger hypotaurine, *PAG* DL-propargylglycine, *PCD* Programmed cell death

9.6.1.1 Interaction of Hydrogen Sulfide with Gibberellic Acid

Gibberellic acid (GA) biosynthesized in the seeds, buds and roots play a major role in germination and cell elongation in plants. Interaction of H_2S with GA has been extensively studied in inducing seed germination (Zhang et al. 2008, 2010b) and in ameliorating abiotic stress. NaHS and GA, either alone or, in combination increased wheat seed germination by modulating the activity of enzyme β-amylase in wheat plants (Zhang et al. 2010b). The activity of antioxidants and amylase was stimulated by NaHS in hypocotyls and radicles also in *Cucumis sativus* under cadmium stress (Yu et al. 2011). NaHS is also known to alleviate GA-triggered programmed cell death (PCD) by increasing the accumulation of endogenous H_2S and reducing the functioning of L-cysteine desulfhydrase (LCD) in the wheat aleurone layer. In *Solanum lycopersicum* plants, boron toxicity leads to an increase in malondialdehyde, hydrogen peroxide, and endogenous H_2S and a decrease in chlorophyll a, chlorophyll b, dry weight, water content, water potential, and photosynthetic quantum yield. But the application of GA ameliorated these negative impacts of boron by reducing the content of malondialdehyde, hydrogen peroxide and increasing levels of endogenous H_2S. Surprisingly, NaHS further enhanced the GA-induced boron tolerance, but H_2S scavenger blocked boron tolerance (Kaya et al. 2020b). These findings suggest that H_2S regulates plant growth and development by interacting with GA under normal as well as under stress conditions.

9.6.1.2 Interaction of Hydrogen Sulfide with Auxin

Auxins, mainly indole-3-acetic acid (IAA), are the most prominent plant hormones that significantly regulate plant growth and developmental processes like root growth, apical dominance, and vascular dominance (Wang et al. 2001; Lymperopoulos et al. 2018). Apart from growth promotion under normal conditions, auxins are also known to modulate plant growth under different stress conditions (Kazan 2013). In-plant cells, IAA homeostasis is maintained by the biosynthesis key enzymes LCD and D-cysteine desulfhydrase (DCD), compartmentalization, degradation, and conjugation of free IAA with soluble sugar, amino acids, etc. (Xu et al. 2010). The biosynthesis of IAA required for rhizogenesis and organogenesis is improved by H_2S. H_2S induces root development by influencing upstream auxin transduction signalling pathways (Zhang et al. 2009b). The interaction between H_2S and IAA is associated with the sustenance of physiochemical processes and stress amelioration in plants (Jia et al. 2015). In *C. sativus* explants, depletion of IAA resulted in the inhibition of adventitious root formation, while treatment with NaHS retrieved the inhibition (Lin et al. 2012). Similarly, in *Ipomoea batatas*, H_2S stimulated the development of adventitious roots, but IAA transport inhibitor enervated H_2S-induced adventitious root formation. These studies indicate that H_2S and IAA in auxin signalling assist in plant growth and development.

Interaction of IAA with H_2S also ameliorates abiotic stress in plants. A study reported that H_2S enhanced IAA content in seedlings of *B. rapa* seeded in normative and cadmium-toxic soil resulting in increased shoot and root fresh weight by 12.98% and 23.65% respectively (Li et al. 2021a). In *C. sativus* auxin acts as a downstream signalling molecule during H_2S-induced chilling tolerance. Relative expression of YUCCA2 (flavin monooxygenase (FMO)-like protein) and the activity of FMO was significantly increased on the application of NaHS which in turn improved cold tolerance by increasing the level of endogenous IAA. Removal of H_2S and application of IAA had hardly any effect on the signalling of other molecules, but defence gene expression and H_2S-induced cold tolerance were inhibited by the IAA polar transport inhibitor NPA (Zhang et al. 2020). Thus, IAA works as a downstream signalling molecule involved in H_2S-mediated stress resistance in plants, while H_2S assists auxin signal transduction through regulation of synthesis of auxin and expression of auxin-related genes thus intensifying the plant resistance to abiotic stresses.

9.6.1.3 Interaction of Hydrogen Sulfide with Melatonin

Melatonin (MEL) is an animal hormone regulating learning, memory, circadian rhythms, mood, retinal physiology, sleep, sexual behaviour, reproductive seasonality, etc. (Reiter 1998). In many plant species endogenous MEL was reported to play a critical role in growth and development of plants, and physiological processes such as seed germination, seedling formation and fruit ripening along with response and resistance to various environmental stresses (Nawaz et al. 2021; Zhang et al.

2021; Zhao et al. 2021). In cucumber (*C. sativus*) and tomato (*S. lycopersicum*), salt stress increased ROS burst and reduced photosynthetic parameters and chlorophyll fluorescence parameters (Fv/Fm), while a foliar spray of MEL in cucumber stimulated the activity of different enzymatic antioxidants like CAT, SOD, APX, and POD which in turn ameliorated negative impacts of salt stress. In addition, exogenous MEL in cucumber and tomato also increased the endogenous H_2S content by significantly enhancing the activity of LCD/DCD but this increase was impaired by hypotaurine (HT; H_2S scavenger) (Mukherjee and Bhatla 2021; Sun et al. 2021). Similarly, in pepper (*Capsicum annuum*), salt stress and iron deficiency reduced plant dry weight, chlorophyll contents, Fv/Fm, and fruit yield and increased oxidative burst, while exogenous application of MEL alleviated this reduction by increasing endogenous H_2S level and stimulating the antioxidants like CAT, SOD, and POD, however, these positive effects of exogenous MEL were depleted by HT (Kaya et al. 2020a). Further, MEL confers Cd tolerance to plants by enhancing H_2S production (Gu et al. 2021). These reports indicate that MEL could ameliorate abiotic stress in plants by interacting with H_2S, where H_2S work as a downstream signaling molecule.

Further, H_2S may also function as an upstream signaling molecule to MEL in ameliorating abiotic stress in plants. In osmotically stressed *A. thaliana* exogenous application of H_2S significantly increased the level of endogenous MEL, thereby increasing relative water content, soluble sugar and proline content, stomatal closure, and reducing MDA content (Wang et al. 2021).

9.6.2 Interaction of H_2S with Inhibitory Phytohormones

In addition to interaction with stimulatory phytohormones, H_2S also interplay with inhibitory phytohormones like abscisic acid (ABA) (Li and Jin 2016; Zhang et al. 2019), ethylene (ET) (Liu et al. 2020a), jasmonic acid (JA) (Deng et al. 2020; Yu et al. 2021) and salicylic acid (SA) (Zanganeh et al. 2019; Pan et al. 2020) and affects the plant growth and development under normal and stressful conditions.

9.6.2.1 Interaction with Abscisic Acid

Abscisic acid (ABA) functions crucially in plant growth, development, and physiological processes such as seed dormancy and plant senescence and also mitigates the negative impacts of different environmental stresses alone or/and by interacting with H_2S (Xuan et al. 2020). Recently, participation of H_2S in ABA-dependent stomatal closure was observed during studies on *Impatiens walleriana, V. faba*, and *A. thaliana*, where the exogenous supply of H_2S enhanced the ABA-induced closing of stomata (Garcia-Mata and Lamattina 2010; Liu et al. 2011). Conversely, ABA was found to activate the expression and activity of LCD required for the generation of H_2S (Jin and Pei 2016). In addition, exogenous treatment of ABA in heat-stressed

Nicotiana tabacum increased the activity of LCD leading to an increase in accumulation of endogenous H_2S, while HT and DL-propargylglycine (PAG; H_2S-synthesis inhibitor) partially blocked this increase (Li and Jin 2016). Li et al. (2016) observed that priming NaHS and ABA reduced the ROS burst by activating the antioxidant defence system in cucumbers under chilling stress. Furthermore, in wheat (*Triticum aestivum*), NaHS treatment modulated biosynthesis and catabolism of ABA leading to an increase in accumulation of endogenous ABA, which sequentially triggered ABA-responsive gene expression, chased by a decrease in hydrogen peroxide content by stimulating antioxidative defense machinery (Ma et al. 2016). Additionally, in *A. thaliana,* exogenous administration of NaHS not just induced the gene expression of ABA receptors but also prompted persulfidation of ABA receptors under drought stress, while in *Arabidopsis* mutants lacking LCD, this induction was reduced (Aroca et al. 2018). Similarly, H_2S deficiency in drought-stressed *A. thaliana* weakened the ABA-induced stomata closing by affecting expression levels of ABA receptors (Jin et al. 2013). Similar findings were reported by Chen et al. (2020a) who also observed that H_2S triggers ABA-induced stomatal closure. These studies indicate that H_2S interacts with ABA and employs its signaling role by locating upstream or/and downstream of ABA in ameliorating abiotic stress in plants.

9.6.2.2 Interaction with Ethylene

Ethylene (ET), is a gaseous plant hormone known to play a pivotal role in seed germination, stomatal movement, flowering, fruit ripening, organ maturation, senescence, and resistance to environmental stress in plants (Iqbal et al. 2017). H_2S, which is also a gaseous signaling molecule, also plays vital role in various physiological activities (Li et al. 2016; Banerjee et al. 2018). The interaction of these two molecules synergizes to benefit growth and development of plants during usual and stressful state. The production rate of ET is determined by 1-aminocyclopropane-1-carboxylic acid (ACC), ACC oxidase, and ACC synthase activities (Barry et al. 2000). In *V. faba* exogenous application of ET donor (ethephon) induced the activity of LCD/DCD leading to an increase in H_2S accumulation in guard cells leading to stomatal closure, while PAG blocked the ET-induced stomata closure (Liu et al. 2012). Similarly, treating *A. thaliana* with ACC leads to accumulation of endogenous H_2S and induced closing of stomata (Hou et al. 2013). In addition, under osmotic stress, ET-induced stomatal closure was mediated by the generation of H_2S inside guard cells of *S. lycopersicum*, while HT and PAG eliminated the positive effects of ET. Similarly, endogenous H_2S was found to play an important role in ET-induced amelioration of chromium stress in *Vigna radiata* and *V. mungo*, while the application of PAG increases Cr toxicity (Husain et al. 2021).

Also, ET fumigation leads to an increase in ROS and malondialdehyde in fruits and results in fruit ripening, while fumigation with H_2S can mitigate ET-persuaded fruit softening. For example, in kiwifruit, fumigation of H_2S and ET effectively controlled the fruit softening by increasing the content of starch, soluble protein, titratable acid, ascorbic acid and reducing sugar. Additionally, H_2S and ET application stimulates the defence system in form of antioxidant enzymes (CAT and APX) and reduces the osmotic stress in plants. Further, it is found that the expression of ET-synthesis genes is inhibited by H_2S (Li et al. 2017). Similarly, exogenous application of H_2S can reverse the ET-induced abscission of the rose (*Rosa rugosa*) floral organ and tomato (*S. lycopersicum* L.) petiole, as well as lily (*Lilium brownii*) anther dehiscence. These results indicate that H_2S interacts with ET and strives its signalling part downstream and/or upstream to ET under various conditions in plants.

9.6.2.3 Interaction with Salicylic Acid

Salicylic acid (SA) plays an important role in various metabolic processes in plants such as photosynthesis (Tang et al. 2017), AsA-GSH cycle regulation (Yan et al. 2018), and ROS detoxification (Li et al. 2019). H_2S is also found to play an important role in ROS detoxification and AsA-GSH cycle regulation (Liu et al. 2019b; Chen et al. 2020b; Li 2020). This overlap of functions between SA and H_2S indicates an interaction between them in some metabolic pathways. Although the picture is not clear, various studies have been done to evaluate the response of plant against SA under various conditions like in maize under water stress (Loutfy et al. 2020), in sunflower under salinity stress (Noreen et al. 2017) and under Pb stress in basil (Padash et al. 2019), *B. juncea* (Kohli et al. 2019) and *N. tabacum* (Halim and Phang 2017). Similarly, studies have been done to evaluate the response of *A. thaliana* and cucumber plants growing under drought and salinity stress, respectively against H_2S treatment (Du et al. 2019; Jiang et al. 2019). H_2S and SA play a key role in mitigating abiotic stresses, especially heavy metal toxicity (Khan et al. 2014; Chen et al. 2017). At the same time, few reports claim that in pepper plants, H_2S and SA work together to mitigate oxidative stress by regulating the AsA-GSH cycle (Kaya 2021). Researchers have also found that the endogenous level of SA remains unaffected upon removal of endogenous H_2S with DL-propargylglycine and hypotaurine but SA induced level of H_2S activated the level of L-/D-cysteine desulfhydrase (L-/D-CD) mRNA; which indicates a role of H_2S in SA signalling. Studies also report that both H_2S and SA enhance the level of mRNA in the case of POD, SOD, CAT, GR, and APX. H_2S modulates chilling-response genes and antioxidant system and acts as a downstream signalling molecule in chilling tolerance induced by SA in cucumber plants (Pan et al., 2020).

9.6.2.4 Interaction with Jasmonic Acid

Jasmonic acid (JA) regulates various processes in plants like growth, germination, development, stimulates senescence, and provides immunity to plants against biotic stress. The downstream activity of JASMONATE ZIM-domain (JAZ) activates transcription factors which lead to JA response (Ruan et al. 2019). Studies have shown that JA induces the activity of DCD or LCD which further promote H_2S content.

Moreover, H_2S also participates in stomatal closure triggered by JA (Hou et al. 2011). The stomatal activity involves five transcription factors namely SPEECHLESS (SPCH) (MacAlister et al. 2007), MUTE (Pillitteri et al. 2007), FAMA (bHLH097), (Ohashi-Ito and Bergmann 2006), SCRM2 and ICE1/SCREAM (SCRM) (Kanaoka et al. 2008). Studies have reported that JA mediates the positive regulation of endogenous H_2S content, *LCD* expression, and L-cysteine desulfhydrases (LCDs) activity. H_2S functions upstream of the SPEECHLESS and downstream of TMM (TOO MANY MOUTHS) and SDD1 (STOMATAL DENSITY AND DISTRIBUTION1) which are important components of signalling pathways related to stomata. This suppression of genes related to stomata by H_2S shows that both JA and H_2S coordinate to regulate stomatal activity (Deng et al. 2020). Studies on leaves of *A. thaliana,* have also shown that regulation of glutathione and ascorbate metabolism by JA is mediated by H_2S (Shan et al. 2018).

9.7 Conclusion

H_2S, a lipophilic gas transmitter plays a pivotal role in various cellular processes of plants. The role of H_2S in germination, root elongation, opening and closing of stomata, growth, and senescence has been well documented. In addition to this, H_2S also ameliorates plant responses against different types of stresses like chilling, high temperature, drought, flooding, heavy metal, and salinity stress. Moreover, H_2S also crosstalks with inhibitory as well as stimulatory phytohormones that helps in regulating different stresses, reduce oxidative damage, and modulates the activity of the antioxidant defense system, thereby helping in the sustainable production of crops even under unfavourable conditions. However, various transcriptomics, proteomics, and metabolomics studies should be performed in the near future to understand the detailed potential of H_2S in modulating plant defense systems against different stressors.

Conflict of Interest Authors declare no conflict of interest.

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Chapter 10
Gasotransmitter Hydrogen Sulfide (H_2S) and Its Role in Plant Development and Defense Responses Against Abiotic Stress

Aditi Sahariya, Chellapilla Bharadwaj, and Afroz Alam

Abstract Abiotic stresses are among the potent threats to plant production and growth. The abiotic stresses are accountable for the obstruction of biological redox homeostasis, oxidative stress, and formation of reactive oxygen species (ROS) in the plants. From sprouting and growth to the reproductive stage, plants are routinely opened to several abiotic challenges, including temperature, heavy metal, salt, and drought pressures. Certain defense mechanisms exist in plants that provide definite and precise signaling in the metabolic pathways to combat and survive. Among these signaling molecules, hydrogen sulfide (H_2S) is recognized as a useful 'gasotransmitter' which has been emerged as a vital gaseous signal in regulating gene expression under various abiotic stresses. Though, the defensive role of this gasotransmitter is almost established, yet its precise role in plants remains a point to discuss more and more considering recent advancements related to plant and environment interactions. Hence, this chapter attempts to provide an insight into the various roles of H_2S as a gasotransmitter to assist plant adaptations under challenging abiotic conditions.

Keywords Abiotic stress · Defense · Gasotransmitter · H_2S · Metabolism · Stress response

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T. Aftab, F. J. Corpas (eds.), *Gasotransmitters Signaling in Plants under Challenging Environment*, Plant in Challenging Environments 5,
https://doi.org/10.1007/978-3-031-43029-9_10

10.1 Introduction

Salt, heavy metals, high or low temperature, UV irradiation, osmotic or drought are some of the abiotic stresses that plants face in nature. Abiotic stress causes changes in plant height, leaf shape, and stomata openness, according to several studies (Shen et al. 2011; Ali et al. 2017; Jin et al. 2017). Abiotic stresses also disrupt plant physiological metabolism, causing changes in the levels of electrolyte leakage, proline, hydrogen peroxide, and malondialdehyde (Chen et al. 2017; Sun et al. 2018). Simultaneously, reactive oxygen species (ROS) production is a common element in plants; reactions to abiotic stresses as the pursuit of some antioxidant enzymes change (Fatma et al. 2016; Wu et al. 2015). Salt, droughts, heavy metal toxicity, and high- and low-temperature stressors are the key environmental conditions that negatively affect plant physiology and output (Paul and Roychoudhury 2019). Drought stress and salt both cause oxidative destruction, also salinity causes significant Na^+ ion toxicity as well as osmotic and nitrosative stresses generated by reactive nitrogen species (Valderrama et al. 2007). Membrane breakdown, electrolyte discharge, and changes in photosynthetic latent cause cellular function to be interrupted. Plant defense processes rely on a vast family of genes that express themselves in response to a variety of stressors (Serra et al. 2015; Yang et al. 2018). H_2S protects plants from salt stress by reducing hydrogen peroxide (H_2O_2) accumulation, regulating membrane stability and antioxidant systems in mitochondria. These reports show that H_2S has a positive effect on plant physiology.

On the other hand, H_2S is involved in the defense mechanisms of plants against various abiotic stresses, including osmotic stress, drought stress, salt stress, extremely high- or low-temperature stress, and metalloids stress, by reducing reactive oxygen species (ROS) accumulation and actively mobilizing bioactive proteins, such as post-translational modification (PTM), as represented by *S*-sulfhydration (Table 10.1).

Small gas molecules produced by organisms and used to transmit biological signals are known as gasotransmitters. Gasotransmitter research is advancing at a rapid pace, and knowledge about their potential in biology and medicine is growing. Gasotransmitters have long been a source of fascination in a variety of professions. The role of gasotransmitters in modulating stomatal closure as part of the plant's innate *immune* response to protect against biotic/abiotic stress conditions has attracted a consideration in the past two decades (García-Mata and Lamattina 2013). Endogenous gasotransmitter emissions in plants have recently been extensively examined and evaluated, yielding knowledge that will aid our comprehension of new gasotransmitter signaling pathways. Plants typically generate these gasotransmitters in response to abiotic stresses, conforming to earlier research (Abdulmajeed et al. 2017; Cui et al. 2017; Xu et al. 2017). Carbon monoxide (CO), hydrogen sulfide (H_2S), and nitric oxide (NO) are the three gasotransmitters that have received the most attention so far in plants (García-Mata and Lamattina 2013). However, unlike NO and H_2S, the ecology of CO in this biological activity is as much well understood. As a result, the focus of this chapter will be on the activity and interaction of H_2S.

Table 10.1 Effects of various stresses on different plant species and its relationship with H_2S

Plant species	H_2S donors	Stresses	Effects on plants	Stress and H_2S relation	References
Alfalfa (*Medicago sativum* L.)	Sodium hydrosulfide (NaSH)	Heavy metals	Produces toxins in plants, which prevents them from growing and developing	Production of cellular enzymatic and non-enzymatic antioxidants	Cui et al. (2013, 2017) and Arif et al. (2021)
Cucumber (*Cucumis sativus* L.)	Sodium hydrosulfide (NaSH)	Salinity	Causes growth retardation resulting into poor development of plants	Reduces salt-stressed plants' oxidative stress	da-Silva and Modolo (2017)
Coriander (*Coriandrum sativum* L.)	Sodium hydrosulfide (NaSH)	Drought	Disrupts cell integrity, causes osmotic and oxidative stress, destroys PS II, and has a undesirable influence on overall progress of the plant	Osmoprotectant accumulation	Thakur and Anand (2021)
				Glutathione production	Calderwood and Kopriva (2014)
Pea (*Pisum sativum* L.)	Sodium hydrosulfide (NaSH)	Radiation	Negatively impacts the general development and growth of plants	Production of UV-absorbing substances, specific ROS scavengers, and other antioxidant enzymes	Jasrotia (2021)
Blueberry (*Vaccinium* sect. *Cyanococcus*)	Sodium hydrosulfide (NaSH)	Low or high temperature stress	Affects gaseous exchange	Helpful in leaf exchange gas, lowering photo-inhibition of PSII and PSI, and boosts proline concentration	Zulfiqar and Hancock (2020)

Recently, H_2S got the recognition as a novel gasotransmitter with many functions that are comparable to those of NO. Depending on their relative concentrations, any of these compounds acts as a signal or as a promoter of damage in plants (Corpas et al. 2019a). Sulfur-containing substance hydrogen sulfide (H_2S) is recognized to partake in plants' responses to a variety of stresses, *viz.*, drought, osmotic, heavy metal, salt, and temperature. Hydrogen sulfide can efficiently mediate numerous pathways of plant response to diverse abiotic stressors and can regulate the antioxidant defence system. H_2S has the ability to move to various portions of the plant cells and balance the antioxidant pools by delivering sulfur to cells because of its gaseous nature and it has also exhibited resistance against plenty of detrimental environmental conditions like drought, salt, high temperature, chilling, heavy metals, and flood through a change in the levels of osmolytes, malondialdehyde (MDA), sodium/potassium ions uptake, the mechanism of H_2S biosynthesis, and the activities of antioxidative enzymes (Pandey and Gautam 2020).

10.2 Hydrogen Sulfide (H_2S)

H_2S as a signalling molecule controls the essential functions and enables plants to withstand harsh environmental situations. This action is caused by its chemical reactivity, and the best-studied mode is persulfidation, which encompasses change in protein thiol groups to produce persulfide groups (Aroca et al. 2021). Continuously, H_2S was thought to be a poisonous gas. It has been noticed that doses as low as 50 ppm are capable to produce tunica conjunctival, and greater quantities can be fatal in human (Reiffenstein et al. 1992). The basic procedure of H_2S lethality is the blockade of cytochrome c oxidase activity, which inhibits mitochondrial respiration (Dorman et al. 2002; Mancardi et al. 2009). H_2S has also been identified as a harmful chemical in plants. Several crop plants that were constantly fumigated with high amounts of H_2S (3000 ppb) displayed leaf damage, death of delicate species, and lessened growth, proving that H_2S is harmful. Nonetheless, fumigation with lower quantities of H_2S, such as 30–100 ppb, stimulated the development of several plant species significantly (Thompson and Kats 1978).

H_2S is regarded to be a critical signaling molecule, and research into its functions in plants is gaining traction (Hancock and Whiteman 2014). Heavy metal stresses, temperature, and drought are only a few of the abiotic factors that cause H_2S production (Hancock 2019). Drought stress causes H_2S generation in *Arabidopsis thaliana*, according to several studies (Jin et al. 2011). Meanwhile, in wheat under drought stress, abscisic acid (ABA) treatment refines endogenous H_2S concentration (Ma et al. 2016). Furthermore, there is rising evidences that temperature stresses cause the delivery of H_2S in grapes (Fu et al. 2013) and cucumbers (Liu et al. 2019). High temperatures quickly induce H_2S production in poplars (Cheng et al. 2018). Shi et al. (2014) observed that the cadmium (Cd) treatment causes H_2S to be produced in Bermuda grass. H_2S generation was similarly stimulated by lead exposure in cauliflower (Chen et al. 2018; Cheng et al. 2018). Nickel (Ni) stress enhanced H_2S levels in zucchini. Under osmotic stress, wheat seedlings emitted H_2S (Khan et al. 2017). H_2S is produced by glyphosate in Arabidopsis (Corpas et al. 2019b). Surprisingly, Aghdam et al. (2018) reported that treating fruits of hawthorn with exogenous H_2S when they are under cold stress can cause H_2S to be released. Therefore, it was postulated that L-cysteine is the source of H_2S in the presence of hydrogen cyanide, in a reaction catalyzed by ß-cyanoalanine synthase in plants. However, its generation under abiotic stress requires further research (Jost et al. 2000).

10.2.1 Chemistry of H_2S

It is crucial to understand the chemistry of H_2S in order to comprehend its various impacts and ways of action. H_2S is in equipoise with its anions, 2 H^+ and S^{2-}, in liquid solution and at physiological pH, however the potency of S is low (Li and Lancaster 2013). Because the active form has yet to be determined, H_2S is used to represent all three species. H_2S is an interesting biological compound because it has two key biochemical features that provide its physiological role: it is both a nucleophile and a reductant (Li and Lancaster 2013). H_2S does not require a receptor to conduct its biological activity because its solvability in lipophilic solvents is five times larger than in water, allowing it to readily permeate lipid membranes (Wang 2002). Almost all physiological processes, such as guard cell movements, seed germination, root growth, fruit ripening, senescence, and methods of response to abiotic and biotic stimuli, are now recognized to involve H_2S (Corpas et al. 2021).

10.2.2 Sources of H_2S

Plants have also been found to produce H_2S from L-cysteine. A cysotolic L-cysteine desulfhydrase 1 enzyme (DES1) has been identified in *A. thaliana* (González-Gordo et al. 2020). It catalyzes the conversion of L- cysteine to pyruvate, ammonia and H_2S (Alvarez et al. 2010). Plants may have additional sources of H_2S since *DES1* null mutants produce about 30% less endogenous sulfide. However, even though the existence of D-cysteine inside the cell is still disputed, two D-cysteine desulfhydrases have been recognized (Papenbrock et al. 2007).

10.2.3 Hydrogen Sulfide Homeostasis in Plants

In addition to carbon monoxide and nitric oxide, hydrogen sulphide is acknowledged as the third endogenous gasotransmitter in plants. A colorless gas with a potent stench, known as H_2S, may be easily detected in the environment. Originally thought to be a phytotoxic gas, its function as a signalling molecule within plant cells has recently gained widespread recognition. H_2S can also be added externally to plants using a contributor such sodium hydrosulfide (NaHS), in addition to its endogenous availability. By maintaining physiological homeostasis through controlled stomatal conductance, photosynthesis, relative water content, respiration, and mineral nutrition, H_2S positively influences plant progress and development (Filipovic and Jovanović 2017).

Low quantities of H_2S can effectively manage the physiological homeostasis. Antioxidant levels frequently change within plant cells in reponse to abiotic and biotic stressors. Under adverse circumstances, external H_2S supply in the form of

NaHS upholds the antioxidants and antioxidative enzyme reservoir. The H_2S homeostasis is influencing the responses of genes and proteins at the cellular level as well as the gene products involved in cell defence, transcription factors, and signal transduction. During post-translational modifications, H_2S also plays a crucial role in the induction of cysteine persulfidation (Filipovic et al. 2018).

L-cysteine desulfhydrase catalyzes the conversion of L-cysteine to pyruvate and ammonia, which results in the formation of H_2S (Alvarez et al. 2010), as indicated above. In *Arabidopsis*, this mechanism is mediated by the *DES1* gene (Alvarez et al. 2010; Scuffi et al. 2014), the *AtLCDES* gene (Jin et al. 2011), and the *L-C Des* gene (Hou et al. 2013). Recent research has shown that the presence of these genes is increased in response to the stomatal motility regulators ABA, ethylene, jasmonic acid, and salicylic acid (Hou et al. 2013). Nevertheless, analysis of the sequence showed that the *DES1* gene promoter contains ABA-responsive regions (Scuffi et al. 2014), To clearly understand how these hormones induce gene expression, more investigation is necessary. It has been confirmed that H_2S balances the movement of K^+ in channels in a way that it is independent of ABA and Ca^{2+} when DES1 has been shown to facilitate ABA-dependent closure of stomata (Scuffi et al. 2014), signifying the presence of an ABA-regulated signalling pathway that can be triggered in reply to other impetuses (Papanatsiou et al. 2015). The amount of H_2S needed for spore germination under challenging conditions was quantified, and it was observed that when seeds and ensuing roots of bean, pea, wheat, and maize were exposed to 10–100 mM H_2S solutions, the germination rates increased with shorter germination times and larger seedlings were developed compared to controls. All H_2S -pretreated plants had larger overall mass, roots, and fruits (Xuan et al. 2020).

10.2.4 H_2S Physiology in Guard Cells

H_2S role in guard cell signalling was first reported in 2010, and further research has shown that it increases guard cells closure in a few plant species (García-Mata and Lamattina 2013; Su et al. 2014; Papanatsiou et al. 2015). DES1 in *Arabidopsis* produces H_2S in response to ABA. H_2S then promotes the synthesis of endogenous nitric oxide (NO) (Scuffi et al. 2014). The process of stomatal closure is defective in the *nia1/nia2* double mutant. Additionally, it was discovered that both genes expression was elevated by H_2S donors, proving that nitrate reductase (NR) is important in NO production which is dependent on H_2S (Scuffi et al. 2014).

H_2S is required for the ABA-induced NO production, and acts as upstream of NO in ABA-dependent stomatal closure. H_2S is required for the ABA-induced NO production, and acts as upstream of NO in ABA-dependent stomatal closure (Lisjak et al. 2010, 2011). Unusually, H_2S has been found to affect stomatal closure, but NO has been proven to work before H_2S (Hou et al. 2013; Liu et al. 2019). Along with NR, a few substances have been discovered to be H_2S targets during the induction of stomatal closure. One of these substances is: (i) AtMRP5, an extreme of the multidrug resistance protein family that has been proposed as a regulator of Ca^{2+} and anion passage (Suh et al. 2007; García-Mata and Lamattina 2010); (ii) K^+ in channels, which are stalled by H_2S in an ABA-independent manner (Papanatsiou et al. 2015); and (iii) 8-nitro cGMP, which interacts with H_2S to produce 8-mercapto CGMP, which is employed to modulate cytosolic [Ca^{2+}] (Honda et al. 2015).

ROS function as second messengers in guard cell reactions to most stimuli that elicit stomatal closure. NADPH oxidase (RBOH) is a key enzyme in the production of ROS from the outer side of the plasma membrane and is a homolog of the mammalian 91-kD glycoprotein subunit of phagocyte oxidase (gp91phox) (Shen et al. 2020). H_2S have signaling properties through protein post-translational modifications (PTMs) and by their crosstalk with other cellular compounds, including phytohormones, hydrogen peroxide, or calcium, among others (Mishra et al. 2021).

10.2.5 H_2S-Based Reactions

There is an inadequate comprehension of H_2S physiological effects, particularly in plants and the proposed mechanism currently relies primarily on its chemical features. Because of its nucleophilic nature, it can react with O_2, H_2O_2 and peroxynitrite indicating a function in lowering cellular oxidative stress (Kabil and Banerjee 2010; Fukuto et al. 2012; Aroca et al. 2015). Persulfidation is a type of PTM that involves cysteine residue and results in the formation of persulfide (Paul and Snyder 2012; Aroca et al. 2015). It can be triggered by a variety of natural actions. The emergence of the SH thiols and SSH persulfide groups is aided by the dissociation constant pKa, which is determined by the ambient environment of the cysteine residue. The latter has an indestructible nucleophilic capacity for considerable chemical tendency. Because of its lower pKa, SSH becomes more active hydrogen donors under physiological pH circumstances than SH (Paul and Snyder 2012; Zhang et al. 2017).

H_2S does not directly interact with the -SH protein; instead, it reacts with sulfenic acids to create RSSH groups, which are identified by protein Tyr phosphatase 1B (PTP1B) during the endoplasmic reticulum stress response (Krishnan et al. 2011). Recent studies have shown that H_2S can interact with -SH proteins by creating an RSSH pathway from the sulfane sulphur that is formed by the interaction of oxygen with oxygen (Toohey 2011, 2012; Aroca et al. 2015). By adding sulfane sulphur to the cysteine residue in the active site, Nagahara and Wróbel (2020) postulated that polysulfides arranged in the NaHS solutions interact as oxidants and trigger the rapid alterable oxidation of lipid phosphatase. According to preliminary studies, S-nitrosation of proteins impairs their functionality (Zaffagnini et al. 2013), whilst persulfidation can operate (Vandiver et al. 2013) or block (Krishnan et al. 2011) the protein's functionality.

10.2.6 Regulation of Photosynthesis

H_2S has a role in several physiological processes, including photosynthesis. However, cyanobacteria *Aphanothece halophytica*, *Synechococcus*, and tobacco chloroplasts are inhibited by sulfide at greater concentrations (1 mM), while red mangrove (*Rhizophora mangle*) development and photosynthesis are significantly impacted at an accumulation of 2 mM (Lin and Sternberg 1992). Conversely, H_2S controls photosynthesis in plants at lower concentrations. Pre-treating fresh vegetables with gaseous H_2S has a considerable impact on ascorbic acid content holding during drying under hot air, including kale (*Brassica oleracea* var. *sabellica*), cabbage (*Brassica oleracea*), and parsley (*Petroselinum crispum*) (Petersen 1948). Later, Joshi et al. (1975) demonstrated that exogenous H_2S decreases the root respiration and oxidative capacity of the rice plant (*Oryza sativa*) as well as altering a variety of physiological parameters in several rice cultivars. However, there has been a significant rise in research into its function in higher plant physiology over the past few years (Corpas and Palma 2020).

10.3 Established Roles of H_2S in Plant Metabolism

To date many important involvements of the gasotransmitter H_2S have been established in the defense strategies of the plant, starting from the germination to the maturation of fruits (Fig. 10.1). Here all those roles are discussed.

10.3.1 Role of H_2S in Fruit Ripening

Fruit ripening is a highly coordinated, genetically programmed, and an irreversible phenomenon involving a series of physiological, biochemical, and organoleptic changes, that finally leads to the development of a soft edible ripe fruit with desirable quality attributes. Hydrogen sulfide (H_2S) in association with the two other potential signaling molecules, *viz.*, nitric oxide (NO), and melatonin plays a vital role in numerous biological events of plants (Liu and Xue 2021). The association of these three molecules interacts with ethylene, an imperative enhancer to complete the process of fruit ripening. Furthermore, all these three molecules also interact with each other in controlling the delay in ripening and subsequent senescence during preharvest and post-storage phases in numerous fruit crops. These molecules are known to be incredible latent to save the economically important postharvest quality as they can conquer the fruit senescence under variable storing environments.

Hydrogen sulfide (H_2S) has been shown to reduce damage induced by biotic and abiotic stresses, regulate the ripening and postharvest storage of climacteric and non-climacteric fruits and vegetables, thus maintaining the nutritional and edible value of postharvest fruits and vegetables. All these characteristics are frequently

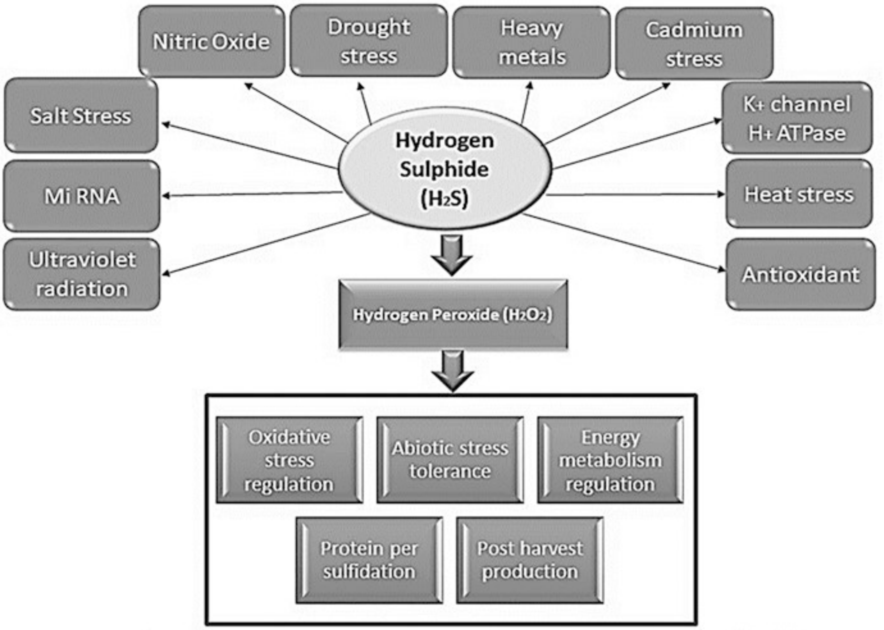

Fig. 10.1 Contribution of gasotransmitter H2S during various stresses and its impact of plant defense. Hydrogen sulfide (H2S) signaling activates a cascade of biochemical events that enhance plants' tolerance to abiotic and biotic stresses, such as controlling reactive oxygen species, activating the antioxidant defense system, cytosolic osmoprotectants accumulation, and induction of Ca^{2+} increase in cytosol

linked to oxidative stress, and numerous studies have demonstrated that the exogenous administration of H_2S could prolong the shelf life of a wide variety of fruits, vegetables, and flowers (Corpas and Palma 2020).

10.3.2 Hydrogen Sulfide in Lessening Oxidative Stress

The adverse abiotic conditions negatively affect the growth and quality of crop plants, this decrease in net yield is well studied? as a global environmental concern. Under abiotic stress, hydrogen sulfide can regulate the plant physiology as a gasotransmitter. Numerous studies have shown that by reducing cellular damage and noxiousness, H_2S could improve plants' ability to adapt to varied environmental pressure scenarios. There have been previous reports on the function of H_2S in specific physiological and metabolic processes, *viz.*, stomatal cycles of opening and closing, seed sprouting, improved photosynthesis, and maturation of lateral roots (Corpas and Palma 2020). It has been shown that H_2S can provoke numerous protection strategies under the conditions of abiotic stress. Furthermore, an exogenous supply of H_2S alleviates plant impairment from oxidative stress by exciting numerous enzymatic and non-enzymatic components. H_2S dealing recovers the action of antioxidative enzymes to counteract excessive production of ROS and shield the cell from oxidative harm.

10.3.3 H_2S Can Regulate the Heavy Metal Stress Toxicity

A significant environmental pollutant that threatens both human health and plant growth is heavy metal contamination. Initially thought to be poisonous to plants, the gasotransmitter H_2S has lately been found to have several functions in the control of healthy plant growth and development (Huang et al. 2021). As a gasotransmitter, H_2S exerts a beneficial influence on the growth, development, and stress tolerance of plants. It skillfully promotes the production of cellular enzyme- and non-enzyme-based antioxidants. Heavy metal stress causes endogenous H_2S production in cases of soil, water, and air contamination with heavy metals, and H_2S lessens the effects of metal toxicity on plants (Arif et al. 2021).

10.3.4 Hydrogen Sulfide and Lateral Root Development in Plants

One of the compounds that helps the plant withstand stressors is H_2S. Plant growth-related H_2S signalling is exceedingly complex. By causing oscillations in the transport and dispersal of auxins, which are responsible for root formation, this gasotransmitter interacts with auxin and actin-binding proteins to regulate root growth. H_2S is therefore crucial for root growth and development under stress as a signalling molecule (Khalid et al. 2021).

10.3.5 Hydrogen Sulfide Interacts with Nitric Oxide under Stress

Like nitric oxide (NO), it has been demonstrated that low levels of H_2S may have positive effects on stressed plants. Additionally, it appears that these gases play a variety of physiological roles during the plant life cycle, including seed sprouting, stomatal activity, and senescence. NO, a messenger molecule, is hypothesized to function in a variety of ways in plants under physiological, pathogenic, and adverse environmental conditions. Recently, it was discovered that H_2S is a novel gasotransmitter with many functions that are comparable to NO. Depending on their relative concentrations, both compounds act as signals or damage promoters in plants either cooperatively or antagonistically. Despite this, research indicates that the complex biological relationships between NO and H_2S involve numerous pathways, depend on plant organ and species, and are affected by experimental conditions. Studies in proteomics and biochemistry have revealed that certain target proteins have post-translational modifications such as S-nitrosation, which is brought on by NO, and persulfidation, which is brought on by H_2S, both of which have an impact on plant functionality (Corpas et al. 2019).

10.3.6 Gene Regulation by H_2S in Plants

It is generally understood that hydrogen sulfide is a crucial, multipurpose gaseous signalling molecule that affects a variety of biochemical and molecular processes in most plants. A key process by which plants manage the systematic programmes of progressive transformation, metabolism, and resistance is the transcriptional regulation of genes (Iranbakhsh et al. 2021). The coordinated systems of several signalling channels cause the desired responses in plants to ecological variables. Perception of an indication and its subsequent transduction are associated with systematic participations of an excess of regulatory components, including transcription factors, miRNAs, and epigenetic chromatin-remodeling systems. The focus of current research is on the role of H_2S in the multilayered pre-, transcriptional, and post-transcriptional stages of gene programme modifications. Discovering how H_2S interacts with miRNAs, transcription factors, epigenetic changes, and signal transmission is equally important. In addition, research is being done on how H_2S interacts with Ca^{2+}, phytohormones, and other signalling molecules (Pandey and Gautam 2020).

10.3.7 Hydrogen Sulfide in Salinity Stress

One of the biggest environmental threats to horticulture and agriculture around the world is salt stress. High salt levels result in phytotoxicity, which is seen as general plant growth slowdown and poor development. It is now well established that H_2S controls plant metabolism and growth. Additionally, H_2S is crucial in protecting plants from the harmful effects of salt pressure (Srivastava et al. 2022).

Plants that can survive a variety of stressors, including excessive salinity, are regulated by H_2S in a few physiological processes. The oxidative burst, which leads to an increase in electrolyte leakage, lipid peroxidation, and protein oxidation, is one of the first processes triggered in plant cells in response to salt stress. In fact, one of H_2S most well-studied effects is its ability to lessen oxidative damage in plants under salt stress. In stressed cucumber seedlings, treatment with sodium hydrosulfide (NaHS) (an H_2S -donor) increased the activity of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), glutathione peroxidases (GPX), and dehydroascorbate reductase (DHAR), but under the same experimental conditions, H_2O_2 and lipid peroxide levels decreased (Da-Silva and Modolo 2017).

10.3.8 Hydrogen Sulfide and Drought Stress

Another significant issue that plants are now dealing with in the current water shortage situation is drought. The overall progression of plants is negatively impacted by this abiotic stress, which ultimately leads to a decline in yield. H_2S regulates vivacious biochemical and physiological processes under drought stress, among a wide range of other physiological activities in plant biology (Bhardwaj and Kapoor 2021).

Plants under drought stress experience osmotic stress, oxidative stress, PS II damage, cell integrity loss, and other adverse effects that hinder their normal way of progression. H_2S acts as a signalling molecule in response to drought stress, inducing a variety of modifications in plant cells, including osmoprotectant accumulation. Under drought-induced water deprivation and osmotic stress, adaptation to plant may be provided due to accumulation of suitable low molecular-weight osmoprotectants like soluble sugars, sugar alcohols, proline, and glycine betaine (GB). Thakur and Anand (2021) proposed a model for the regulation of drought stress tolerance by H_2S that involved the synthesis of polyamines and sugars (Chen et al. 2016). Additionally, exposure to H_2S makes it easier to produce glutathione, which is essential for stress defence because it scavenges reactive oxygen species (ROS) (Calderwood and Kopriva 2014).

10.3.9 H_2S against Radiation Stress

Reactive oxygen species are produced as a result of UV-B radiation exposure, which causes oxidative damage (Rostami et al. 2019). Plants are adversely affected by UV radiation, which has been proven to have detrimental effects by preventing plant growth and development. By promoting the accumulation of specific ROS scavengers, UV-absorbing molecules, and other antioxidative enzymes, H_2S has been revealed to be involved in the amelioration of UV radiation stress (Jasrotia 2021).

10.3.10 Crosstalk Amid Hydrogen Sulfide and Phytohormones in Plant Defense

The plant cells contain hydrogen sulfide, which serves as a messenger for an assimilatory sulphate reduction. Despite H_2S natural release, it has been suggested that its exogenous use is useful for reducing a variety of abiotic stressors. It is well established that plants are highly dependent on phytohormone signaling during any physiological process. This area of research is a rapidly developing field, since a well-defined interactome of H2S with other phytohormones can present novel signaling nodes which can be genetically targeted for yielding multiple stress-tolerant traits in the susceptible cultivars. Under varied stress conditions, phytohormones are also engaged in controlling the defensive reactions. Plants' tolerance to abiotic stress is expressly improved by H_2S in combination with these phytohormones (Banerjee et al. 2018).

10.3.11 Consequence of H_2S and Ca^{2+} Signaling in Abiotic Stress Tolerance

It has been revealed that calcium and hydrogen sulfide-mediated signaling pathways control several biochemicals, physiological and molecular courses in plants. These signaling molecules also support the plants to fight against stresses and play indispensable parts in growth and development of plant. Ca^{2+} and H_2S signaling triggers a torrent of biochemical processes that improve plants' ability to cope up stresses, *viz.*, cytosolic osmo-protectants accumulation, activating the antioxidant defense system, controlling reactive oxygen species and induction of Ca^{2+} increase in cytosol (Zulfiqar and Hancock 2020).

10.3.12 H_2S and Temperature Stress

Molecules based on H_2S might be helpful in protecting against temperature changes. Because they are sessile organisms, plants must withstand the temperature that is imposed on them by their environment, which is usually not ambient. Numerous horticulture crops have been shown to have low and high temperature tolerance in part as a result of H_2S. For instance, a recent study investigated whether blueberry seedlings could benefit from the application of exogenous H_2S and hypotaurine, an H_2S scavenger, to help them withstand cold temperatures. By controlling leaf gas exchange, reducing photo-inhibition of PSII and PSI, and increasing proline content, they discovered that administering H_2S (in the form of NaHS) increased tolerance (Zulfiqar and Hancock 2020).

10.4 Concluding Remarks

Plants produce a remarkable diversity of structurally and functionally diverse natural chemicals that serve as adaptive compounds throughout their life cycles. Due to their several functions in controlling the progression of the plants, gases like H_2S, which were once thought to be detrimental to plants, are now considered as beneficial signalling gaseous molecule. NO and CO are now regarded as the two most significant endogenous gasotransmitters in plants. Plant scientists face, as far as characterizing the effects of climate change on plant food quality and then helping to develop efficient ways to mitigate the decline of key nutrients in plant tissue due to eCO_2. (e.g., with crop breeding or genetic engineering, fertilizer applications, or changes in cultivation techniques). H_2S was primarily produced *in vivo* as an endogenous brain active substance, but it is also showing promise as one of the signalling molecules at the moment. H_2S regulates an extensive range of biochemical and physiological processes in plants, including germination, growth and development, modulation of defence mechanisms, senescence, and fruit maturation. Aside from protecting plants from osmotic pressure, H_2S also increases the production of osmoprotectants, changes the cell cycle, induces apoptosis, and lessens the oxidative damage brought on by reactive oxygen species. H_2S enhances plant responses to a variety of stressful environmental conditions by lessening the harm and toxins already inflicted on them. In signalling pathways, H_2S also interacts with other signalling molecules such as CO, H_2O_2, Ca^{2+}, NO, etc. Persulfidation, a cysteine-dependent post-translational alteration, is the main signalling mechanism through which H_2S regulates the activities of proteins.

Despite encouraging results under controlled conditions, a complete methodological and financial feasibility investigation is still required before exogenous H_2S may be applied widely in the field. Even so, there is a widespread misconception that plants are toxic to H_2S and their growth is stunted by it. H_2S forceful effects in plants have been linked to crucial procedures like the control of senescence and maturity, the modulation of defensive responses, and plant growth and development.

Recently, it has been discovered that H_2S affects the signal transduction pathways of phytohormones, which can help plants fight off various abiotic stressors like salinity, drought, cold, and heavy metals. H_2S may also have the ability to postpone the ripening and senescence of fruits during postharvest procedures. There are few publications on the H_2S-mediated control of plant metabolism during biotic stress, which is controlled via phytohormonal interactions, even though the main emphasis is typically placed on its utility under abiotic challenges. Therefore, there is a lot of room to figure out how H_2S helps plants defend themselves against different biological challenges.

Acknowledgements The Vice Chancellor of Banasthali Vidyapith (Rajasthan), India, Prof. Ina Aditya Shastri, is acknowledged by the authors for her encouragement and all other assistance required to complete this work.

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Chapter 11
Hydrogen Sulfide (H_2S) Signaling in Plants Responding to Abiotic Stresses

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Abstract Hydrogen sulfide (H_2S) is an endogenous gasotransmitter that is engaged in a variety of plant physiological functions. In recent years, plant scientists are giving more attention to this gaseous molecule which at first instance was found and recognized as a gasotransmitter in mammals. This chapter is mainly focused on the importance of H_2S as a key mediator in plants against abiotic stresses. The exogenous application of H_2S in plants increases adaptation against different abiotic stresses like temperature, salt, heavy-metals, and moisture stress. Hydrogen sulfide appears in the current decade as an innovative signal mediator which is involved in many plant systems. Also, H_2S plays a crucial part in regularization of plant systems which assures normal plant development, protection against pathogens, senescence and maturity. Furthermore, we summarized here, how H_2S, as a signaling molecule in plants, responds to different abiotic stresses in many cases through a protein post-translational modification designated persulfidation.

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T. Aftab, F. J. Corpas (eds.), *Gasotransmitters Signaling in Plants under Challenging Environment*, Plant in Challenging Environments 5,
https://doi.org/10.1007/978-3-031-43029-9_11

Keywords Gasotransmitters · Hydrogen sulfide · Abiotic stress · Signaling pathway · Stress response

11.1 Introduction

Hydrogen sulfide (H_2S) has been postulated as an important molecule of the gasotransmitters family that additionally incorporates nitric oxide (NO) and carbon monoxide (CO). Hydrogen sulfide-related research was only published in toxicology journals until the early 1990s, indicating that the general bioscience community had little interest in this molecule. H_2S studies are now published in a variety of biological and medical publications covering areas such as physiology, pharmacology, and biochemistry. H_2S modulates smooth muscular strength, metabolic functions, and growth, demise, and motility in the central nervous system as a secondary messenger (Li et al. 2011; Wang 2012). A computationally intensive pathway including several secondary mediators such as calcium ions (Ca^{2+}), hydrogen peroxide (H_2O_2), abscisic acid (ABA), and nitrous oxide (NO_2) as well as their interactions was revealed to be involved in the development of stress responses, especially cross-adaptation in numerous investigations (Wang et al. 2016). Mechanical stimulation in tobacco plants can cause H_2O_2 and NO signaling, whereas, at the same time, heat shock lead to signaling by Ca^{2+} and ABA (Gong et al. 1998), that ultimately results in cross-adaptation of plants to such kinds of stresses. In maize seedlings, Gong et al. (2001) also reported comparable findings, which depict that the signal crosstalk between abscisic acid (ABA), Ca^{2+}, NO, and H_2O_2 in plants is mainly due to cross-adaptation. Calderwood and Kopriva (2014) discovered that H_2S is a part of this signaling network in plants, which thereafter was also confirmed by Guo et al. (2016). Subsequently, the results of many studies established H_2S as a key mediator in plants. H_2S has a wide range of beneficial impacts on plant stress tolerance performance and physiological functions, including root organogenesis, seed germination, stomatal activities, photosynthesis, leaf senescence, and yield (Zhang et al. 2009; Papanatsiou et al. 2015). For instance, H_2S enhanced barley's resistance to chromium stress by increasing the rate of photosynthesis and lowering Cr uptake (Ali et al. 2017). Under salt stress, H_2S boosted chlorophyll and soluble protein levels while inhibiting the buildup of reactive oxygen species (ROS), considerably improving rice salt tolerance (Mostofa et al. 2015). Under drought stress, the activity of the enzymes L-desulfhydrase and D-desulfhydrase were up-regulated in *Arabidopsis thaliana*, increasing the endogenous H_2S generation (Jin et al. 2011). Furthermore, there are substantial proofs that gasotransmitters can help plants become more tolerant (Jin et al. 2017; Maryan et al. 2019). Vishwakarma et al. (2017) reported that the rise and fall in the level of salinity, temperature, moisture or osmotic pressure, heavy metals, and UV-irradiation are only a few of the abiotic stresses that plants face in nature. Abiotic stress causes changes in plant height, leaf shape, and stomatal openness, according to several studies (Jin et al. 2017; Shen et al. 2011). Abiotic stresses are the most significant restrictors of plant progression

(Ashraf et al. 2018). It has been generalized the protective effect of H_2S in developing resistance in plants through the knowledge of H_2S-linked genes which regulate plant functioning during heat, low temperature, drought, waterlogging, and metal toxicity. In addition, the significance of H_2S as a signaling molecule is addressed to answer questions about how it interacts with the other signaling molecules in plants (calcium ion, methylglyoxal, and nitric oxide) (Fu et al. 2018). The responses of H_2S in plants under abiotic stress are discussed in this chapter. Meantime, we concentrated on the current developments in H_2S roles and interactions with the other gasotransmitters under abiotic stresses (Yao et al. 2019).

11.2 Abiotic Stresses and H_2S

Global food security is becoming increasingly challenged by climate change. Plants are subjected to a range of stress conditions, including salinity, high temperatures, drought, and heavy metals, all of which can have a significant impact on a farmer's income. As a result, much effort has gone towards reducing the negative impacts of environmental extremes on plants by better understanding the processes and signaling mechanisms involved in plant protection. Plant scientists are increasingly interested in discovering chemicals that can defend plants against the adverse impact of climate change (Hossain and Fujita 2013). Exogenous treatment of phytohormones, osmo-protectants, trace elements and signaling molecules have been proven to protect plants from abiotic stresses, because of their antioxidant and growth-promoting properties (Savvides et al. 2016). H_2S is associated with various physiological functions in plants, according to growing evidence (Kimura 2014). Multiple environmental stresses have been identified to stimulate H_2S production in plants which directly or indirectly increased stress tolerance in susceptible plants (Guo et al. 2016). Under abiotic stress, endogenous H_2S levels in several species of plants increase by approximately 2–2.5 fold on average (Shi et al. 2015). The effect of sulfur fertilization was linked to the first findings on the biological action of H_2S in plants (Rennenberg 1983, 1989). Later research linked H_2S to other signaling mechanisms in plants, primarily those related to oxidative stress (He et al. 2019), and H_2S finally broke into the stomatal signaling system exactly a decade ago (Lisjak et al. 2010). Overall, it can be assumed that the release of H_2S is critical for the control of a variety of plant functions to stimulate the defensive machinery against these situations (Bloem et al. 2011). Plant cells have long been known to produce and emit H_2S due to the reduction of SO_4^{2-} to SO_3^{2-} and further catalysis of sulfite reductase converts it from SO_3^{2-} to H_2S (Filipovic and Jovanovic 2017). Wilson et al. (1978) measured H_2S emissions from the leaves of cucumber pumpkin (*Cucurbita pepo* L.), (*Cucumis sativus* L.), cantaloupe (*Cucumis melo* L.), cotton (*Gossypium hirsutum* L.), soybean (*Glycine max*), and corn (*Zea mays* L.). As the light intensity increased the emission increased as well. Rennenberg et al. (1987) reported the first evidence for the presence of D- cysteine desulfhydrases (DCDs) and L-cysteine desulfhydrases (LCDs) which trigger the generation of H_2S in the mitochondria and chloroplasts, and the cytoplasm, respectively, of cucurbit plants or cultivated tobacco cells. Several studies have recently shown that exogenous H_2S administration improves plant resilience against abiotic stresses such as drought, salinity, heavy metals concentration and extremes temperature. The following sections discuss reports that suggest possible methods by which H_2S priming orchestrates plant defense responses when faced with numerous abiotic stress conditions (Fotopoulos et al. 2013). Plants produce H_2S via LCD or DCD in response to stress in the environment and encourage the development of stress responses. Furthermore, surplus hydrogen sulfide can be discharged into the atmosphere (Li and Gong 2013; Hancock and Whiteman 2014).

11.3 H_2S Signaling Pathway

H_2S is a signal molecule that plays an important role in all physiological functioning from germination to the flowering and fruiting of higher plants (González-Gordo et al. 2020), and in response to a variety of abiotic stimuli (Singh et al. 2015). It is evident that in jatropha seeds, when immersed in H_2O_2, there was a rise in the LCD activity, and a buildup of H_2S was detected (Li et al. 2012a), implying that H_2S-mediated signal transduction is involved. It also disrupts NO signaling by raising and reducing NO levels depending on the situation (Lisjak et al. 2010). In addition to Lisjak's study, H_2S has also been shown to have a role in ABA (abscisic acid) -dependent NO generation (Scuffi et al. 2014). H_2S is produced primarily in plant systems in the chloroplast through the process of photosynthetic sulfate assimilation which is governed by the sulfite reductase enzyme (Garcia et al. 2015). The enzyme cyanoalanine synthase-C1 synthesizes hydrogen sulfide in mitochondria by mashing up β-cyanoalanine enzyme (Yamaguchi et al. 2000). Nevertheless, L-cysteine desulfhydrase-1 enzyme is required for the majority of intercellular cytosolic H_2S synthesis (Alvarez et al. 2010). Enzymes like D-cysteine desulfhydrase and Nifs-like proteins have also been reported to produce H_2S (Heidenreich et al. 2005; Riemenschneider et al. 2005). Persulfidation is thought to have a role in H_2S-based signaling in plants, according to several studies (Yang et al. 2013, Paul and Snyder 2015). In another study, Sen et al. (2012) revealed H_2S-induced cysteine-38 persulfidation of the P65 subunit of NF-κB, which may provide insight into the likelihood of H_2S-induced changes in protein activity in terms of structural and functional aspects. The effect of reactive nitrogen and oxygen species (RNS and ROS, respectively)-induced protein persulfidation on protein activity is a well-known phenomenon (Aroca et al. 2017). As a result, ROS and H_2S -induced persulfidation can be linked and H_2S may modulate ROS and NO signaling at various levels (Hancock and Whiteman 2016). The persulfidation process helps to study the physio-pharmacological changes that occur in the plant due to H_2S (Filipovic et al. 2018). Surprisingly, the persulfidation of proteins includes a set of protein phosphatases and kinases which bring about ABA signaling in guard cells (Zhou et al. 2020). Significant progress has been made in understanding the complexities of persulfidation processes involved in modifying plant ABA signaling using the model plant Arabidopsis. ABA, a crucial plant growth hormone, plays a vital role in regulating plant developmental mechanisms, particularly in response to fluctuating climatic conditions. (Aroca et al. 2021). H_2S interacts with ABA as well with as other hormones and ionic compounds like auxin, ethylene, calcium, oxides of carbon, and nitrogen (Xuan et al. 2020). ABA suppresses the activity of clade by binding to the ABA receptors pyrabactin resistance/pyr-like/ controlling constituent of ABA receptor (Gong et al. 2020). Thereafter, Sucrose non-fermenting (SNF1) related protein KINASE_2.6 SnRK_2.6/open stomata-1 [OST1] is then activated, causing several downstream signaling cascades to be triggered (Gong et al. 2020). In guard cells, H_2S modulates ABA signaling by the persulfidation of SnRK-2.6 (Chen et al. 2020b). SnRK-2.6's activity of kinase and its integrity with ABA Response Element-Binding Factor-2 is enhanced by persulfidation of the Cys-137 and Cys-131 residues (Chen et al. 2020b). The activation of downstream target genes through the phosphorylation of ABF2 plays a critical role in the regulation of stomatal closure. Furthermore, recent research has demonstrated that, preceding ABA, H_2S triggers the persulfidation of DES-1 at Cys-44 and Cys-205, leading to an increase in H_2S levels within stomatal cells (Shen et al. 2020). Through the persulfidation of specific residues, Cys-825 and Cys-890, in the NADPH respiratory burst oxidase homolog-D (RBOHD), the temporary increase in H_2S levels triggers an excessive accumulation of reactive oxygen species (ROS), leading to stomatal closure. Consequently, the accumulated endogenous ROS in guard cells can hinder the ongoing activation of ABA signaling, establishing a negative feedback loop that involves the per-sulfide oxidation of RBOHD and DES1. (Shen et al. 2020). H_2S-mediated protein persulfidation could be used in a variety of ways, such as thiol group of cysteine residues (Corpas et al. 2021) and alter the abscisic acid signaling in a specific tissue and complex manner.

11.4 Adaptive Response of H_2S to Abiotic Stress

The adaptive mechanism of H_2S in the reduction of environmental stresses are classified below into the following subgroups.

11.4.1 Heat Stress and H_2S Signaling

High-temperature stress has already become a notable abiotic stress around the world as a result of global warming, and the process of heat stress damage and tolerance to this stress has gained great popularity (Wahid et al. 2007; Emmati et al. 2015). After being treated with H_2S, plants' heat tolerance improves as well. Pretreatment with NaHS improves the high-temperature tolerance of wheat seedlings in such a way that it is specific to H_2S, but not to other sulfur-containing compounds

(Yang et al. 2016). High temperatures, like other stresses, can cause endogenous production of hydrogen sulfide in many plant species. High-temperature treatment at 35 °C boosted LCD activity, which stimulated the formation of cellular H_2S in 3-week-old seedling of tobacco, and this is continuously increased till the third day of heat application (Chen et al. 2016). More intriguingly, high-temperature H_2S generation can cause the buildup of jasmonic acid, which promotes the synthesis of nicotine. These findings imply that nicotine and H_2S production are related under high-temperature-stressed tobacco plants. Furthermore, heat stress generated a significant rise in H_2S concentration in strawberry seedlings after the first, fourth and eighth hours of exposure to 42 °C in contrast to untreated plants. After the first hour of high-temperature stress, 0.1 mM NaHS pretreated plants had a significant increase in H_2S concentration, which then is slowly minimized to untreated regimes (Christou et al. 2014a). Pretreatment of NaHS helps to the enhancement of seedling emergence and seedling aliveness, on the other hand reducing enhanced the leakage of electrolytes in the roots of plant and MDA gathering in the heat-stressed coleoptiles of *Zea mays* (Li et al. 2013). In addition, H_2S increased the performance of 1-pyrroline 5-carboxylate synthetase (1P5CS) at the time of lowering the performance of proline dehydrogenase (PD), resulting in proline buildup. These findings suggest that the production of proline may be included in *Z. mays*' increased heat tolerance when treated with H_2S (Li et al. 2013). Meanwhile, due to increased LCD activity, pre-treated with the exogenous NO sodium nitroprusside (SNP) donor, dramatically enhances the surviving percentage of *Zea mays* seedlings in the presence of high-temperature stress. While the treatment with the H_2S donor GYY4137 improved *Z. mays* seedlings' NO-induced heat tolerance, inhibitors of H_2S production and H_2S scavengers inhibited this activity. These findings showed that H_2S works as a downstream signaling molecule of NO to protect *Z. mays* seedlings from oxidative damage produced by heat stress (Li et al. 2013). It is uncertain what function H_2S plays in plant priming for the acquisition of systemic heat stress resistance. Application of H_2S levels on temperature endurance in tobacco cells as well as connected with Ca^{2+} in protecting responses was studied by Li et al. (2012b). The observations showed that pre-treated NaHS improved the proportion of tobacco mixture cultured cells that survived heat stress and their ability to re-grow afterward. Furthermore, H_2S decreased the activity of *S*-nitrosoglutathione reductase (GSNOR) and downstream antioxidant enzymes, allowing poplars to withstand higher temperatures (Cheng et al. 2018) (Table 11.1).

11.4.2 Drought Stress and H_2S Signaling

Low moisture is a key abiotic limitation that has an impact on plant output and quality all over the world. When leaf water loss exceeds root water intake, membrane damage, turgor loss, lowering yield, and cell death occur (Santisree et al. 2018). Drought has an impact on crops in both arid and semiarid climates. Plants have evolved different systems to sense and send signals that communicate information

Table 11.1 Effect of exogenous H_2S application against heat stress on various crops

Plant species	H_2S roles under stress	Source of H_2S	Reference
Zea mays	Salicylic acid (SA) promotes H_2S synthesis; increases the germination of seed, survival percentage and tissue vitality; increased capability of antioxidants	1.5 NaHS (mM)	Li et al. (2013) and Li (2015)
Nicotiana tabacum	Increasing antioxidant activity	0.05 NaHS (mM)	Li et al. (2012b) and Li and He (2015)
Fragaria × *ananassa*	Controlling homeostasis of ascorbate/glutathione homeostasis. Expression of genes related to heat-shock proteins, antioxidants and aquaporins	0.1 NaHS (mM)	Christou et al. (2014a)
Zea mays	Increasing antioxidant activity	0.7 NaHS (mM)	Li et al. (2014b)
Poplar	Enhancement of GSNOR activity and lower the reactive NO alteration	_	Cheng et al. (2018)
Fragaria ananassa	Betterment in plant phenotypic damage created by high temperature, which improve antioxidants capacity, improve HSP and AQP production	100 mM NaHS	Christou et al. (2014b)

about water scarcity throughout evolution and diversity (Gong et al. 2020). In this cascade, H_2S plays a crucial role. The role of H_2S in drought tolerance is centered on the regulation of guard cell mobility, which is primarily controlled by alterations in their turgor condition, which affects both transpiration and gas exchange (Zhang et al. 2010a). Drought stress tolerance is improved by H_2S in two ways. The first is via promoting stomatal closure, which reduces water loss, and two is by increasing antioxidant enzyme levels (Li et al. 2017). H_2S is known to cause fatty acid breakdown, cyanoamino acid metabolism, endoplasmic reticulum (ER) related amino acids activities, and the Kyoto encyclopedia of gene & genome (KEGG) route of ribosomal production in large vacuole containing cells when they are stressed by drought (Li 2015; Li and He 2015). H_2S regulates seed germination, stomatal closure, and root growth, in addition, it increases low moisture stress tolerance and heat shock resistance and reduces metal and oxidative stress (Jin et al. 2011, Wang et al. 2012). By modulating the energy currency binding cassette (EcBC) movement in the ABA-dependent signaling pathway in stomatal cells, H_2S increases drought tolerance in *Vicia fabia*, Impatiens walleriana and *Arabidopsis thaliana* by promoting stomatal closure and lowering the loss of water (Garcia-Mata and Lamattina 2010).

Furthermore, Shen et al. (2013) reported that H_2S regulates the look of miR-393 and its specific genetic movement inhibiting reaction-1 and auxin hormone signaling F box amino acids (AFB-1, AFB-2 and AFB-3) in Arabidopsis under drought conditions. In guard cells, H_2S is necessary for the ABA signaling cascade to work. During stomatal closure, the gas combines with ROS and NO (Scuffi et al. 2014). The dynamic activation of ion channels and ionic movable proteins located at the vacuolar and plasma membranes causes guard cells to open and close (Gong et al. 2020). Scuffi et al. (2018) found that hydrogen sulfide drives various ion fluxes that cause the closing of stomata in Arabidopsis during drought stress conditions. Furthermore, Zhang et al. (2010a) demonstrated that 0.1 mM NaHS concentration of H_2S modifies the activity of antioxidant enzymes in soybean seedlings, effectively boosting chlorophyll content, lowering MDA content, and increasing the level of O_2 and H_2O_2, resulting in increased drought tolerance. In the presence of the water shortage effect, the expression arrangement of L/DCD in Arabidopsis seedlings was comparable to those genes linked with drought, whose expression was highly boosted due to H_2S (Jin et al. 2011).

Similarly, pretreatment of *F. ananassa* roots with NaHS prevented oxidative and nitrosative stress, implying that H_2S plays an essential function in moisture stress mitigation (Christou et al. 2013). Exogenous treatment of wheat seedling leaves with an H_2S giver NaHS increased ABA-biosynthesis, its reactivation genetic look, and relative water content (Ma et al. 2016). In *A. thaliana*, *I. walleriana*, and *Vicia fabia*, H_2S-induced stomatal closure was seen (Garcia-Mata and Lamattina 2010). In summary, the above discussion clearly highlights that H_2S as a signaling molecule shows a vital part in crops opposite to the drought stress. H_2S as a signaling molecule in plants also increases tolerance in plants against drought stress (Table 11.2).

Table 11.2 Effect of exogenous H_2S application against drought stress in various crops

Plant species	H_2S roles under stress	Source of H_2S	Reference
Glycine max	Improvement in leaf green pigment and reduction in the synthesis of H_2O_2	0.1 mM NaHS	Zhang et al. (2010a)
Arabidopsis thaliana	Stimulating the look of moisture stress related genes	0.08 mM NaHS	Jin et al. (2011)
Triticum. aestivum	Improved capability of enzymatic and non-enzymatic antioxidants, enhance membrane stability (MS), close the stomata, up regulation of ABA-biosynthesis	500 µM & 1 mM, NaHS	Ma et al. (2016)
A. thaliana	Closed stomata; increase the production of H_2S, as well as survival rate	GYY-4137 80 mM NaHS	Garcia-Mata and Lamattina (2010)
Ipomoea batatas	Increasing antioxidants capability enhance MS of cell	0.8 mM of NaHS for 8 days	Zhang et al. (2009)
Vicia fabia	Guard cell closure in dose dependent way	500 mM NaHS	Garcia-Mata and Lamattina (2010)
Impatiens walleriana	Decrease in water wastage by 20% and induction in guard cell closure	500 mM NaHS	Garcia-Mata and Lamattina (2010)
F. ananassa	Enhancement in RWC and stomatal conductance (SC)	100 mM NaHS	Christou et al. (2013)

11.4.3 Salt Stress and H_2S Signaling

Salinity is a major worldwide problem that affects the plant at different growth stages from sowing to harvesting including germination, vegetative and reproductive stages. Salt overload disrupts ion equilibrium and redox equilibrium, resulting in the production of free radicals (Kabil et al. 2014). Salinity stress depolarizes membranes by activating outward rectifying potassium (K^+) channels, which results in (potassium) K^+ loss (Hirsch et al. 1998). A growing body of research suggests that preventing sodium-induced K^+ leakage is crucial for plant salinity tolerance (Shabala and Cuin 2008). Different transport systems at the plasma membrane (PM) and vacuole membranes (VM), including the H^+ ATP and ion channeling, appear to finely regulate this specific intake and movement of K^+ and Na^+ (Morsomme and Boutry 2000). Similar to drought stress, salt stress causes an osmotic stress response, which outcomes in rapid production of H_2S signaling molecules. The activation of total LCD activity and increased endogenous H_2S generation (30–70 nmol/g FW) in alfalfa seedlings were progressively produced by increasing the quantity of (Sodium chloride) NaCl (50–300 mM) (Lai et al. 2014). Furthermore, abundance research investigations on H_2S have revealed that H_2S is implicated in different environmental stresses like salt stress (Jiang et al. 2019).

Hydrogen sulfide protects plant against salinity stress-induced oxidative damage through improving the effectiveness of antioxidative defense systems (Guo et al. 2016). Priming of seeds with H_2S for 1/2 day considerably reduces the effect of salt stress on seedling growth and the emergence of seedlings, as evidenced by enhanced germination percentage, seed vigour indices, and the development of wheat seedlings in a concentration-dependent fashion (Bao et al. 2011). In salt stress, H_2S could protect cucumber seedlings by stabilizing Na^+/K^+ maintenance, modulating endogenous H_2S treatments, and boosting the anti-oxidative system (Jiang et al. 2019). Exogenous H_2S induces osmoregulation in *Oryza sativa*, *C. sativus*, and *C. dactylon* in reply to saline stress, according to a growing number of studies (Shi et al. 2013, Mostofa et al. 2015). NO creates different types of oxidative and cellular changes in plants (Corpas 2019). Exogenous H_2S can alleviate both nitrification and oxidative stress in strawberry (*F. ananassa*) plants in the presence of saline or non-ionic osmotic variations by preserving the huge redox state of glutathione (GSH) and ascorbic acid (AA) (Christou et al. 2013).

Surprisingly, NO and H_2S are the two gasotransmitters, helpful in almost all physiological and stress related functioning of plant (Mishra et al. 2021). Up-stream or down-stream of NO is controlled by H_2S (Corpas 2019). NO has a role in H_2S-enhanced salt tolerance in plants. The buildup of H_2S in tomato leaves and roots is promoted by NO (Silva et al. 2017). H_2S, on the other hand, cannot induce the accumulation of NO, indicating that it operates downstream of NO and hence helps tomato plants in tolerating excessive salt (Silva et al. 2017). All of these studies support that H_2S plays a role in in reestablishing redox homeostasis at the time of responding plant to extreme saline levels by activating the antioxidant status, maintaining a high K^+ to Na^+ ratio (Lai et al. 2014) and accumulating osmolytes (Shi

Table 11.3 Effect of exogenous H_2S application against salt stress in various crops

Plant species	H_2S roles under stress	Source of H_2S	Reference
Medicago sativa	Activating antioxidant enzyme	0.1 mM NaHS	Wang et al. (2012)
Triticum aestivum	Increase germination rate and growth	0.13 mM NaHS for 12 h	Bao et al. (2011)
Arabidopsis thaliana	Keep out minimum sodium to potassium ionic ratio, enhance the genetic look and the phosphorylation of H^+ ATPase and sodium to potassium ionic antiporter	0.2 mM NaHS	Li et al. (2014a,b)
Cucumis sativus	Maintaining Na^+ and K^+ homeostasis	–	Jiang et al. (2019)
Oryza sativa	Improved capability of antioxidants both non-enzymatic and enzymatic; ionic balance	50 mM NaHS	Mostofa et al. (2015)

et al. 2013). Furthermore, various investigations and the preceding arguments demonstrate that H_2S is a plant signaling molecule resistance to extreme salinity stress (Table 11.3).

11.4.4 Heavy Metal and Other Metalloids Stress and H_2S Signaling

Heavy metals (HMs) are metal elements with densities larger than 6 g mL^{-1}, such as chromium, copper, and Zinc (Gupta et al. 2013). HMs have become significant type of abiotic stresses in plants because of high toxicity and persistence, and it even poses a concern to human health via the food chain. Heavy metal stress frequently causes oxidative stress, or an overabundance of reactive oxygen species (ROS), which causes protein oxidation, peroxidation of lipid, DNA damage, and enzyme inactivation (Yadav 2010). On the other hand, lengthy plants have developed a complex antioxidative defending mechanism to hunt excess (ROS) and protect plant equilibrium (Foyer and Noctor 2009, 2011). Cd is an extremely poisonous, non-soluble heavy metal that interferes with plant physiological systems (Yasir et al. 2022). It is absorbed by the plant roots and delivered to the plant's areal sections, producing enzyme inactivation, chlorosis and necrosis by replacing critical cofactors and disrupting the plant's mineral homeostasis (Sandalio et al. 2001). Furthermore, recent research has demonstrated the relevance of D-CDes and L-CDes in the modulation of plant responses to HM stress (Fang et al. 2016). Moreover, H_2S generated by L-CDes modulates Cd tolerance in Arabidopsis as a downwind signaling molecule of salicylic acid (SA) (Qiao et al. 2016). Although SA significantly increases L-CDes enzyme activities, it does not protect L-CDes knockout mutants from Cd stress (Qiao et al. 2016). In comparison to Cd treatment alone, Cd stress increased H_2S generation in *Oryza sativa* seedlings, which was further improved by exogenous administration of sodium hydrosulfide (NaHS). The increase in endogenous H_2S levels observed during elevated Cd stress was reduced by HT, showing the specificity of H_2S build-up under high cadmium stress (Mostofa et al. 2015). A lot of findings indicate that H_2S is not acting on its own. It's important to look at how H_2S interacts with other signaling molecules including NO, Ca^{2+}, and salicylic acid (SA). Plants' endogenous synthesis of NO and H_2S responds to Cd stress in a significant way (Shi et al. 2014). Exogenous NO and H_2S boosts *Cynodon dactylon* tolerance to Cd stress (Shi et al. 2014). NO triggered H_2S generation is required in Cd stress responses, according to pharmacological investigations (Shi et al. 2014). Meanwhile, H_2S maintained mineral homeostasis, reduced oxidative stress, elevated numerous enzymatic antioxidants, and enhanced the phenotypic expression of *S. italica* under Cd stress conditions (Tian et al. 2017).

Aluminum (Al), an unnecessary metal for plants, has a negative effect on crop production and even its survival, particularly in acidic soils. Al stress decreased root elongation in barley seedlings but pretreatment with NaHS partially restored the root elongation inhibition generated by Al and this recovery was strongly connected with the decline in Al build-up in seedlings (Chen et al. 2013). Furthermore, by activating the antioxidant system, NaHS dramatically reduced citrate release and oxidative stress (as evidenced by lipid oxidation and ROS blast) caused by Al (Chen et al. 2013). Zhang et al. (2010b) obtained comparable results in *Triticum aestivum*. The use of H_2S donor increased the activity of the enzymatic ascorbate-glutathione (AG) cycle while reduced the formation of ROS, allowing the cell to retain its redox status and reduce arsenate toxicity in peas (Singh et al. 2015).

Cr is a typical HM that has a negative impact on plants. Cr^{3+} and Cr^{6+} are the most common forms, which have been collected from the tanning, leather and textile sectors as well as the painting and steel sectors and became the main cause of pollutants in the environment (Gupta et al. 2009). The production of H_2S is induced by 10 mM Cr^{6+} in a dosage and time depending orders, with a maximum after a day of Cr^{6+} treatment. Elevated appearance of H_2S biosynthesis associated genes DCD, DES, and LCD preceded by enhancing H_2S generation, showing that Cr^{6+} stress triggers endogenous H_2S synthesis via startup its outflow mechanism in Foxtail millet. Further research revealed that H_2S reduced Cr accumulation in cells not only by downregulating the appearance of genes involved in HM intakes, such as *ZIP1*, *ZIP4*, *ZIP3*, and *ZIP6* and also by upregulating the appearance of genes involved in HM efflux, such as *MTPC* 1, *MTPC* 2, *HMA* 3–1, *HMA* 3–2, in a Ca^{2+} depending order. Exogenous administration of NaHS might boost the wheat emerging seedling by enhancing the performance of esterase, amylase, and key enzymatic antioxidants while lowering the performance of lipoxygenase in a dose-dependent order. As a result, NaHS treatment reduced the amounts of malondialdehyde and H_2O_2 generated by Cr while maintaining the high endogenous H_2S level (Zhang et al. 2010b). Cr toxicity in the soil can cause chlorosis, necrosis, plant development disturbance, and plant mortality (Gupta et al. 2009). Cr, for example, caused cell demise in the root end of *Setaria italica* due to an excess of ROS (Fang et al. 2016). Recent studies and previous research, as well as the explanation above, show that H_2S as a secondary messenger reduces oxidative stress and improves the plant defense system of plant against HM stress.

Kaya and Ashraf (2019) looked into the role of NaHS as an H_2S donor on strawberry seedlings in the presence of iron deficit. Exogenous application of NaHS to plant leaves by spraying a 0.2 mM sodium hydrosulfide solution over them. Under iron deficiency, strawberry plants developed leaf interveinal chlorosis. The application of sodium hydrosulfide foliar, on the other hand, was able to alleviate these problems. In young leaves, sodium hydrosulfide enhanced chlorophyll concentration, and available iron and iron enhancement. Under iron deficiency, the amounts of H_2O_2, EL, and malondialdehyde (MLD) in plant leaves get improved. Exogenous application of NaHS reduced H_2O_2, EL, and malondialdehyde (MLD) while also enhancing the performance of important enzymatic antioxidants. This results in NaHS being found to be effective in treating chlorosis caused by an iron deficiency (Table 11.4).

Table 11.4 Effect of exogenous H_2S application against Heavy metals stress in various crops

Plant species	Stress applied	H_2S roles under stress	Sources of H_2S	Reference
Triticum aestivum	Al-stress Al stress	Lowering Al increment, relieving the secretion of citrate, and oxidative variations	0.6 mM NaHS	Zhang et al. (2010b)
Hordeum vulgare		Lowering Al increment, relieving the secretion of citrate, and oxidative variations	0.2 mM NaHS	Chen et al. (2013)
Setaria italica	Cd stress	Lowering EL and improving photosynthetic activity	–	Tian et al. (2017)
Pisum sativum	As stress	Enhanced level of nitrogen mono-oxide, relieve oxidative stress	–	Singh et al. 2015
Setaria italica	Cr stress	Improved seed germination	50 mM NaHS	Fang et al. (2016)
Zea mays L.		Plasma membrane integrity	500 μM NaHS	Kharbech et al. (2020)
		Modulate H_2S metabolism	500 μM NaHS	Kharbech et al. (2022)
Oryza sativa	Hg stress	Improving the transcription of bZIP60, alleviating Hg toxicity	–	Chen et al. (2017)
Brassica oleracea var. botrytis	Pb stress	Upgrading non-protein thiols and total GSH steps	–	Chen et al. (2018)
Strawberry (*Fragaria × ananassa Duch.*)	Iron deficiency	Reduces hydrogen peroxide, malondialdehyde, and EL. enhance iron intake. Up-regulating performance of enzymatic antioxidants	0.2 mM NaHS	Kaya and Ashraf (2019)

11.4.5 Cold Stress and H_2S Signaling

During exposure of plants to low temperatures stress (> 0 °C) and extremely lower temperatures (< 0 °C), they experience cold stress. Cold stress frequently causes cell membrane failure, as well as oxidative and osmotic impairment (Chongchatuporn et al. 2013). Examined plants protect themselves from cold stress harm by adjusting their osmotic pressure and activating their antioxidant system. Exogenous treatments such as methyl jasmonate (MeJA) and SA have been projected in the past to help plants recover from cold stress damage (Wang et al. 2009). Chilling treatment at 4 °C increased the activities of L.DCD-1 and boosted the expression of *L.DCD-1* genes which raised the accumulation of endogenous H_2S (7–15 mol/g FW) in seedlings of grape vine (Fu et al. 2013). Furthermore, the latest study on banana fruit found that H_2S spraying preserved peel hardness, increased lightness values, and reduced MDA accumulation during cold stress (Luo et al. 2015). Meanwhile, H_2S reduced cold stress injury in *Musa* fruits by increasing antioxidant capacity by encouraging the manufacture of both enzymatic and non-enzymatic substances, such as important phenylalanine ammonia-lyase, PPO, antioxidant enzymes, and total phenolics (Luo et al. 2015). H_2S fumigation also inhibited ethylene synthesis, implying a negative connection between H_2S and ethylene in preserving post-harvest traits quality at the time of storage at low temperature. Further research revealed that H_2S spraying increased the contents of proline by activating the activity of P5CS and decreasing proline dehydrogenase (ProDH), which could be connected to cold damage tolerance (Luo et al. 2015). In bermuda grass, Shi et al. (2013) discovered that low-temperature stress levels as 4 °C might promote the buildup of intercellular H_2S levels. Ma et al. (2015) used comparative proteomics to explore the vibrant structures of amino acid outlook in a herbaceous plant like *Lamiophlomis rotata* (Benth.) cultivated at three fluctuating altitudes 4350 m, 4800 m, and 5200 m, and found that the treatments and enzymatic performance of proteins involved in H_2S bio-synthesis (OAS-TL, CAS, and L/DCD) improved markedly with increasing altitudes from 4800 m to 5200, indicating that H_2S performs a key function in *L. rotate* adaptations to environmental stress at higher elevations. Hydrogen sulfide increased total phenolic content, the activity of phenylalanine ammonia-lyase, and antioxidant capacity in banana fruit, reducing ROS formation and enhancing chilling tolerance (Luo et al. 2015).

H_2S improves the chilling tolerance of hawthorn fruit, according to Aghdam et al. (2018) by improving the performance of enzymatic antioxidants and encouraging phenol accumulation. As a result of these findings, H_2S appears to be able to influence the look of the associated genome, boost the enzymatic antioxidant activities, and stimulate the build-up of phenolic compounds in plants that are subjected to low-temperature stress (Li and Jin 2016). H_2S (0.5 mM NaHS) significantly increased the performance of cytochrome c oxidase, H^+-ATPase, and succinate dehydrogenase, resulting in improved chilling tolerance and energy status in banana fruiting (Luo et al. 2015). In conclusion, the above discussions and many research findings clearly show that H_2S as a signaling molecule in plants increases cold stress adaption (Table 11.5).

Table 11.5 Effect of exogenous H_2S application against cold stress on various crops

Plant Species	H_2S response to stress	Sources of H_2S	Reference
Lamiophlomis rotate	Improving antioxidant enzyme activity, as well as proline and sugar build up	0.05 mM NaHS	Ma et al. (2015)
Vitis vinifera	Increasing SOD activity and $VvCBF_3$ and $VvICE_1$ gene expression	0.10 mM NaHS	Fu et al. (2013)
Cynodon dactylon	Regulate non-enzymatic antioxidant and antioxidant enzymes	0.50 mM NaHS	Shi et al. (2013)
Musa paradisiaca L	Higher lightness and peel firmness values; formation of MDA	1.00 mM NaHS	Luo et al. (2015)
Musa paradisiaca L	Decrease ethylene production and preventing electrolyte leakage	–	Li and Jin (2016)
Crataegus monogyna	Boosting antioxidant enzyme activity and promoting phenols build-up	–	Aghdam et al. (2018)
Musa sp.	Decrease build-up of MDA, maintaining a higher peel firmness		Luo et al. (2015)

11.5 Mode of Action of H_2S

Hydrogen sulfide performs its various physiological activities primarily by the oxidative post-translational oxidation of cysteines (RSH) to persulfides (RSSH), according to new research (Filipovic et al. 2018). Multiple persulfidated amino acids have really been found in mammalian investigations to date (Krishnan et al. 2011) and the biomolecular process of protein persulfidation in mammals has been extensively documented and discussed further (Paul and Snyder 2015). The SSH has higher nucleophilicity and also can combine with electrophilic molecules as compared to the SH group. Such reagents are more readily available (Zhou et al. 2020). Methyl sulfonyl benzo thiazole (MSBT), S4bromobenzyl methanethiosulfonate (BBMTS), and methanethiosulfonate (MMTS) are only a few of the reagents available. As a result, by the combination of nucleophilic affinity-based screening, bioinformatics analysis, and mass spectrometry detection, the protein persulfidation proteome may be characterized (Aroca et al. 2015).

11.5.1 Protein Persulfidation

In *A. thaliana*, Aroca et al. (2015) presented the very first report of 106 proteins with persulfidation modifying cysteines. To improve on its previous investigation, the same group has done a logo test with MSBT as a barrier and found proteins in des1 mutant plants and in wild-type plants, from which most of them were involved in glycolysis, amino acid metabolism, protein biosynthesis, redox, and stress responses (Aroca et al. 2017). This elevated persulfidation sample is the largest persulfidation dataset to date, and it fully recapitulates prior proteomics results. Furthermore, the findings also made us aware of persulfidating amino acids in crops. A range of essential proteins, including actin, are found in the persulfidome (Aroca et al. 2017). Eight *ACTIN* genes are found in the Arabidopsis genome, which are classified into two groups depending on their roles in the reproductive and vegetative systems (Mcdowell et al. 1996). H_2S modulates actin dynamics and influences root hair formation, according to Li et al. (2018). The overabundance of H_2S promotes f ACTIN2 (vegetative group) persulfidation at Cys287, resulting in actin cytoskeleton depolymerization and root hair development suppression (Li et al. 2018). Complementation of *actin* (2–1) mutant with an ACTIN-2 variant with a Cys-287 mutation specifically inhibited the H_2S depending on suppression of root fibers development (Li et al. 2018). In the case of ethylene signaling, an H_2S-related persulfidation feedback loop inhibits the action of ACC oxidases, the limited rate catalysts in ethylene production, which affects root hair elongation (Bleecker and Kende 2000).

Surprisingly, the persulfidation proteome includes a group of protein phosphatases and kinases that are implicated in abscisic acid signaling in guard cells (Aroca et al. 2017). Using the model plant Arabidopsis, recent advancement has been achieved in understanding the complication of persulfidation in modifying plant ABA signaling processes. ABA inhibits the activity of clade A protein phosphatases by binding to the ABA receptors Pyrabactin Resistance/ PYR-Like/ ABA Receptor Regulatory Component (PYR/ PYL/RCAR) (Gong et al. 2020). SNF-1 Related Protein Kinase-2.6 (SnRK2.6)/ Open Stomata-1 (OST1) is then activated, causing several downstream signalling cascades to be triggered (Gong et al. 2020). In guard cells, H_2S modulates ABA signaling by persulfidating SnRK-2.6 according to Chen et al. (2020b). SnRK-2.6's kinase action and association with ABA Response Element-Binding Factor-2 are enhanced by persulfidation of the Cys-131 and Cys-137 residues (Chen et al. 2020b). After then, phosphorylated ABF2 activates its downstream target genes, which is important in stomatal closure controls. In addition, current research shows that in the presence of ABA, H_2S activates DES1 by persulfidation at Cys44 and Cys205, resulting in a rupture of H_2S in guard cells (Shen et al. 2020). By persulfidating the NADPH oxidase also called respiratory burst oxidase homolog-D (RBOHD) on Cys890 and Cys825 residues, this temporary rise in H_2S increases the over-accumulation of ROS, resulting in guard cell closure (Shen et al. 2020). Endogenous ROS build-up in guard cells may hinder continued ABA signaling activation, resulting in a negative feedback loop involving DES1 and RBOHD persulfide oxidation (Shen et al. 2020). Overall, H_2S-mediated protein persulfidation has the potential to modify ABA signaling in a tissue-specific and complex way.

11.6 Conclusions and Future Perspective

In conclusion, our understanding of H_2S is rapidly catching up fast with that of NO and H_2S is becoming a key signaling modulator involved in a variety of biological activities, including the regulation of multitudinous stress responses. Understanding of the complexities of signaling cascades and hydrogen sulfide regulation systems in crops has advanced to unprecedented levels in recent years (Fig. 11.1).

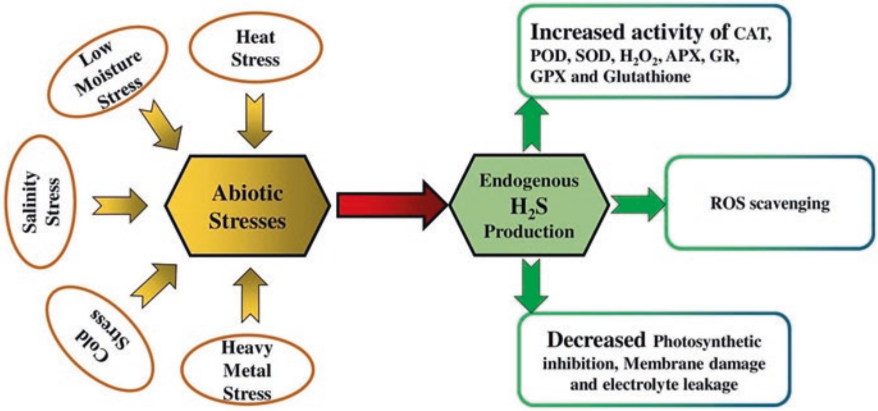

Fig. 11.1 The mechanism of H_2S-mediated abiotic stress tolerance in plant. *CAT* catalase, *POD* peroxidase, *SOD* superoxide dismutase, H_2O_2 Hydrogen per oxide, *ROS* Reactive oxygen species, *APX* ascorbate peroxidase, *GPX* glutathione peroxidase, *GR* glutathione reductase, H_2S hydrogen sulfide

There is no doubt that future research developments in this field will pique people's interest. Scientists are presently striving to comprehend the inherent regulatory mechanisms of H_2S on the modulation of biochemical functions, thanks to the advancement of more modern bimolecular biology approaches. Despite the existence of many persulfidation proteomes (Wang et al. 2021) and the hundreds of proteins that undergo persulfidation in plants body, whereas the purpose and mechanism of persulfidated proteins still are unknown. The strong interaction of persulfidation and S-nitrosation on cysteines in response to environmental conditions warrants additional study. Comparative proteome investigations of H_2S-mediated persulfidation in plant cells will undoubtedly give useful insight into the regulation mechanisms of H_2S-mediated protein persulfidation in plant tissues under certain developmental processes or environmental alterations. Progress in the study of plant S-nitrosation will have significant implications for persulfidation studies in general. In Arabidopsis, the non-canonical catalase Repressor OF Gsnor-1 (ROG-1)/CAT3 has been identified and characterized as just a "transnitrosylase" that especially rearranges S-nitrosoglutathione reductase-1 (GSNOR-1) and modulates NO-based redox signaling revealing that enzymatic contributors for protein S-nitrosation status exist (Chen et al. 2020a). Undoubtedly, an equilibrium relationship of protein persulfidation is required for cellular physiological functions. Additionally, many proteins are more susceptible to persulfidation than others, depending on their redox state (Fu et al. 2019). A sulfur-dioxygenase (ETHE-1), which oxidizes persulfides with in the mitochondrial matrix and considered to be involved in protein persulfidation and sulfur signaling, is an interesting find (Lorenz et al. 2018). Investigation and identification of persulfidated-functioning proteins, as well as the underlying preferential persulfidation pathways, will help us more detailed and extensive understanding of how H_2S works in plant biology. Exogenous administration of fairly modest levels of H_2S donor via fumigation or spray has routinely demonstrated the beneficial role of H_2S on plant growth parameters under a wide range of environmental conditions. H_2S may have a great value-added capability for the use and growth of modern farming, due to the low expense of these chemicals and the ease with which they can be administered. As a result, it will be critical to investigate how H_2S might be used in agricultural productivity.

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Chapter 12
Hydrogen Sulfide Metabolism and Its Role in Regulating Salt and Drought Stress in Plants

Akankhya Guru, Kundan Kumar, and Padmanabh Dwivedi

Abstract Environmental stresses such as salinity and drought have a disastrous impact on the agricultural sector affecting crop performance, growth, and development. Salinity stress minimizes plant growth by means of osmotic stress followed by ion toxicity, nutrient imbalance, and oxidative stress. Drought is the predominant abiotic stress factor that reduces crop productivity by creating water deficit conditions. To combat salt and drought stresses, numerous defense mechanisms including the accumulation of osmolytes, activation of stress-responsive genes, transcription factors, and antioxidant defense machinery are involved. Hydrogen sulfide (H_2S) has turned up as a new gaseous signaling molecule that favors various physiochemical events and helps in acclimatization to variations due to stress in plants. The present chapter focuses on exploring the H_2S-induced tolerance mechanism of action at physicochemical and molecular levels in salt and drought-affected plants.

Keywords Hydrogen sulfide · Salt stress · Drought stress · Metabolism

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T. Aftab, F. J. Corpas (eds.), *Gasotransmitters Signaling in Plants under Challenging Environment*, Plant in Challenging Environments 5,
https://doi.org/10.1007/978-3-031-43029-9_12

12.1 Introduction

Since plants are sessile, they are easily subjected to several abiotic stresses including salinity and drought (Li 2013). This leads to the production of reactive oxygen species, (ROS) such as superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), and singlet oxygen ($^1O_2$) in plant cells (Choudhary et al. 2017), which exert negative influences on the overall growth of plants. To improve crop performance, antioxidative enzyme (superoxide dismutase, peroxidase, catalase, etc) profiles are increased and thereby imparting stress tolerance in plants via scavenging ROS (Guo et al. 2016).

Hydrogen sulfide (H_2S) is currently being evaluated as a signaling molecule under changing environmental conditions in plants. Apart from nitric oxide (NO) and carbon monoxide (CO), H_2S has been regarded as the third most important gasotransmitter that controls cell-mediated functions in both plants and animals (Williams et al. 2015). Because of its unpleasant and pungent smell, it has been treated as an environmental pollutant for so long (Jin and Pei 2015). In the past, H_2S was believed to be phytotoxic owing to its blocking action on cytochrome c oxidase, a central enzyme involved in the mitochondrial electron transport system. Later, its role in plant growth, development, fruit maturation, seed germination, stomatal closure, senescence control, and alleviation of different abiotic stresses in plants was confirmed (Table 12.1). H_2S, as a stress tolerance molecule, promotes crop growth by controlling ROS generation. The use of H_2S may enhance the levels of several antioxidant components, leading to increased tolerance (Corpas 2019; Corpas and Palma 2020). It is currently being used exogenously for extra protection because of its key regulatory function in abiotic stress. Currently, this chapter discusses H_2S biosynthesis, the impact of H_2S applied exogenously/endogenously on plants facing salinity and drought stress. Furthermore, the fundamental objective of this chapter is to deliver enough knowledge about the mechanism involved in H_2S signaling-induced stress tolerance in crops (Table 12.1).

12.2 Hydrogen Sulfide Metabolism in Plants

The biosynthesis of hydrogen sulfide takes place through different enzymatic pathways (Fig. 12.1). The majority of H_2S synthesis eventuates in the chloroplast, however, cytosol and mitochondrion are involved to a certain extent (Aroca et al. 2018). Five enzymes namely L-cysteine desulfhydrase, (L-DES), D-cysteine desulfhydrase (D-DES), l-3-cyanoalanine synthase (CAS), cysteine synthase (CS), and sulfite reductase (SiR) contribute to H_2S biosynthesis in plant cells (Yamasaki and Cohen 2016). The cytoplasmic enzyme L-DES depicts a principal involvement in the H_2S biosynthesis by converting L-cysteine to pyruvate along with the release of NH_4^+ and H_2S, utilizing pyridoxal 5′-phosphate (PLP) as a cofactor to quicken this reaction. Similarly, in the cytoplasm, D-DES catalyzes the formation of pyruvate, NH_3,

Table 12.1 Effects of exogenous application of H_2S on various abiotic stresses in plants

H_2S dose	Abiotic stress	Crop	Protective function	Reference
15 mM	Salinity	*Cucumis sativus*	Maintains Na^+/K^+ balance, regulates H_2S metabolism and ROS homeostasis	Jiang et al. (2019)
0.05 mM	Salinity	*Triticum aestivum*	Maintenance of lower Na^+ content	Deng et al. (2016)
0.05 mM	Drought	*Triticum aestivum*	Increased antioxidant levels, reduced sugar, and starch contents, decreased lipid peroxidation	Ding et al. (2018)
100 mM	Drought	*Spinacea oleracea*	Altered glycine betaine content, increased soluble sugars, and polyamines levels	Chen et al. (2016)
0.2 mM	Cadmium	*Triticum aestivum*	Reduced oxidative damage	Kaya et al. (2020)
0.5 mM	Alkalinity	*Brassica oleracea*	Regulation of glutathione (GSH) homeostasis	Montesinos-Pereira et al. (2018)
0.1 mM	Hypoxia	*Zea mays*	Elevated endogenous H_2S level, enhanced alcohol dehydrogenase (ADH) activity	Peng et al. (2016)
0.15 mM	Heat	*Triticum aestivum*	Elevated gene expression levels of antioxidant enzymes	Min et al. (2016)
0.1 and 0.5 mM	Chilling	*Triticum aestivum* and *Secale cereale*	Decreased malondialdehyde content	Kolupaev et al. (2019)
100 mM	Nickel	*Oryza sativa*	Prevented chloroplast damage, improved nitrogen metabolism	Rizwan et al. (2019)

and H_2S from D-cysteine. The mitochondrial enzyme CAS is responsible for catalyzing the condensation of L-cysteine to cyanide (CN^-) to produce H_2S. Due to the phytotoxic nature of CN^-, chloroplast and mitochondrial electron transfer chains are hindered. Plant cells can control the toxic levels of CN^- with the help of CAS enzymatic activity (Li 2015a). Another enzyme, cysteine synthase (CS), found in the cytoplasm, chloroplast and mitochondria is chiefly associated with the production of O-Acetyl-L-serine and H_2S from L-cysteine and acetate (Li 2015a). In cytoplasm, mitochondrion, and plastid, inorganic sulfur is the source of production of cysteine and H_2S in presence of the enzyme cysteine synthase (Jez and Dey 2013). H_2S biosynthesis in chloroplast can be mediated by PLP-dependent transferase, although the pathway is yet to be prospected (Majtan et al. 2018). Besides these sources, plants can also synthesize H_2S by reducing sulfite (SO_3^{2-}) in the presence of ferredoxin and a chloroplast enzyme, SiR. In plants, SO_3^{2-} may be produced from either sulfur dioxide (SO_2) taken up from the atmosphere or sulfate (SO_4^{2-}) via sulfur nutrition. SO_4^{2-} produces adenosine 5′-phosphosulfate (APS) in presence of ATP sulfurylase. Then, APS reductase catalyzes the further reduction of APS to SO_3^{2-} (Li 2015b; González-Gordo et al. 2020). H_2S mediates post-translational

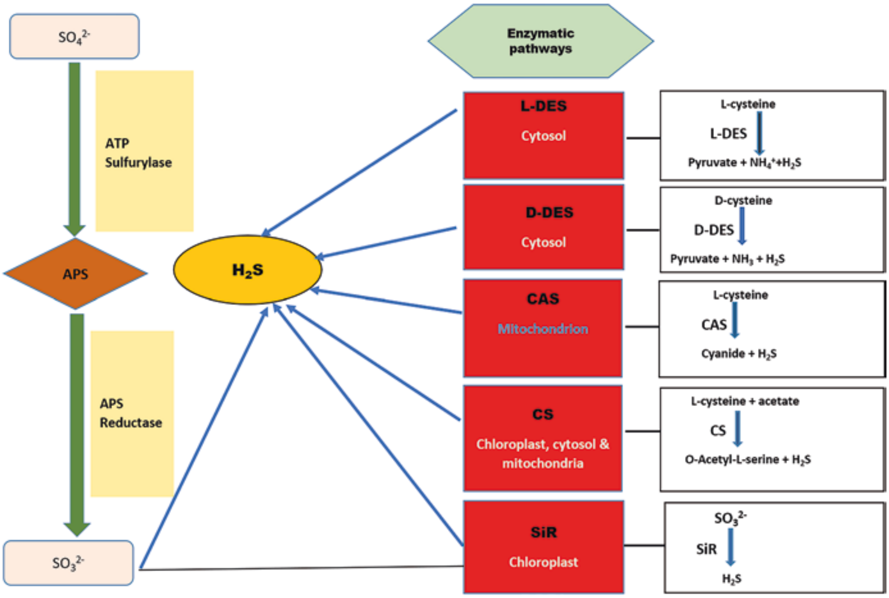

Fig. 12.1 Biosynthesis of hydrogen sulfide through different enzymatic pathways. *APS* Adenosine 5′-phosphosulfate, *L-DES* L-cysteine desulfhydrase, *D-DES* D-cysteine desulfhydrase, *CAS* l-3-cyanoalanine synthase, *CS* cysteine synthase, *SiR* sulfite reductase

modification known as persulfidation which is a process involving the transformation of cysteine thiol group (-SH) into its corresponding persulfide form (-SSH) (Corpas et al. 2021). This may lead to changes in the function, subcellular localizations and structures of target proteins. It has been reported that persulfidation acts as an important mechanism in preventing protein damage against oxidative stress (Fig. 12.1).

12.3 Insights into H_2S - Induced Salinity Tolerance in Plants via Modulation of Physico-Chemical Responses

H_2S regulates many physio-chemical processes in response to various concentrations of salt treatments in plants. High salinity disintegrates the photosynthetic apparatus and reduces the photosynthetic efficiency of crops. The levels of chlorophyll pigment as well as other accessory pigments in plants reduces in response to salt stress resulting in depreciated crop yield and productivity (Mbarki et al. 2018). Application of sodium hydrosulfide (NaHS), a donor of H_2S, preceding salt treatment in rice and other crops significantly increased the levels of photosynthetic pigments and many other metabolic events (Fig. 12.2). Total chlorophyll and carotenoid contents were comparatively higher in NaHS pretreated salt-stressed leaves

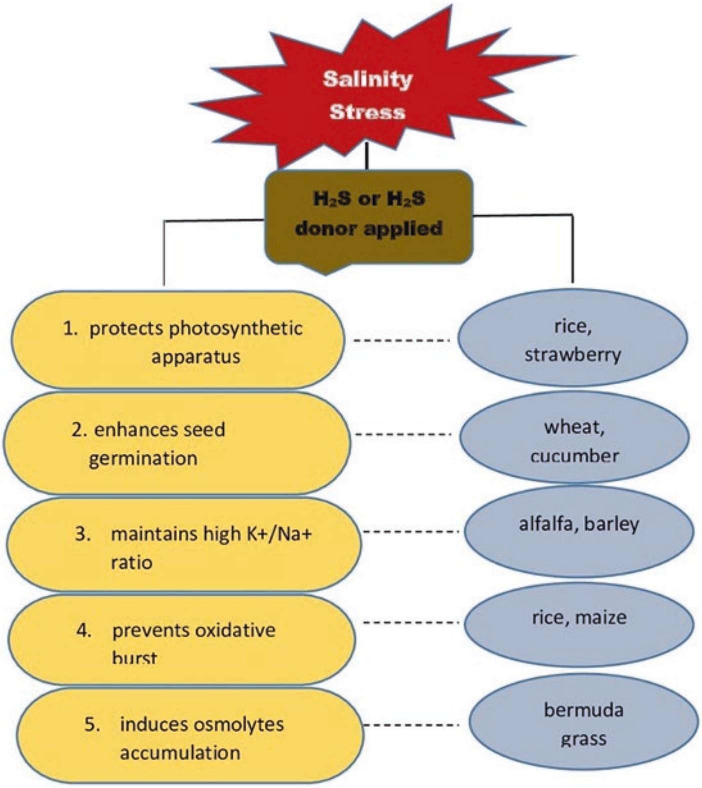

Fig. 12.2 Application of sodium hydrosulfide (NaHS), a donor of H_2S, preceding salt treatment significantly enhances photosynthetic pigments and many other metabolic events

than that of plants without NaHS treatment. Therefore, H_2S plays a pivotal role in triggering salinity tolerance in rice by protecting photosynthetic pigments, improving photosynthetic efficiency, and promoting chloroplast biogenesis (Mostofa et al. 2015a). Chlorophyll fluorescence is also affected negatively due to salt stress in plants. In strawberry plants susceptible to salinity stress condition, F_v/F_m ratio decreased dramatically, which was alleviated by treating plants with NaHS before NaCl application (Christou et al. 2013). Pre-treatment of strawberry roots with NaHS ensured maximum photosystem II efficiency, electron transport rate and hence improved photosynthetic performance. Further, stomatal conductance of H_2S pretreated and stressed plants was found to be higher as compared to non-stressed and plants without pre-treatment. H_2S promoted salt tolerance in wheat seedlings by improving the photosynthetic activity of leaves (Ding et al. 2019). Plants exposed to NaCl treatment alone showed decreased values for gaseous exchange parameters (photosynthesis, transpiration, and stomatal conductance) and leaf chlorophyll content. The addition of NaHS exogenously to salt-stressed wheat seedlings reduced the inhibiting effects of salt stress on leaf photosynthesis and chlorophyll content.

This led to the alleviation of foliar injury and the promotion of overall plant height. The application of 50 μM NaHS enabled wild-type *Arabidopsis* to preserve chlorophyll and carotenoid pigments in salt-affected leaves (Yastreb et al. 2020) (Fig. 12.2).

H_2S could significantly depress the knock-down effect of salt treatment on seed germination in wheat. Pre-treatment of wheat grain with NaHS showed higher amylase and esterase activities under stressed conditions (Ye et al. 2015). Under salinity stress, seed priming using NaHS successfully ameliorated the suppression of traits such as germination rate, germination index, and vigor index in the wheat genotype LM15 (salt-sensitive) (Bao et al. 2011). Salt stress reduced the rate of seed germination in cucumber but the exogenous application of 400 $\mu molL^{-1}$ NaHS could ameliorate this effect by stimulating the germination rate of cucumber seeds which could be attributed to an elevated level of amylase activity (Yu et al. 2013). Additionally, NaHS increased the length of hypocotyl and radicle under salinity stress conditions in cucumber. Increased amylase activity facilitated the breakdown of starch to form glucose and hence enabling the allocation of carbohydrates to the embryonic axis which led to the promotion of hypocotyl and radicle growth. H_2S rescued salinity-induced suppression of plant growth. In barley, 50 or 100 μM NaHS treatment significantly increased root length while on the other hand, root length was inhibited due to exposure of plants to high NaHS concentration (Chen et al. 2015). Moreover, the addition of low NaHS (50 or 100 mM) treatments enhanced the biomass of barley seedlings and leaf relative water content in comparison to 150 mM salt treatment alone. Pre-treatment of 100 μM NaHS mitigated the suppressive effect of salinity stress on root elongation in alfalfa (Lai et al. 2014). NaHS abolished salinity-mediated retardation of root growth in *Arabidopsis thaliana* (Li et al. 2014a, b).

On the other hand, H_2S alleviated salinity stress by deterring K^+ outflow in alfalfa seedlings (Lai et al. 2014). NaHS pre-treated alfalfa plants showed a phenomenal enhancement in K^+ concentration in roots along with an insignificant increase in Na^+ content and thus maintaining a high K^+/Na^+ ratio in stellar cells subjected to salt stress. Further, the electron-physiological analysis revealed that endogenous form of H_2S helps to maintain K^+ homeostasis by preventing the salt-activated K^+ efflux in the root maturation zone of alfalfa seedlings. However, the mechanism of H_2S-mediated ion homeostasis maintenance in *Arabidopsis* is different from that of alfalfa under salinity stress.

When plants are exposed to salinity stress, they face oxidative bursts usually caused due to the accumulation of toxic molecules such as ROS, which results in oxidative stress causing an impairment to nucleic acids, lipids, proteins, and carbohydrates. To overcome ROS burst, a complex ROS detoxification system, involving enzymatic antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and glutathione reductase (GR) among others as well as non-enzymatic antioxidant molecules (ascorbate, glutathione, etc.), has evolved by plants to maintain the survival of cells. H_2S is capable of alleviating oxidative bursts and improving crop responses to salinity stress tolerance. In Bermuda grass, exogenous application of NaHS could effectively protect plant cells by modulating ROS accumulation and associated damage due to oxidative stress accompanied by salinity (Shi et al. 2013). Under salinity stress, Bermuda grass treated with 500 μM

NaHS manifested significantly reduced H_2O_2, $O_2^{\bullet-}$ and malondialdehyde (MDA) concentrations as compared to untreated plants, endowing minimal oxidative damage. NaHS treatment also alleviated salt-induced decrease in glutathione redox state via increasing GR activity. Increased CAT and POD values might have contributed to lower H_2O_2 and $O_2^{\bullet-}$ content and hence assisted in plants adaptation to salt stress.

Exogenous H_2S application regulated ascorbate and glutathione metabolism, which, consequently, strengthened the antioxidant mode of action and allowed maize plants to combat oxidative stress triggered by salt stress (Shan et al. 2014). In wheat, NaHS pretreatment promoted salinity tolerance by stimulating antioxidant enzymatic activities and decreasing MDA content (Ye et al. 2015). When salt-stressed wheat plants were exposed to NaHS treatment, the activities of SOD, CAT, and POD increased significantly following the decline in H_2O_2 and MDA contents. This confirmed that H_2S treatment assuaged salinity stress in wheat leaves by improving the antioxidant defense system (Ding et al. 2019). Unexpectedly, hypotaurine-mediated infiltration of tobacco leaves suppressed the endogenous level of H_2S which had a negative impact on the profile of antioxidative enzymes (SOD, CAT, and APX) under NaCl stress (da-Silva et al. 2017).

To withstand devastating effects of salinity stress, osmolytes such as proline, total soluble sugars, and sucrose are accumulated to ease the survival of plants by offsetting osmotic pressure and adjusting the stability of the cell membrane. Exogenous application of NaHS induced production of osmolytes (L-proline, sucrose) under NaCl stress condition, conferring salt tolerance in Bermuda grass (Shi et al. 2013). However, proline synthesis does not always correspond with abiotic stress tolerance in plants, that somewhat, is dependent on proline turnover (Kishor and Sreenivasulu 2014). It has been observed in numerous studies that proline imparts tolerance to numerous abiotic stresses (Loutfy et al. 2012; Mostofa et al. 2015b). In rice, a high amount of proline synthesis was not desirable because prior application of NaHS in salt-stressed plants decreased the rate of water loss (increased relative water content) by decreasing the concentration of Na^+ (Mostofa et al. 2015a, b). Analogously, in cucumber, NaHS application receded the proline pool induced by the salinized condition in radicles and hypocotyls (Li-Xu et al. 2013).

H_2S interacts with another gasotransmitter molecule, NO, in response to different abiotic stresses including salinity and drought (Karle et al. 2021). The interaction of H_2S and NO promotes root development via auxin-dependent pathways in stressed plants (Mishra et al. 2021). For example, H_2S enhances adventitious root formation with the help of NO in plant species such as *Ipomoea batatas, Salix matsudana, G. max* and *C. sativus* under external stress conditions. It has been reported that the signaling molecule H_2S acts through two distinct pathways i.e., HO-1 (heme oxygenase 1)/CO and Ca^{2+}/CaM1 (calmodulin) which are then passed on to NO via feedback control to trigger lateral root development. Furthermore, H_2S and NO are actively involved in the adjustment of stomatal movement. In salt-treated tomato plants, H_2S stimulates NO concentration and positively balances stomatal closure to prevent the loss of water and to hold osmotic balance at cellular and tissue levels.

Also, H_2S-NO interaction participates in ameliorating heavy metal stress-induced cell death in plants. In mung bean plants, H_2S and NO prevent damage due to Cd^{2+} stress by repairing photosynthetic apparatus (Khan et al. 2020). The coordination between NO, H_2S, and melatonin improves gaseous exchange parameters and photochemical efficiency in cucumbers under salinity stress. In addition, melatonin induced H_2S when interacts with NO reduces the accumulation of salt-induced ROS and hence prevents oxidative damage in cucumber leaves (Sun et al. 2021).

12.4 Gene Regulation by H_2S During Plant Salinity Stress

H_2S is considered as a master regulator of abiotic stress tolerance mechanism through up-regulation and down-regulation of various stress-responsive genes and thus transits relevant signaling networks (Karle et al. 2021; Shi et al. 2015). H_2S-mediated regulation mechanism is complex and the regulatory loop involves stress stimulus perception from external circumstances accompanied by activation of signaling route entangling interactions among proteins, transcription factors, and promoters and modified protein genesis integrating the defense framework of antioxidative enzymes in plants (Aroca et al. 2018).

We know that salinity results in excess buildup of Na^+ in plant cells which in turn causes K^+ deficiency due to less K^+ uptake (Munns and Tester 2008). There are several mechanisms evolved by plants to tolerate salinity, among which reduction of Na^+ accumulation in plants by preventing entry of Na^+ into roots primarily ranks the best. To combat salt stress and maintain ionic homeostasis in the cytosol, SOS (salt overly sensitive) pathway is necessary (Zhu 2002). In cucumber roots, plasma membrane *PM H + -ATPase, SOS1*, and potassium channel *SKOR* genes were upregulated during salt stress in contrast to the control, although the reverse was noticed in leaves (Jiang et al. 2019). The gene expression might be affected as a consequence of root irrigation treatment procedure or induction of any signal in roots. H_2S mitigated the elevation in transcript level of the above-mentioned genes in roots induced by salt stress and thus maintained Na^+/K^+ balance for the improvement of salinity tolerance. H_2S pretreatment controlled the expression of SOS pathway genes (*SOS2, SOS3,* and *SOS4*) and preserved Na^+/K^+ homeostasis in salt-stressed strawberry plants (Christou et al. 2013). H_2S induced the up-regulation of *SOS1*, which participated in the removal of excess Na^+ and, therefore, enhanced endurance to salinity stress in wheat (Deng et al. 2016).

H_2S treatment displayed elevated expression of *Triticum aestivum* glutathione synthetase (*TaGS*) and *Triticum aestivum* dehydroascorbate reductase (*TaDHAR*) genes, enhancing ascorbate and glutathione activity in wheat leaves, and in addition to that, SOS pathway genes- *TaSOS1, TaSOS2, and TaSOS3* showed upregulation in their expression levels (Ding et al. 2019). This implies that H_2S protected wheat seedlings against salt stress via modulating the metabolism of ascorbate and glutathione and the SOS pathway. Plant adaptation to salinity stress can be improved by the involvement of MAPK (mitogen-activated protein kinase) cascades (Rodriguez et al. 2010). The elevated expression of the *MPK4* gene in NaHS treated wheat seedlings imparted salt tolerance by regulating osmolytes production (Ding et al. 2019). Under salinity conditions, H_2S enhanced the expressions of *PM-H*$^+$ *ATPase* genes namely *AHA3 and AHA4* in *Arabidopsis* roots, thereby improving plants tolerance to salt stress via modulating PM-H^+ ATPase activity both at the phosphorylation and transcription levels and inducing high protein activity of the PM Na^+/H^+ antiporter which led to the maintenance of ionic homeostasis at the intracellular level (Li et al. 2014a, b). In alfalfa seedling roots, samples treated with NaCl and NaHS showed increased endogenous H_2S levels which in turn up-regulated the transcript levels of *GR, DHAR,* and monodehydroascorbate reductase (*MDHAR*) genes, strengthening the AsA-GSH metabolic pathway and further, partly hindering salt-triggered oxidative damage (Lai et al. 2014).

12.5 Physico-Chemical and Molecular Effects of H_2S Under Drought Stress

Drought is the most predictable and damaging among all abiotic stresses, that severely affect plant yield. It prompts desiccation, osmotic imbalance, and wilting in various crops and varieties around the world (Banerjee and Roychoudhury 2017). Drought leads to the overproduction of ROS that triggers disturbance in cellular redox homeostasis. Exogenous application of H_2S or H_2S donors alleviates the harmful consequences of drought stress in plants. For example, exogenous treatment of NaHS in wheat seedlings subjected to drought stress caused by polyethylene glycol 6000 (PEG 6000) increased seed germination depending upon concentration (Zhang et al. 2010). Drought stress was relieved by treating seedlings with solely NaHS but no other S_2, SO_4^{2-}, SO_3^{2-}, HSO_4^-, or HSO_3^- containing compounds. This underlined the importance of H_2S in reducing drought vulnerability (Zhang et al. 2010). Lowered lipoxygenase (LOX) activity and malondialdehyde (MDA) concentration along with the enhanced ascorbate peroxidase (APX) and CAT activity were observed in treated seeds (Zhang et al. 2010). Plant survival under drought stress requires the retention of cellular redox equilibrium and the triggering of antioxidative defense pathway (Gong et al. 2020). H_2S helps plants improve their antioxidant responses through glutathione and ascorbic acid metabolism, which ameliorates tolerance against drought (Hancock and Whiteman 2015). Moreover, the physiological production of H_2S is rather limited, it is doubtful that these positive antioxidant effects can directly scavenge ROS by H_2S synthesis. MicroRNA (miRNA) pathway modulation might potentially be a suitable choice (Shen et al. 2013).

In plant-cells, the ascorbate-glutathione (AsA-GSH) cycle is one of the most important antioxidant replenishment mechanisms (Banerjee and Roychoudhury 2016). During drought stress, Shan et al. (2011) reported that NaHS-treated wheat seedlings increased the activities of AsA-GSH cycle enzymes such as APX, GR, and DHAR with respect to non-treated samples. Significant increases in cellular osmolyte accumulation, such as AsA and GSH limits MDA generation and electrolyte leakage in drought-stressed plants (Shan et al. 2011). H_2S has improved drought tolerance in rice possibly via two mechanisms i.e., (1) enhancing the antioxidant capacity to re-establish redox balance; and (2) modulating the abscisic acid (ABA) signaling pathway and activating downstream drought linked genes (Zhou et al. 2020). H_2S-mediated drought tolerance is relatable to the induced expression of genes along with the up-regulation in the transport of ions especially iron in wheat (Li et al. 2017). It has been reported that the pre-treatment with H_2S triggered the synthesis of novel proteins as well as other macromolecules to prevent membrane and protein damage during drought stress. In addition, acceleration of genes related to sugar metabolic pathways resulted in adjusting osmotic pressure and thereby reducing the damage due to drought stress in wheat plants. Also, H_2S influenced signaling pathways of plant hormones, transcription factors, and protein kinases (Li et al. 2017). H_2S seems to have a wide range of effects on plant survival and growth, as evidenced by these research findings.

According to Wei et al. (2017), H_2S increases senescence-associated gene (*SAGs*) expression followed by inhibition of chlorophyll degradation in detached leaves, and the scientists hypothesized that S-nitrosoglutathione reductase was involved, implying the active participation of NO metabolism. In certain cases, H_2S causes stomatal closure, whereas, in others, the stomatal opening is influenced (Garca-Mata and Lamattina 2013; Lisjak et al. 2011). This obvious abnormality has lately been studied further (Honda et al. 2015). These authors discovered that brief exposure to an H_2S donor resulted in stomatal closure, whereas long-term exposure led to larger stomatal apertures. It was revealed that 8-mercapto-cGMP mediates the effects of H_2S, which is very significant. Ca^{2+}, cADP ribose, and slow anion channel 1 were discovered to be implicated downstream of this signaling molecule. Jin et al. (2013) investigated the role of H_2S in controlling stomatal movement as well as the relationship between H_2S and ABA metabolism in signal transduction in *Arabidopsis* plants by knocking down the L-cysteine desulfhydrase (*LCD*) gene. They also discovered that H_2S regulates the expression of ABA receptor candidates, potassium ions, and calcium ion channels in guard cells. H_2S may be implicated in ethylene signaling, which leads to the stomata closure (Liu et al. 2011). The importance of H_2S and its interplay with NO and ABA were studied further using an L-cysteine desulfhydrase (*DES1*) mutant in *Arabidopsis* (Scuffi et al. 2014). Upon H_2S treatment under drought stress, the expression profiles of genes that control ABA metabolism were changed in roots and leaves in various ways, however, the expression levels of ABA receptors were upregulated in both roots and leaves. In addition to stomatal activity, multiple studies have found that H_2S aids drought tolerance by accumulating osmolytes such as proline and interacting with the calcium messenger network (Li et al. 2014a, b).

Various cellular processes are linked by mitogen-activated protein kinases (MAPKs), which are stimulated by developmental and environmental inputs form cellular signaling systems (Danquah et al. 2015). In MAPK signaling channels, H_2S plays a crucial function. Drought-induced stress responses are regulated by the

H_2S-MAPKs cascade, which is implicated in ABA-dependent stomatal movement (Du et al. 2019). Drought stress also stimulates H_2S biosynthesis as well as *MAPK* gene expression (Samajová et al. 2013). In the *lcddes1* double mutant lacking endogenous H_2S production, *MAPK* expression is reduced (Du et al. 2019). Furthermore, in contrast to wild-type *Arabidopsis*, the *mpk4* mutant's growth is hampered during drought stress, and H_2S-induced stomatal closure is hindered in the *mpk4* mutant.

Chen et al. (2016) created a model to highlight the role of H_2S in regulating drought stress tolerance via modulation in the accumulation of polyamines and sugars. Exogenous application of NaHS to drought-stressed *Spinacea oleracea* seedlings led to the elevated levels of expression of genes for polyamine biosynthesis, such as ornithine decarboxylase (*ODC*), N-carbamoyl putrescine amidohydrolase (*CPA*), and arginine decarboxylase (*ADC*), and downregulation in the expression of S-adenosyl-Met-decarboxylase (*SAMDC*). The recent findings indicated the ability of H_2S to give considerable drought stress protection in *Medicago sativa,* resulting in severe cellular damage and nitro-oxidative stress in the treated samples (Antoniou et al. 2020). This defense appears to be accomplished by a combination of increased physiological activity, equilibrium of reactive oxygen and nitrogen species, and transcriptional control of defense-related pathways. In conclusion, the foregoing discussion clearly demonstrates the extensive effect of H_2S on plant drought tolerance and serves as a useful reference for boosting crop resilience and output (Fig. 12.3).

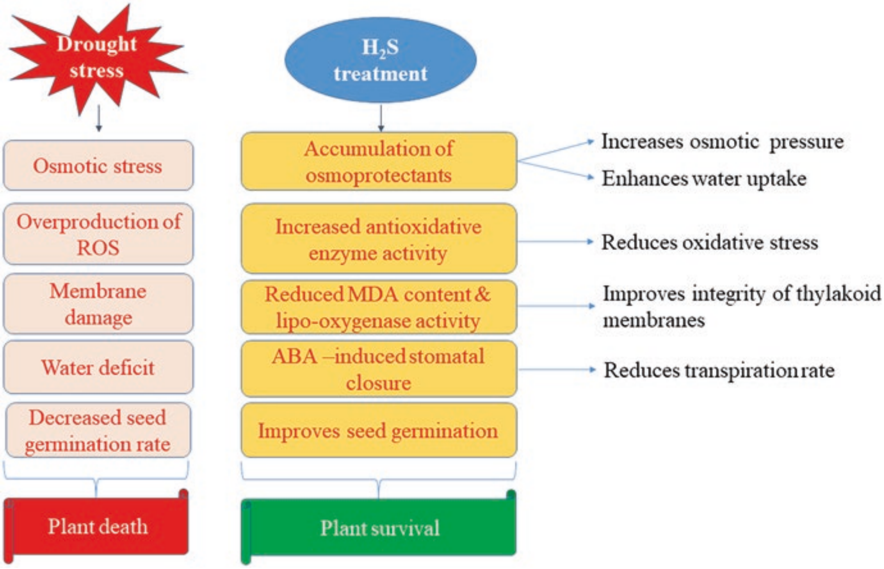

Fig. 12.3 Ameliorative actions of H_2S in plants under drought stress. *ABA* abscisic acid, *MDA* malondialdehyde, *ROS* reactive oxygen species

12.6 Conclusion and Future Perspectives

Plant cells emit H_2S as a survival signal under salinity and drought stress. H_2S promotes systemic resistance against stressed environment by re-establishing redox equilibrium, increasing osmolyte accumulation, ensuring ion balance, and modulating gene expression, along with many other factors. With its ability to undergo reaction with thiol groups, it also boosts plant resistance to soil salinity and drought stress. However, most of the physiological studies in plants are based on exogenous treatment with H_2S or H_2S donors. Genetic researches on H_2S metabolism are very poor. Thus, the process of endogenous H_2S generation in plants, as well as the signal transduction channels between plant cells and tissues, must be investigated in future. Additionally, H_2S interacts with other signaling compounds to protect plants against salinity and drought stress conditions. How plants respond to this interaction needs to be clearly understood. As we know that omics play a major role in abiotic stress adaptation in plants, future research should be focused on a systematic analysis of omics-based approaches to critically understand the H_2S signaling pathway for improving salt and drought stress in crop cultivars.

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Chapter 13
Functional Roles of Hydrogen Sulfide in Postharvest Physiology of Fruit and Vegetables

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Abstract Hydrogen sulfide (H_2S) is a naturally occurring gaseous molecule in plants and a potential signalling molecule that regulates many physiological processes in the plant system. Various studies have reported the beneficial effect of H_2S in delaying the fruit ripening, senescence, and better fruit quality during storage. It reduces oxidative damage, membrane permeability, and lipid peroxidation by boosting the antioxidant defence mechanism in many fruits and vegetables. It has a greater potential for use in the postharvest industry for reducing postharvest decay and improving fruit quality with extended storage life. This chapter is mainly focused on the role of H_2S in postharvest physiology, its signalling action, and cross-talk with other hormones viz. ethylene, abscisic acid, and nitric oxide during fruit ripening.

Keywords Gasotransmitters · Eco-friendly · Postharvest · Shelf life · Storage

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T. Aftab, F. J. Corpas (eds.), *Gasotransmitters Signaling in Plants under Challenging Environment*, Plant in Challenging Environments 5,
https://doi.org/10.1007/978-3-031-43029-9_13

13.1 Introduction

Consumer awareness of nutritional and safe food has increased the demand for fresh consumption of fruits and vegetables. It has also increased the need for better production strategies, management practices, and fewer post-harvest losses in fruits and vegetables (Kader 2005). A report of various studies conducted by FAO indicated that a huge quantity of horticultural products is wasted due to improper post-harvest handling (Porat et al. 2018). To reduce the postharvest losses, traders use many synthetic chemicals which have raised several issues such as pathogen resistance, residual effect, and other food safety issues (Deng et al. 2013). Therefore, there is a greater need for establishing an eco-friendly and safe alternative to the commercially used synthetic harmful chemical compounds. Toward this goal, several naturally occurring compounds, such as salicylic acid (Reddy et al. 2016; Reddy and Sharma 2016), methyl jasmonate, hydrogen sulfide, nitric oxide, etc. have been used as a postharvest treatment for different fruits and vegetables (Gong et al. 2018; Lata et al. 2018, 2021; Siddiqui et al. 2021a, b).

Hydrogen sulfide (H_2S) is a colorless gaseous molecule having a foul smell and is present naturally in plants. It is well known for its various signalling actions and regulation of numerous physiological functions in plants (Li et al. 2017a; Lata et al. 2022). In the bygone era, H_2S was known for its phytotoxic effect on plants and animals but previous studies had proven the beneficial effect of H_2S as a signalling molecule during pathogenic and environmental stresses (Chen et al. 2016; Fang et al. 2017; Paul and Roychoudhury 2020; Corpas and Palma 2020). Postharvest application of H_2S delayed tissue browning, ripening, softening, maintained green color, and inhibited postharvest decay in various horticultural crops (Gao et al. 2013; Fu et al. 2014; Al Ubeed et al. 2017; Deshi et al. 2020; Yao et al. 2020; Molinett et al. 2021; Lata et al. 2022). In this chapter, the effect of H_2S application on postharvest physiology and the storage life of various fruits and vegetables have been discussed.

13.2 Role in Plant Physiology, Biochemistry and Signalling

H_2S is confirmed as the third gasotransmitter next to nitric oxide (NO) and carbon monoxide (CO) because of its major role in various physiological processes in both plants as well as animals (Tan et al. 2010; Corpas and Palma 2020; Mishra et al. 2021). The presence of H_2S in plants had been known for many years but very few studies have been conducted on its role in plant physiology. Recently, many studies have reported the contribution of H_2S in signalling pathways of different processes during growth and development, for example, germination, root initiation, autophagy, movement of stomata, photosynthesis, etc. (Scuffi et al. 2014; Papanatsiou et al. 2015; Jia et al. 2015; Duan et al. 2015; Jin and Pei 2016; Aroca et al. 2017; Liu and Xue 2021). Additionally**,** H_2S enhanced the tolerance level of different abiotic stresses such as moisture stress, osmotic imbalance, salt stress, low-temperature stress, heavy metal stress, etc. (Fu et al. 2013; Lai et al. 2014; Ali et al. 2015; Chen et al. 2016; Jin et al. 2017; Khan et al. 2017; Fang et al. 2017). Various studies showed that H_2S enhanced the product quality and nutritional value during abiotic stresses in various horticultural crops (Qian et al. 2014; Reich et al. 2016; Kaya et al. 2018). Besides, the signalling action of H_2S on plant defense responses against pathogens and other developmental processes, for example, fruit ripening, senescence, and softening had been well proven (Corpas and Palma 2020; Zhang et al. 2021; Mishra et al. 2021).

Biosynthesis of H_2S in plants occurs due to the catalysis of sulfite to sulfide by sulfite reductase. This process involves two cysteine-dependent reactions in which O-acetylserinelyase gene family takes part. At first, L-cysteine is converted into H_2S, NH_3, and pyruvate by the enzyme L-cysteine desulfhydrase (LCD) and the second process consists of conversion of cysteine to cyanide using β-cyanoalanine synthase which produces H_2S (Hatzfeld et al. 2000; Riemenschneider et al. 2005). In Arabidopsis, catalysis of cysteine to H_2S is governed by two genes namely *AtNFS1* and *AtNFS2* and other genes namely *CYSC1*, *CYS-D1*, and *CYS-D2* which were identified to encode β-cyanoalanine synthase (Jost et al. 2000; Leon et al. 2002).

Biochemical aspects of H_2S are studied extensively and proposed that it shows signalling activities mainly through the oxidation of cysteine residues into persulfides during post-translation process (Filipovic and Jovanović 2017; Ruetz et al. 2017; Kimura 2020). It has been studied that a small concentration of H_2S can also exhibit signalling properties along with other substances such as hormones, free radicals or reactive oxygen species (ROS), nitric oxide (NO), etc. during biotic and abiotic stresses and various physiological processes (Hancock and Whiteman 2014; Christou et al. 2014; Ziogas et al. 2015; Antoniou et al. 2016).

13.3 Role in Postharvest Physiology

Postharvest losses in horticultural crops are a serious global concern that is more prevalent in developing countries. In this aspect, various approaches have been implemented to reduce the postharvest losses of fresh fruits and vegetables. In this regard, H_2S can be a useful tool in maintaining the postharvest quality and storage life of perishable produce. It delays color change, respiration, ethylene production rate, ripening, senescence, and softening and also alleviates chilling injury during cold storage. Exogenous application of H_2S prevents oxidative stress, microbial infection, regulates postharvest ripening, and senescence in many horticultural crops (Gao et al. 2013; Hu et al. 2014b; Ni et al. 2016; Ge et al. 2017; Siddiqui et al. 2021a).

13.3.1 Effect on Postharvest Ripening and Senescence

Postharvest ripening and senescence of horticultural products are directly related to respiration and ethylene production rate. Higher respiration rate and production of ethylene promote overripening and senescence in harvested fruits and vegetables (Razzaq et al. 2013). H_2S acts as an ethylene antagonist and inhibits ethylene biosynthesis by down regulating the expression of genes responsible for ethylene biosynthesis and signal transduction (Luo et al. 2015; Li et al. 2015b; Zheng et al. 2016; Ge et al. 2017). In the plants, cytochrome c oxidase and succinate dehydrogenase play a key role in cellular respiration, subsequent energy metabolism, and the tricarboxylic acid cycle (TCA) (Brunori et al. 1987; Affourtit et al. 2001). H_2S increases the cytochrome c oxidase and succinate dehydrogenase activities thus producing higher energy which leads to lesser consumption of energy and finally reduce the respiration and senescence processes (Henriksson and Reitman 1977; Li et al. 2016).

In climacteric fruits like bananas, postharvest application of H_2S slowed down the ripening and senescence process by constraining the effect of ethylene and also maintained the green color, soluble protein content, and total antioxidant activity (Ge et al. 2017; Siddiqui et al. 2021a). H_2S treatment down-regulated the gene expression associated with ethylene biosynthesis such as *MaACO1*, *MaACS1*, *MaACS2*, and *MaPL* (pectate lyase) and also enhanced the expression of *MaETR*, *MaERS1*, and *MaERS2* (ethylene receptors). Similarly, H_2S suppressed the expression of genes *MdACS1*, *MdACS3*, *MdACO1*, *MdACO2*, *MdETR1*, *MdERS1*, *MdERS2*, *MdERF3*, *MdERF4*and *MdERF5* involved in ethylene biosynthesis and signal transduction (Zheng et al. 2016). Postharvest application of H_2S reduced the ethylene production in kiwi fruit, which resulted in delayed senescence and softening and led to the longer storage life of fruits (Gao et al. 2013). H_2S suppressed the gene expression responsible for ethylene biosynthesis (*AdACS1*, *AdACS2*, *AdSAM*, *AdACO2*, and *AdACO3*) and also *AdCP1* and *AdCP3* (cysteine protease related genes) which reduced the ethylene production in kiwi fruits (Li et al. 2017b).

In non-climacteric fruits like strawberries, mulberry, and grapes, postharvest application of H_2S reduced the respiration rate, ethylene-associated softening, and senescence of fruits (Zhang et al. 2014; Hu et al. 2014b; Ni et al. 2016). Various studies have suggested that H_2S effectively delayed the ripening and senescence process in different horticultural crops (Table 13.1).

13.3.2 Effect on Visual Appearance, Nutritional Quality and Shelf-Life

Harvested fruits and vegetables are living entity and use carbohydrate reserves as a substrate during storage. Thus, respiration rate is a crucial factor that determines the quality and shelf life of perishable products during storage. A higher respiration rate

Table 13.1 Effect of H_2S on ripening and senescence of harvested fruits and vegetables

Crop	Concentration	Storage conditions	Effect	Reference
Apple	0.4 mmol/L H_2S	25 ± 0.5 °C & 85–90% RH	Expression of *MdACO1*, *MdERS1*, *MdETR1* and *MdPG1*genes that are responsible for ethylene biosynthesis and signal transduction were suppressed thus delaying the senescence up to 5 days.	Zheng et al. (2016)
Banana cv. Brazil	Ethephon 1.0 g/L + NaHS (sodium hydrosulphide) 1 mM	25 °C & 85–90% RH	Improved the expression of *MaETR*, *MaERS1* and *MaERS2* (ethylene receptor genes) and suppressed the expression of *MaACO1*, *MaACS1*, *MaACS2* (ethylene synthesis genes) and pectate lyase *MaPL*, thus antagonizing the effect of ethylene and delaying the senescence	Ge et al. (2017)
Broccoli	0.8 mM NaHS	20 °C & 85–90% RH for 4 days in dark	Postponed senescence up to 4 days by maintaining higher ATP contents and energy charges by increasing the activity the enzymes involved in glycolysis, tricarboxylic acid cycle, electron transport chain etc.	Li et al. (2017a)
Chilean strawberry	0.2 mM NaHS	20 °C	Delayed the pectin degradation by decreasing the activity of pectate lyase, polygalacturonase and expansion thus delaying the senescence and extending the shelf life up to 6 days.	Molinett et al. (2021)
Kiwi fruit	Ethephon 0.4 g/L + NaHS 1 mM	25 °C & 85–90% RH	Down regulated the expression of genes *AdACS1*, *AdACS2*, *AdSAM*, *AdACO2* and *AdACO3* involved in ethylene biosynthesis, thus inhibiting ethylene synthesis and alleviating the ripening process.	Li et al. (2017b)
Kiwi fruit cv. Jinkui	45 and 90 μmol/L H_2S	25 °C & 85–90% RH	Delayed the maturity and senescence up to 18 days	Zhu et al. (2014)
Kiwi fruit	1.0 mM NaHS	20 ± 0.5 °C & 85–90% RH	Delayed the senescence by enhancing the antioxidant activity.	Gao et al. (2013)
Mulberry	0.8 mM NaHS	2.0 ± 0.2 °C & 85–90% RH	Attenuated the senescence by reducing the respiration rate, delaying the increase of soluble pectin and anthocyanin content.	Hu et al. (2014a)
Navel and Valencia oranges	100 μL/L H_2S	20 °C & 65–70% RH for 5 weeks	Reduced the accumulation of ethanol and delayed the senescence till 5 weeks	Alhassan et al. (2020)

(continued)

Table 13.1 (continued)

Crop	Concentration	Storage conditions	Effect	Reference
Pak choy	250 μL/L H_2S	6 days at 10 °C	Reduced the respiration rate and ethylene production, delayed senescence, degradation of chlorophyll, weight loss and ionic leakage	Al ubeed et al. (2018)
Peach cv. Dahong	NO 15 μL/L + H_2S 20 μL/L	25 ± 0.5 °C & 85–90% RH	Reduced the softening by inhibiting cell membrane polysaccharides solubilisation and de-polymerization, ethylene biosynthesis and activity of cell wall degrading enzymes (PG, PE and EGase).	Zhu et al. (2019)
Pear	2.0 mM NaHS	20 °C	Delayed the senescence up to 8 days by reducing the softening and decay.	Hu et al. (2014b)
Strawberry	0.8 mM NaHS	20 ± 0.5 °C & 85–90% RH	Improved postharvest shelf life by decreasing the respiration rate and inhibiting the activity of PG	Hu et al. (2012)
Tomato 'Micro Tom'	Ethephon 1.0 g/L + NaHS 0.90 mM	25 °C & R.H 85–90%	Delayed the senescence up to 7 days by antagonizing the ethylene effect and decreasing ROS level (MDA, H_2O_2 and $O_2^{\bullet -}$)	Yao et al. (2018)
Water spinach	2.4 mM NaHS	12–14 °C & R.H 85–95% in dark	Delayed the senescence by reducing the respiration rate and enhancing antioxidant enzyme activity (SOD, CAT, POD)	Hu et al. (2015)

deteriorates the quality and shortens the shelf-life of the produce (Hu et al. 2012). H_2S reduced the respiration rate in many horticultural crops (Chang et al. 2014; Li et al. 2016; Al Ubeed et al. 2017; Ali et al. 2019). It helps to maintain adequate energy levels in fresh produce, which leads to better quality in terms of freshness and higher shelf life (Aghdam et al. 2018). The external colour of fruits and vegetables is an important criterion for marketing and consumer acceptability of produce. Thus, discoloration is a major limitation during storage of perishable produce. Postharvest treatment with H_2S showed positive results in maintaining the color of different horticultural crops (Al Ubeed et al. 2017; Yao et al. 2020; Deshi et al. 2020; Siddiqui et al. 2021b). Li et al. (2015b) suggested that postharvest application of NaHS (H_2S releasing compound) down regulated the expression of genes for example *BoCLH1*, *BoNYC*, *BoRCCR*, *BoSGR*, and *BoPPH* which are responsible for chlorophyll degradation in broccoli and maintained 6 days' shelf life. H_2S plays a key role in reducing the enzymatic browning in fresh produce by inhibiting the activities of phenylalanine ammonialyase (PAL), polyphenol oxidase (PPO), and peroxidase (POD) enzymes (Hu et al. 2014b; Ali et al. 2018; Deshi et al. 2020). However, fruits and vegetables are rich source of nutrition like vitamins, essential amino acids, phenols, flavonoids, antioxidants, etc. Therefore, retention of these nutritional components during storage is a major objective. H_2S as postharvest treatment influences the concentration of these components during storage. Application of H_2S retained higher phenolic compounds, anthocyanins, total carotenoids, ascorbic acid, and flavonoids during storage in different horticultural crops and maintained better quality and longer marketable life (Table 13.2).

Biochemical parameters such as TSS (total soluble sugars), acidity, and sugars contribute majorly to the organoleptic quality of fresh produce (Fallik and Ilic 2018). Similarly, rapid loss of firmness accelerates softening and postharvest decay. Therefore, retention of higher firmness during storage in fruits and vegetables is a crucial factor for better quality and storage life of produce (Barrett et al. 2010; Saei et al. 2011). H_2S retained better firmness of fruits and vegetables during storage by reducing the activity of pectin methylesterase (PME), endo-β-1,4-glucanase (EGase) and polygalacturonase (PG) (Hu et al. 2012; Chang et al. 2014). Postharvest application of H_2S maintained higher firmness, sugars and titratable acidity, ascorbic acid, phenols, flavonoids, and sensory quality in fruits and vegetables (Table 13.2).

13.3.3 Effect on Membrane Permeability and Lipid Peroxidation

During storage, temperature stress and microbial infections lead to membrane disintegration and permeability. Membrane permeability occurs due to lipid peroxidation and ionic leakage. In lipid peroxidation, lipoxygenase (LOX) is the major factor that induces alteration in cell membrane lipids and promotes higher electrolytic leakage and malondialdehyde (MDA) accumulation (Imahori et al. 2008). Higher concentration of electrolytic leakage and MDA damage the cell membrane integrity (Aghdam et al.2018). Many studies have reported that H_2S reduced lipid peroxidation and membrane permeability in several fruits and vegetables (Chang et al. 2014; Zhu et al. 2014; Yonggen et al. 2015; Ni et al. 2016; Siddiqui et al. 2021a, b). Postharvest application of H_2S inhibits the activity of LOX enzyme and reduces electrolytic leakage and MDA concentration in different horticultural crops (Table 13.3).

13.3.4 Effect on Chilling Injury Alleviation

Cold storage of fruits and vegetables prolongs their storage life and maintains their quality. However, storage of perishable produce at low temperatures often results in chilling injury (Wang 1994; Hakim et al. 1999). Chilling injury (CI) causes surface pitting, internal breakdown, discoloration of pulp, uneven ripening or failure to ripen, imbalance metabolism, electrolytic leakage, and peroxidation of lipids in fruits and vegetables (Wang 1994; Aghdam et al. 2013; Garcia-Pastor et al. 2020).

Table 13.2 Effect of H_2S on visual appearance, nutritional quality and shelf life of fruits and vegetables

Crop	Concentration	Storage conditions	Effect	Reference
Apple	0.4 mM NaHS	25 ± 0.5 °C & 85–90% RH	Maintained higher levels of soluble protein and reducing sugars and lower concentration of free amino acids and protease.	Zheng et al. (2016)
Banana cv. Brazil	Ethephon 1.0 g/L + NaHS 1 mM	25 °C & 85–90% RH	Improved flavonoid content, decreased the yellow colour development, sugar conversion and maintained firmness of fruits.	Ge et al. (2017)
Broccoli	2.4 mM NaHS	25 °C & 85–90% RH	Maintained higher soluble protein, reducing sugar, soluble solid, anthocyanins carotenoids and ascorbic acid.	Li et al. (2014)
Tomato 'Micro Tom'	Ethephon 1.0 g/L + NaHS 0.90 mM	25 °C & 85–90% RH	Delayed colour transition and softening, maintained higher flavonoids, reducing sugar, ascorbic acid antioxidant activity	Zhong et al. (2021)
Tomato var. 'Micro Tom'	Ethephon 1.0 g/L + NaHS 0.90 mM	25 °C & 85–90% RH	Maintained higher soluble proteins, sugars, anthocyanins, ascorbic acid and flavonoids	Yao et al. (2020)
Valencia orange	100 µL/L H_2S	20 °C & 65–70% RH for 5 weeks	Improved the shelf life by maintaining lower soluble sugars and higher acidity.	Alhassan et al. (2020)
Brinjal	Phenylalanine 7.5 mM and NaHS 3 mM/L	7 ± 1 °C 85% & RH for 21 days	Exhibited lower weight loss and higher fruit firmness, anthocyanin, vit C, titratable acidity.	Najafi et al. (2021)
Broccoli	0.96 mmol/L H_2S	25 °C & 85–90% RH	Inhibited the yellowing and degradation of chlorophyll by down-regulating the expression of genes (*BoSGR, BoNYC, BoCLH1, BoPPH*, and *BoRCCR*) and reduced ethylene synthesis (*BoACS2* and *BoACS3*) up to 5 days	Li et al. (2015a)
Pear	2.0 mM NaHS	20 °C	Alleviated the browning by decreasing the activity of PAL and PPO. Maintained higher level of reducing sugar, soluble protein and amino acids.	Hu et al. (2014b)
Passion fruit	2 mM NaHS	5 ± 1 °C for 35 days	Improved the quality and shelf life by maintaining better anthocyanin, fresh weight and higher acidity, soluble solid, sugar and vit C.	Liu et al. (2019)
Litchi cv. Purbi	2 mM NaHS	28 °C & 70–75% RH	Inhibited the pericarp browning by alleviating the oxidative damages and enhanced antioxidant activity.	Deshi et al. (2020)

(continued)

Table 13.2 (continued)

Crop	Concentration	Storage conditions	Effect	Reference
Litchi cv. Purbi	2 mM NaHS	7 ± 1 °C & 85–90% RH	Retained higher membrane integrity, TSS, titratable acidity, anthocyanin, total phenolics and antioxidant activity.	Siddiqui et al. (2021b)
Strawberry	0.8 mM NaHS +5 μmmol/L nitric oxide	(20 ± 1) °C	Preserved the fruit quality by reducing the rate of respiration, maintained firmness and red colour of peel.	Chang et al. (2014)
Grape Kyoho	1.0 mM NaHS	25 °C & 85–90% RH	Inhibited rachis browning, softening and berry rotting by improving the activity of APX, CAT and maintaining higher levels of ascorbic acid, flavonoids and phenols.	Ni et al. (2016)
Water spinach	2.4 mM NaHS	12–14 °C & R.H 85–95%	Alleviated the yellowing of leaves up to 8 days by decreasing chlorophyll degradation	Hu et al. (2015)
Carrot	0.4 mM NaHS	5 ± 2 °C for 10 days	Reduced surface discoloration by lower accumulation of peroxide, MDA and inhibiting PPO, POD activity.	Chen et al. (2018)

PAL phenylalanine ammonialyase, *PPO* polyphenol oxidase, *POD* peroxidase, *CAT* catalase, *APX* ascorbate peroxidase, *MDA* malondialdehyde

Table 13.3 Effect of H_2S on membrane permeability and lipid peroxidation in different fruit crops

Crop	Concentration	Storage conditions	Effect	Reference
Grape cv. Kyoho	1.0 mM NaHS	25 °C & 85–90% RH	Inhibited the activity of LOX, lowered MDA content, lipid peroxidation and ionic leakage.	Ni et al. (2016)
Kiwi fruit cv. Jinkui	45 μM H_2S	25 °C & 85–90% RH	Increased SOD, CAT and POD activity, thereby reducing ROS damage and membrane permeability	Zhu et al. (2014)
Litchi cv. Purbi	2 mM NaHS	7 ± 1 °C and 85–90% RH	Reduced the accumulation of MDA and membrane leakage, and improved membrane integrity.	Siddiqui et al. (2021b)
Pear	2 mM NaHS	20 °C	Reduced the activity of LOX, and lipid peroxidation, maintained higher membrane integrity.	Hu et al. (2014b)
Sweet potato	2.0 mM NaHS	20 ± 0.5 °C & 85–90% RH	Activated antioxidant enzymes and attenuated LOX activity and resulted in reduced peroxidation of lipids.	Tang et al. (2014)

LOX lypoxygenase, *MDA* malondialdehyde, *SOD* superoxide dismutase, *CAT* catalase, *ROS* reactive oxygen species

Table 13.4 Effect of H_2S on chilling injury alleviation in fruits and vegetables

Crop	Concentration	Storage conditions	Effect	Reference
Banana	0.5 mM NaHS	7 °C for 14 days +20 °C for 6 days	Alleviated CI by maintaining the cell membrane integrity, decreased MDA level and electrolyte leakage, maintained higher activity of CCO, SDH and ATPase, which enhanced energy status and chilling tolerance	Li et al. (2016)
Banana	0.5 mM NaHS	25 °C & 85–90% RH	Ameliorated the chilling injury by enhancing the activity of CAT, SOD, APX and improving proline content (by promoting P5CS activity).	Luo et al. (2015)
Brinjal	Phenyl alanine 7.5 mM and NaHS 3 mM	7 ± 1 °C & 85% RH for 21 days	Reduced the discolouration and surface pitting by increasing proline, phenols, flavonoids and the activity of POD, CAT, SOD and APX.	Najafi et al. (2021)
Hawthorn	1.5 mM NaHS	1 °C for 20 days	Enhanced chilling tolerance and maintained membrane integrity by reducing the production of ROS and enhanced CAT, APX and SOD activity.	Aghdam et al. (2018)

CI chilling injury, *MDA* malondialdehyde, *CCO* cytochrome c oxidase, *SDH* succinate dehydrogenate, *CAT* catalase, *APX* ascorbate peroxidase, *SOD* superoxide dismutase

CI also reduces the fruit quality, and storage life and makes produce susceptible to postharvest decay (Aghdam and Bodbodak 2013). H_2S as postharvest treatment alleviates the CI in many horticultural produces during low-temperature storage (Table 13.4). It enhances proline accumulation by increased activity of 1-pyrroline-5-carboxylate synthetase and decreased activity of proline dehydrogenase (Luo et al. 2015). Proline helps in osmotic balance by maintaining the high concentration of ions in the cytosol which enhance the chilling tolerance capacity of production. H_2S also boosts the defence mechanism by increasing the activity of catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APX) which reduces oxidative damages and accumulation of malondialdehyde (MDA) in fresh fruits and vegetables (Aghdam et al. 2018). Antioxidant enzymes increase chilling tolerance and reduce the extent of chilling injury symptoms. Thus, H_2S can effectively alleviate the chilling injury during cold storage of different fruits and vegetables (Table 13.4).

13.3.5 Effect on Postharvest Disease Resistance

Postharvest decay of horticultural produce during storage and handling is one of the main reasons for postharvest losses. As a sulfur-containing compound, H_2S induces “sulfur induced resistance” in plants and plays a key role in reducing postharvest decay (Bloem et al. 2012). H_2S signaling is associated with the resistance response of plants against pathogen infections. H_2S activates the enzyme L-cysteine desulfhydrase (LCD) against microbial infection (Bloem et al. 2012). The growth of fungal pathogens like *Aspergillus niger*, *Penicillium expansum*, *Monilinia fructicola*, *Penicillium italicum*, *Rhizopus nigricans*, etc. was inhibited by H_2S fumigation during storage (Hu et al. 2014b; Tang et al. 2014; Wu et al. 2018). H_2S created an intracellular burst of harmful free radicals and reduced the genes expression coding antioxidant enzymes inside the microbial cells (Fu et al. 2014). Postharvest application of NaHS as an H_2S donor effectively reduced the fungal decay in several horticultural crops (Table 13.5).

Table 13.5 Effect of H_2S on postharvest diseases resistance of fruits and vegetables

Crop	Concentration	Storage conditions	Effect	Reference
Banana	1.0 mM NaHS	22–25 °C and RH 60–65%	Reduced decay by inhibiting germ tube elongation and cytoplasm fragmentation.	Su mon et al. (2021)
Strawberry	0.8 mM NaHS & 5 µmmol/L NO	(20 ± 1) °C	Enhanced the activities of CHI and GNS, which are responsible for weakening fungal cells decay, thus enhanced disease resistance and reduced decay.	Chang et al. (2014)
Sweet Cherry Lapins & Regina	1 mM NaHS	0 °C and >90% RH	Improved resistance to pitting injury, which is the result of chilling injury by reducing the cell wall disassembly through inhibiting β-GAL and PG activity	Zhi and Dong (2018)
Sweet Potato	2.0 mM NaHS	20 ± 0.5 °C & RH 85–90%	Reduced the fungal growth of *Rhizopus nigricans*, *Mucor rouxianus* and *Geotrichum. candidum* that is responsible for black or soft rot.	Tang et al. (2014)
Peach	50 mM NaHS	25 °C & 80% RH	Pathogenicity of *Monilinia fructicola* responsible for brown rot was reduced by inhibiting the spore germination and mycelial growth	Wu et al. (2018)
Pear	0.5 mM NaHS	20 °C	Reduced the growth of *aspergillus Niger* and *Penicillium expansum*	Hu et al. (2014b)
Apple, kiwifruit, pear, mandarin, sweet orange and tomato	0.5 mM NaHS	25 °C for 4 days	Postharvest decay caused by *aspergillus Niger* and *Penicillium italicum* was reduced by inhibiting the activity of SOD and CAT in microbial cell, which led to increased ROS level and oxidative damage, resulting in inhibition of spore germination and growth of mycelia.	Fu et al. (2014)

CHI chitinase, *GNS* glucosamine N-acetyl-6-sulphate, *β-GAL* β galactase, *PG* polygalacturonase, *SOD* superoxide dismutase, *CAT* catalase, *ROS* reactive oxygen species

13.3.6 *Effect on Antioxidant System*

Antioxidants help to cope with oxidative damages caused due to the imbalance metabolism of horticultural produce during storage. Oxidative stress results in the production of harmful ROS that cause oxidative injury and accelerates senescence and ripening in fruits and vegetables. Various studies have suggested that postharvest application of H_2S inhibited ROS production and delayed the senescence and ripening during storage. H_2S scavenges ROS such as superoxide ion, hydrogen peroxide, peroxynitrite, etc. produced during senescence, lipid peroxidation, and chilling injury (Nagy 2015; Hu et al. 2014b). The possible mechanism is the role of H_2S in counteracting the ROS effect through sulphur metabolism which maintains redox homeostasis (Hancock and Whiteman 2014). This mechanism regulates the gene expression and protein activities related to ROS production. Another possibility is that redox balance is maintained by H_2S through increased antioxidant enzymes activity (CAT, SOD, POD, APX, glutathione reductase, etc.,) during postharvest storage (Gao et al. 2013; Ni et al. 2016; Liu et al. 2017; Yao et al. 2018; Siddiqui et al. 2021a, b). Higher antioxidant capacity enhanced by postharvest treatment of H_2S delayed ripening, senescence, and decay and enhanced the low-temperature tolerance in many fruits and vegetables (Table 13.6).

Table 13.6 Effect of H_2S on antioxidant system

Crop	Concentration	Storage conditions	Effect	Reference
Apple	0.4 mM NaHS	25 ± 0.5 °C & 85–90% RH	Inhibited ROS damage by promoting the antioxidants content (ascorbic acid, total phenolics, and flavonoids), antioxidant enzyme activity (CAT, SOD, POD, GR, and APX).	Zheng et al. (2016)
Broccoli	2.4 mM NaHS	25 °C & 85–90% RH	Enhanced the activity of GR, APX, POD and CAT.	Li et al. (2014)
Carrot	0.4 mM NaHS	5 ± 2 °C for 10 days	Improved the antioxidant capacity and enzyme activity (CAT, APX GR).	Chen et al. (2018)
Strawberry	0.8 mM NaHS	20 ± 0.5 °C & 85–90% RH	Elevated the activity of POD, CAT, GR and APX, against the damaging effects of ROS. Lowered H_2O_2 and $O_2^{\bullet-}$ content.	Hu et al. (2012)
Tomato 'Micro Tom'	Ethephon 1.0 g/L + NaHS 0.90 mM	25 °C & 85–90% RH	Increased the activity of APX, CAT and POD. Upregulated the expressions of the antioxidant genes *SlCAT1*, *SlAPX2*, *SlPOD12* and *SlCuZnSOD*.	Yao et al. (2018) and Zhong et al. (2021)

GR glutathione reductase, *POD* peroxidase, *CAT* catalase, *APX* ascorbate peroxidase, *SOD* superoxide dismutase, *ROS* reactive oxygen species

13.4 Cross-Talk of H_2S with Phytohormones and Signalling Molecule During Ripening

H_2S coordinates with other phytohormones like ethylene and abscisic acid (ABA) and other signaling molecules including nitric oxide during the ripening process. It regulates the feedback mechanism of ethylene biosynthesis and suppresses ethylene signalling which delays ethylene-mediated ripening, senescence, and abscission in fruits and vegetables (Gao et al. 2013; Ge et al. 2017; Yao et al. 2020). H_2S inhibited the ethylene production by downregulating the expression of genes (*ACO1*, *ACO4*, *ACS6*, *ERF1*, and *ETR4*) related to ethylene biosynthesis. (Li et al. 2015b; Zheng et al. 2016; Liu et al. 2020).

It has been proven that ABA triggers ethylene biosynthesis and can alter the gene expression associated with H_2S biosynthesis (Mou et al. 2016; Tayal et al. 2021). H_2S balances ABA signalling by accelerating the persulfidation of respiratory burst oxidase protein D (NADPH oxidase isomer) which produces higher ROS. Overproduction of ROS results in negative feedback and inhibits ABA signaling (Shen et al. 2020). However, the interaction between H_2S and ABA in the fruit ripening process is not very clear.

Both H_2S and NO are identified as important signaling molecules that inhibit ethylene biosynthesis and down-regulate its signalling action (Corpas et al. 2020). H_2S regulates many physiological processes in plants that are linked with NO signaling (Corpas et al. 2020; Mishra et al. 2021). H_2S mediated persulfidation (conversion of cysteine, a part of thiol group to persulfide) is linked with NO which modifies the target proteins using the S-nitrosation process (Corpas et al. 2019). These authors studied that H_2S and NO regulate fruit ripening in *Capsicum* fruit. Results indicated that H_2S and NO regulated the redox balance in fruits by reducing the activity of the enzyme NADP-isocitrate dehydrogenase (substrate of persulfidation). In the ripening process, both H_2S and NO modulate the production of NADPH by regulating the enzymes involved (NADPH oxidase, 6-phosphogluconate dehydrogenase, glucose-6-phosphate dehydrogenase, and NADP-malic enzyme). This mechanism helps in redox homeostasis and higher energy production in harvested produce during ripening (Muñoz-Vargas et al. 2018). H_2S and NO also regulate the antioxidant defense system which indicates their key role in fruit ripening and senescence (Palma et al. 2020; Tayal et al. 2021).

13.5 Conclusion and Future Aspects

The application of H_2S has a key role in the postharvest physiology of harvested fruits and vegetables. The signalling action of H_2S includes delay in ripening, senescence, softening, and alleviation of chilling injury and postharvest decay by higher antioxidant activity. Future work may include the study of combining the effect of H_2S with other postharvest treatments to improve the quality and storage life of harvested produce. The effect of H_2S on ripening-related signaling molecules, gene expression, and antioxidant mechanisms should be studied in-depth. In terms of food safety, residual effect or allergies caused by sulfur need a thorough detail. Crosstalk of H_2S with other phytohormones and signalling molecules should be paid more attention.

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Chapter 14
Carbon Compounds as Gasotransmitters in Plants Under Challenging Environment

Swarnavo Chakraborty and Aryadeep Roychoudhury

Abstract The sessile nature of plants compels them to experience a series of environmental stresses regularly. These stressors are the prime threats to plant growth and yield, due to alterations in the redox homeostasis of the cells, an increase in the production of toxic metabolites, and a rise in the level of oxidative damage within the plant system. Hence, plants have to develop robust mechanisms for sustenance against different stressors and also for the re-establishment of the homeostatic balance within the stressed cells. Currently, the study of different gasotransmitters in association with environmental stress tolerance in plants has attracted much of the attention of the scientific world. Moreover, various stressors have been found to induce the levels of these gasotransmitters within the plants. These gaseous signaling molecules function via up-regulation of anti-oxidative machinery, which in turn can render the stress-associated toxic metabolites harmless and also mediate tolerance against different forms of plant stress. This review mainly focuses on the role of some gasotransmitters in plant stress tolerance, which constitute the element carbon as an important component.

Keywords Gasotransmitters · Environmental stress tolerance · Carbon · Redox homeostasis · Anti-oxidative machinery · Plant stress

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T. Aftab, F. J. Corpas (eds.), *Gasotransmitters Signaling in Plants under Challenging Environment*, Plant in Challenging Environments 5,
https://doi.org/10.1007/978-3-031-43029-9_14

14.1 Introduction

Gasotransmitters can be defined as a group of small gaseous signaling molecules, responsible for the transmission of biological signals within the organism, where the gas molecule has been generated. These molecules have the power to alter the functions of cells capable of producing a target protein having an important physiological role inside the cell. These gaseous molecules have to fulfill certain criteria, like the potential to freely traverse across biological membranes, small size, specificity in terms of function at certain physiological levels, specificity of the molecular and cellular targets, regulated and endogenous enzymatic production by specific enzymes, and the functions can be mimicked via administration of a donor (Wang 2002). Nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfide (H_2S) are the most recognized gasotransmitters in biological systems. However, other gaseous molecules like ammonia, methane, ethylene, ozone, carbon dioxide, etc. are not traditionally considered gasotransmitters, but these molecules can potentially carry out the roles of gasotransmitters. Currently, there has been a rapid expansion in research on these molecules, owing to the extensive role of various gasotransmitters in biological systems and medicinal sectors as well (Wang 2018). The study of the role of different gasotransmitters and their biological applications as signaling molecules has been one of the key fields of research for the past few decades. Moreover, to aid in a better understanding of new signaling cascades, the production of endogenous plant gasotransmitters has been meticulously analyzed.

Plants are regularly challenged by a wide range of biotic and abiotic stressors, including heavy metals, extreme temperature, involved in many cell signaling pathways salinity, drought, ultraviolet rays, etc. These stressors can have serious implications on the plants, via alterations in leaf morphology, plant height, stomatal movements, etc. (Shen et al. 2011; Ali et al. 2017; Jin et al. 2017). Moreover, these alterations can result in the disruption of plant physiological mechanisms, which involves changes in the levels of certain stress-related molecules like hydrogen peroxide, malondialdehyde, proline, and certain electrolytes. As a part of the protective response, anti-oxidative enzymatic activities change which mediate the detoxification of the toxic metabolites produced as a result of stress imposition and re-establishment of redox homeostasis in plants. In addition, most of the gasotransmitters tend to up regulate the activities of a series of protective anti-oxidative enzymes and reduce the toxic effects of the detrimental Reactive Oxygen Species (ROS) produced in response to stress imposition. Existing studies on gasotransmitters indicate the generation of these signaling molecules in response to various plant abiotic stressors (Abdulmajeed et al. 2017; Cui et al. 2017; Jia et al. 2018a, b) and also aid in the development of plant tolerance against the detrimental effects of these stressors (Jin et al. 2017; Xu et al. 2017; Maryan et al. 2019). Therefore, these biological gaseous signaling molecules and mediators generated via complex intracellular and extracellular pathways help in the synergistic or antagonistic regulation of various important plant processes.

14.2 Carbon Monoxide: A Ubiquitous Carbon Gasotransmitter in Plants

Carbon monoxide (CO) has a wide range of plant physiological functions, including the induction in CO production in response to different environmental stressors. This gaseous molecule is odorless, tasteless, and colorless in nature, and comprises one atom of oxygen and one atom of carbon. CO is considered a poisonous gas, generated from forest fires, volcanic eruptions, incomplete combustion of certain organic compounds, etc. Wilks (1959) first reported the existence of biosynthetic mechanisms of CO in plants. CO is involved in many cell signaling pathways and intercellular communication at physiological concentrations. The production of CO in terrestrial plants has been widely reported by different groups (Siegel et al. 1962; Fischer and Luttge 1978). In addition, Siegel and Siegel (1987) demonstrated the presence of a robust light-independent CO source, in association with soil-air and soil-surface interface of smaller plant groups. Upon application of ascorbic acid or hydrogen peroxide, the methylene bridges in heme break, resulting in the release of CO (Dulak and Józkowicz 2003). Apart from this non-enzymatic metabolism of heme, another potentially active and ubiquitous enzyme, heme oxygenase (EC 1.14.99.3; HO) is involved in the enzymatic production of CO in plants as well as animals (Bilban et al. 2008; He and He 2014a, b). This key enzyme catalyzes the cleavage of heme into CO, upon exposure to reducing agents (Gisk et al. 2010).

In plants, the expressions and subsequent enzymatic activities of HOs have been detected in several plant species. HO1 is one such enzyme in plants, displaying induction upon exposure to different environmental stressors, like ultraviolet-B (UV-B) rays, heavy metal toxicity, salinity, drought-induced osmotic stress, wounding, low-temperature imposition, and deficiency of certain nutrients (Yannarelli et al. 2006; Han et al. 2008; Xie et al. 2008, 2015; Liu et al. 2010; Lin et al. 2014a, b; Zhang et al. 2015). Figure 14.1 depicts the role of CO in plant stress tolerance under challenging environmental conditions. Shekhawat and Verma (2010) explained the role of the HO1/CO system in a wide range of plant abiotic and oxidative stress tolerance. The most potent mechanism of CO-mediated protection in plants is the regulation of the antioxidant systems to render tolerance against salinity stress. Liu et al. (2007) demonstrated the alleviation of salt imposed inhibition of germination and subsequent lowering of lipid peroxidation and oxidative damage parameters in rice, via exogenous supply of CO aqueous solution. Moreover, during salt stress in *Cassia obtusifolia*, both the exogenous supply of CO solution and endogenous generation of CO relieved the toxic effects of salt exposure (Zhang et al. 2012). In addition, Ling et al. (2009) have also shown that CO aqueous solution induced repression of toxic superoxide production in wheat plants, which eventually resulted in suppression of programmed cell death and improvement of root growth parameters.

Polyethylene glycol-6000 (PEG-6000)-induced osmotic stress in plants leads to the decline of seed germination percentage and a higher rate of peroxidation of membrane lipids. The HO1/CO system resulted in the potential amelioration of

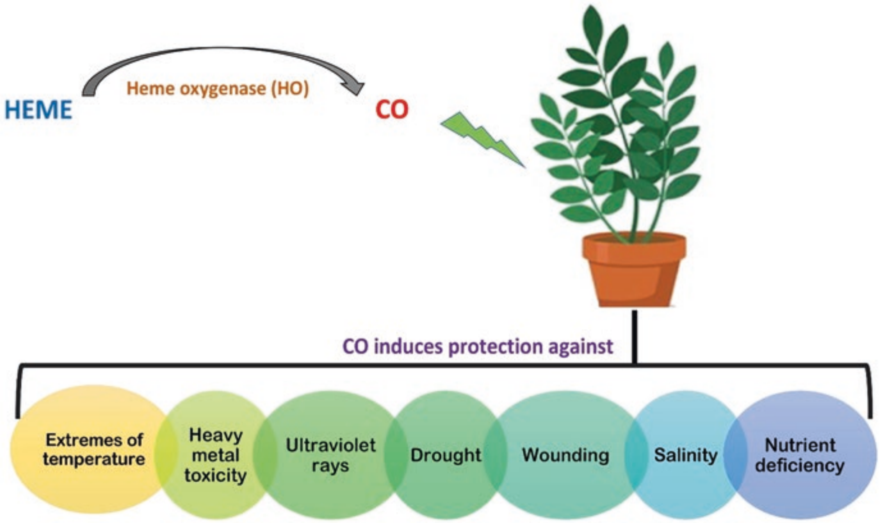

Fig. 14.1 Carbon monoxide (CO) provides plant tolerance against a myriad of environmental stressors

PEG-induced damage in plants (Liu et al. 2010). Interestingly, this system also led to the improvement of drought-imposed restrictions on stomatal movements in many plants (Song et al. 2008; She and Song 2008). CO has been found to induce the activation of glutathione metabolism in *Medicago sativa* plants, thereby reducing the toxic effects of heavy metals like cadmium (Han et al. 2008). Similarly, in *Arabidopsis thaliana* plants, CO production was found to be directly related to the generation of tolerance against cadmium toxicity via regulation of iron homeostasis and reduction in NO production (Han et al. 2014). Mercury toxicity induced damages in alfalfa, *Brassica napus* and Indian mustard, including inhibition of growth of roots and enhanced peroxidation of lipids have also been found to be repaired upon CO administration (Han et al. 2007; Shen et al. 2011; Meng et al. 2011). In addition, Cui et al. (2013) have shown the potential of CO in amelioration of oxidative damages imposed due to aluminum toxicity in *Medicago sativa* plants.

Certain environmental conditions like high or low temperatures are the prime factors that limit plant growth and yield, including the germination of seeds. CO administration led to a marked increase in the anti-oxidative enzyme activities associated with the glutathione-ascorbate cycle, accumulation of the reduced form of glutathione, and also reduced the levels of toxic hydrogen peroxide (H_2O_2), thereby generating tolerance amongst plants against extremes of temperatures like cold stress (Bai et al. 2012). In addition, pre-treatment with 5-aminolevulinic acid (ALA) resulted in up-regulation of HO1 and CO production in soybean, thereby rendering tolerance against chilling stress via induction of heme catabolic pathways (Balestrasse et al. 2010). Moreover, HO1 expression has been reported upon irradiation with UV-B radiation in soybean plants. Such irradiation was associated with the accumulation of toxic ROS, ultimately leading to the imposition of oxidative damages in plants. However, the HO1 accumulation in response to UV-B stress led to potential amelioration of the damages incurred by the plants and thereby rendering cellular protection from oxidative injuries (Yannarelli et al. 2006). Xie et al. (2012) mentioned the possible role of CO in UV-C tolerance in plants as well. An Arabidopsis mutant *HY1*, exhibited hypersensitivity to UV-C marked by impaired biosynthesis of flavonoids and carotenoids and poor anti-oxidative defenses, probably due to disruption of CO production in these plants. In addition, induction of endogenous CO production has also been found during deficiency of iron in certain plants, thereby pointing at the possibility of CO in the mitigation of iron deficiency. This was further demonstrated by Kong et al. (2010), where exogenous CO application resulted in the mediation of iron homeostasis in iron-starved Arabidopsis seedlings. Interestingly, the *HO1* gene in *Brassica napus* has been reported to regulate lateral rooting during the imposition of osmotic and salt stress (Cao et al. 2011). In rice and tomato plants, this gene was also found to control the formation of lateral roots in response to cobalt chloride toxicity (Xu et al. 2011).

Toxicity due to different environmental stressors resulted in oxidative stress in plants, owing to the generation of ROS within the system. These toxic species are formed via the transfer of high-energy electrons to molecular oxygen (Mittler 2002), along with H_2O_2 and superoxide radicals ($O_2^{\bullet-}$) as intermediates, but certain peroxides and singlet oxygen ($^1O_2$) are also considered as ROS which are generated in plants. The primary targets of these species are cellular macromolecules, like lipids associated with membranes, proteins and nucleotides, either in DNA or RNA. Upon interaction with these macromolecules, these harmful ROS lead to the generation of peroxides as the end products, thereby resulting in serious damage at the cellular level. CO has the potential to regulate the levels of intracellular ROS by a range of mechanisms displaying significant consequences within the system. Wu et al. (2011) demonstrated lowering of the production of H_2O_2 and subsequent delaying of programmed cell death in the aleurone layers of wheat, due to up-regulated *HO1* expression in response to some environmental cues. Moreover, the HO1/CO system has probable roles in the modulation of the NADPH oxidase proteins and is commonly considered the potent source of toxic ROS involved in a diverse range of signaling cascades in plants. In addition, CO induced lowering of superoxide radical production, probably due to the downregulated activity of NADPH oxidase, resulting in delaying of programmed cell death (PCD) during salinity stress in wheat (Ling et al. 2009). Also, in *Brassica juncea*, amelioration of cadmium toxicity has been reported to be mediated by HO1 regulated strengthening of the antioxidant system, leading to H_2O_2 detoxification (Li et al. 2012). Therefore, it can be concluded that ROS activities generate a positive feedback signal that results in the up-regulated generation of CO molecules. For instance, higher levels of detrimental H_2O_2 molecules resulted in up-regulation of HO activity due to higher expression of HO1 mRNAs (Wei et al. 2013). Similarly, UV-B irradiation also resulted in up-regulated HO1 mRNA expression, due to toxic ROS generation (Yannarelli et al. 2006).

CO can also induce the closure of stomata in a dose-dependent fashion and is associated with the process of abscisic acid-mediated stomatal movements upstream of NO production (She and Song 2008). CO-induced stomatal closure has been reported to be modulated by hydrogen peroxide (the key ROS generated during plant stress) signaling in plants (She and Song 2008). Wang and Liao (2016) explained the increase in production of CO and subsequent up-regulation of HO activity upon exogenous abscisic acid administration, leading to the closure of stomata in the leaves of *Vicia faba*. In addition, CO-mediated regulation of stomatal movements is operational in plants via an elaborate cross-talk of CO with two other key plant gasotransmitters like H_2S and NO. These gaseous molecules tend to regulate the enzymatic biosynthesis of each other and ultimately form a part of a common signaling mechanism, which needs further scientific elaboration. CO as a gaseous signaling component has already been studied in quite detail in animals, but information regarding the physiological role of this gasotransmitter in plants is rather in their early stages. The potency of CO to act as a protective molecule against plant stress via interplay with other signaling molecules is well established. However, the detailed mechanisms, governing the transduction events in CO-mediated signaling, need greater clarification. Therefore, the improvement of molecular, pharmacological, and physiological methods for a better view of the CO transduction pathways is mandatory.

14.3 Other Forms of Carbon Gasotransmitters in Plants

14.3.1 Methane (CH_4)

CH_4 was the first gas identified to be produced in rice by Nouchi et al. (1990). In addition, CH_4 production has been observed under anaerobic conditions by paddy cultivations. Several reports indicate the production of this gaseous molecule upon exposure to UV radiation in a range of plants, including tobacco, *Crataegus laevigata*, *Betula populifolia*, *Malus domestica*, *Quercus robur*, *Plantago lanceolata*, *Salix caprea*, *Salix alba*, *Brassica oleracea* and certain citrus fruits (McLeod et al. 2008; Bruhn et al. 2009, 2014; Messenger et al. 2009). Under low light conditions, poplar plants have been found to produce CH_4 while in alfalfa, induction of gas production has been reported under salinity and heavy metal stress like copper, aluminum, cadmium, etc. (Brüggemann et al. 2009; Zhu et al. 2016; Samma et al. 2017; Gu et al. 2018). It is known that PEG induces osmotic stress or water shortage situation within the plant system. Hence, PEG treatment in maize seedlings demonstrated an up-regulation in the production of CH_4 (Han et al. 2017). In addition, high-temperature exposures led to the generation of CH_4 in pea plants (Abdulmajeed et al. 2017). In alfalfa seedlings, application of methane solution (containing 0.39 mM CH_4) aided in the re-establishment of the redox homeostasis of the cells during copper toxicity, via enhancement of the activities of amylase enzymes, increment of total sugar levels, alteration in the proline metabolism and reduction of the levels of thiobarbituric acid reacting substances (Samma et al. 2017). Similar restoration of cellular redox homeostasis and maintenance of organic acid levels during aluminum stress has also been reported, via the administration of methane-rich water (Cui et al. 2017). Aluminum toxicity-triggered oxidative damages in plants have also been found to be reduced due to the up-regulation of methane-induced antioxidative machinery. Moreover, treatment with 1.3 mM CH_4 helped in the restoration of cellular homeostasis and glutathione metabolism during cadmium toxicity in plants (Gu et al. 2018). Genetic evidence also indicates that the application of methane solution in plants helped in the modulation of certain important heavy metal transporters during cadmium stress (Gu et al. 2018). Zhu et al. (2016) have shown the use of 50% methane-rich water in alfalfa in the reduction of salt-induced peroxidation of lipids and excess accumulation of ROS, leading to the establishment of ionic homeostasis, optimization of seed germination, and lowering of oxidative damage indices within the system. Application of 0.65 mM CH_4 during osmotic stress in maize seedlings resulted in the up-regulation of levels of essential sugars and modulation of ascorbate metabolism, generating overall plant stress tolerance (Han et al. 2017). Hence, it is evident that CH_4 plays a rather positive role in the establishment of abiotic stress tolerance in different plants.

14.3.2 Ethylene (C_2H_4)

C_2H_4, as an important gaseous phytohormone, has also been considered an outstanding candidate for the gasotransmitter family. Apart from its conventional role as a phytohormone, it has also been widely studied for its importance as a gasotransmitter within the plant system. Plant responses to a myriad of environmental stressors are modulated via the cross-talk between ethylene with other members of the gasotransmitter family including NO and H_2S. Enrichment of UV-B rays and exposure to excess light intensity, results in detrimental implications on the photosynthetic efficiency of plants, due to over-accumulation of toxic ROS within the system (Takahashi and Badger 2011; Demarsy et al. 2018). The absence of optimal light intensity also leads to the reduction of photosynthetic efficiency. Interestingly, both light insufficiency as well as excessive light exposure in plants, triggers the metabolic pathways associated with NO and C_2H_4 cross-talk, thereby regulating the light-induced senescence in plants (Kolbert et al. 2019). Light-imposed stress triggers the generation of both NO and C_2H_4 in Arabidopsis plants (Magalhaes et al. 2000). Both higher light intensity and light exposure for a short time resulted in up-regulation of cascades associated with C_2H_4 and NO interplay mainly in the shoot tissues of Arabidopsis. Similarly, exposure to UV-B radiation triggers the production of C_2H_4 and NO in various plant organs and species (Mackerness et al. 2001; Vanhaelewyn et al. 2016). Exogenous application of NO donors in various plants induced the UV-B regulated generation of C_2H_4 even in the seedling stage. During UV-B triggered stomatal closure, C_2H_4 seems to act as a signal upstream of NO. This can be implicated by the fact that UV-B induced closure of stomata was promoted due to the accumulation of NO in the guard cells of *Vicia faba*, followed by the C_2H_4 evolution peak (He et al. 2011). Moreover, depending on the light intensity available, C_2H_4 can induce the opening or closure of stomata, via modulation of NO accumulation in the guard cells. The effects of NO and C_2H_4 on the movement of stomata rather show a positive correlation under dark conditions.

Both cold and heat stress have been reported to have serious ill effects on the plant system, which can adversely affect plant development and growth. To bypass these ill effects, plants tend to adopt certain mechanisms, which involve alteration in the molecular, physiological, and biochemical plant processes, in which the interaction between C_2H_4 and NO forms a crucial part (Majláth et al. 2012; Parankusam et al. 2017). Alteration in the emission of C_2H_4 is associated with plant cold stress tolerance, via activation of robust anti-oxidative machinery (Guo et al. 2014). Interestingly, it has been observed that the C_2H_4 and NO interaction in cold stress tolerance display a rather antagonistic relationship during fruit ripening. Cold stored mangoes upon exposure to different concentrations of NO fumigation, exhibited a lowering of C_2H_4 production, associated with delaying of fruit ripening, softening, and color development, along with a marked increase in the level of tolerance to chilling injury (Zaharah and Singh 2011). In addition, this antagonistic relationship between these two gasotransmitters, also enhanced fruit chilling tolerance due to regulation of the anti-oxidative enzymatic and sugar metabolism, thereby delaying senescence of fruits and improving their quality. On the other hand, heat stress can also negatively affect the rates of respiration and photosynthesis, membrane stability, membrane fluidity and stability, overall metabolism, and cytoskeletal movements, mostly due to the accumulation of toxic ROS and unfolded proteins. In alfalfa, exposure to heat stress resulted in increased NO and decreased C_2H_4 production (Guo et al. 2014). However, the link between C_2H_4 and NO during heat exposure in plants is rather poorly addressed.

Alteration in osmotic homeostasis and loss of turgor of cells are the common outcomes of drought stress in plants. In addition, osmotic stress can also reduce photosynthetic efficiency and affect stomatal movements. Synthesis of osmoprotectants and antioxidants at the cellular level, regulated by the NO-C_2H_4 cross-talk, forms the basis of drought stress tolerance in various plants. For instance, in Arabidopsis, the production of these two gasotransmitters enhanced the endurance potential of plants against drought stress (Nabi et al. 2019). Moreover, this interaction of NO-C_2H_4 plays an important role in the regulation of plant cell death and defense responses due to drought-induced osmotic shock. In addition, the role of other phytohormones like abscisic acid tends to regulate the levels of these two gasotransmitters in an antagonistic manner, i.e., up-regulation of NO generation and down-regulation of C_2H_4 production within the stomata (Sós-Hegedus et al. 2014; Wilkinson and Davies 2010). However, time dependent C_2H_4-NO interaction in different plant organs tends to display a range of intolerance parameters against drought. In addition, during hypoxic conditions, aerenchyma formation has been reported to be induced due to NO production in wheat plants (Wany et al. 2017). NO production in these oxygen-deprived roots triggered an over-production of ethylene. This in turn helped in the modulation of stress responses in plants like lipid peroxidation, ROS generation, DNA damage, nitration of proteins, and induction of certain important enzymes like cellulases.

One of the most detrimental forms of abiotic stress is salt stress, which leads to disruption of physiological functions including disturbance in growth parameters, early senescence, reduction in yield and fertility, induction of programmed cell death (PCD), and alterations in cellular structures (Munns and Tester 2008; Peleg and Blumwald 2011; Zhu 2016). The association helps in the activation of defense pathways that mediate salt tolerance among plants. This interaction has been first demonstrated in the callus of Arabidopsis, where a considerable reduction in the electrolyte leakage has been reported as a protective response against salt stress (Wang et al. 2009). In callus cultures, salt stress imposition resulted in the rapid accumulation of NO and C_2H_4 production. Moreover, these two gasotransmitters functioned in a dose/concentration- as well as time-dependent manner, thereby leading to progressive reduction in ROS accumulation in the stressed cells. In addition, the NO-C_2H_4 interaction triggered protective strategies against salt stress, including regulation of PCD, cell viability, cellular K^+/Na^+ ratio, developmental and metabolic processes, and rate of protein and DNA degradation; all these responses rather varied from plant cell organs and types, thus generating an array of responses due to NO-C_2H_4 interaction (Poór et al. 2015).

Sometimes, an inadequate supply of certain important plant nutrients like phosphorus, iron, magnesium, etc. can seriously affect overall plant growth and productivity. Ethylene application indicated the positive induction of certain iron-acquisition genes. Similarly, these genes also demonstrated an up-regulation upon NO treatment, indicating a possible link between these two gasotransmitters, as a part of iron insufficiency response. Also, NO application has been involved in the regulation of expression of many ethylene biosynthetic genes and vice-versa in tomato and Arabidopsis, again pointing to the robust interplay between these two gases (García et al. 2010; Romera et al. 2011). However, during phosphorus deficiency in rice, roots demonstrated a rapid production of NO, then followed by a rather slower emission of C_2H_4, suggesting an upstream action of NO, as compared to that of C_2H_4 in the cascades involved in plant phosphorus insufficiency. However, this interaction amongst these gases helped in the increment of phosphorus levels in deficient rice plants (Zhu et al. 2017). A similar increase in C_2H_4 and NO accumulation has also been observed in the case of magnesium deficiency in Arabidopsis, and such accumulation tends to be accompanied by enhanced development of root hairs. Interestingly, these two gasotransmitters tend to mutually and synergistically modulate each other's response, as inhibition of either of the gasotransmitter prevented the development of root hairs in magnesium-deprived plants (Liu et al. 2017). On the other hand, contamination with heavy metals also induces detrimental consequences within the plants. The primary theme of any form of heavy metal toxicity in plants is oxidative stress-induced disrupted cellular redox homeostasis and a series of morphological and physiological alterations in plants leading to extensive cellular and tissue damage. The role of C_2H_4 and NO interplay during exposure to heavy metals like cadmium, arsenate, arsenite, copper, zinc, lead, nickel, etc. have been reported in plants (Zhu 2016; Sahay and Gupta 2017). Preliminary studies on cadmium stress in pea indicated the triggering of ROS metabolism as a result of C_2H_4 and NO interactions. In addition, this gaseous interplay could also regulate the levels of other hormones like salicylic acid and jasmonic acid in plants. However, most of the suggested models hint that C_2H_4 and NO function in an antagonistic fashion, with C_2H_4 levels increasing and NO accumulation decreasing, as a result of cadmium toxicity in plants (Rodríguez-Serrano et al. 2009). Interestingly, upon short-term imposition of cadmium stress in soybean, up-regulated expressions of both C_2H_4 and NO biosynthetic genes were observed (Chmielowska-Bak et al. 2013). Hence, the interactions of these gaseous molecules at the molecular and biochemical levels in plants need to be deciphered in a better way.

Ethylene has been found to play a crucial role in the mechanism of stomatal closure. The participation of H_2S has been reported in the processes associated with C_2H_4 induced closing of stomata. However, a clear view depicting to entire signaling involving C_2H_4 and H_2S has not still been deciphered properly. The central theme of any form of abiotic stress is osmotic stress and H_2S governs the feedback regulation of the biosynthesis of ethylene in plants during osmotic stress. Similarly, ethylene administration also induced the generation of H_2S within the stressed guard cells (Jia et al. 2018a, b). This H_2S-C_2H_4 interplay tends to form a downstream component of the signaling cascades involved in osmotic stress response, particularly associated with the C_2H_4 regulated stomatal closure during stress imposition in plants.

14.3.3 Carbon Dioxide (CO_2)

Stomatal closure is one of the most common adaptations in response to osmotic stress induced due to any form of stress imposition in plants. Many gasotransmitters are involved in the modulation of stomatal movements in plants. Carbon dioxide (CO_2) is one of such gaseous molecules, which has often not been considered an important gasotransmitter in plants, also triggering stomatal closure. The ability to close and open in response to different environmental cues makes the stomatal apparatus a fascinating and dynamic system. The closing and opening of the stomata are under the direct control of the turgidity and flaccidity of the guard cells, which has an essential role in photosynthesis and transpiration. Higher levels of CO_2 induce the closure of the stomatal apparatus, along with the toxic ROS serving as one of the key components in the regulatory cascades. ROS plays a key role in the mediation of the acclimation against different stressors in plants, and ROS generation is commonly found to be associated with the principle of oxidative stress induced as a result of plant stress. Just like CO_2, ROS also acts as major modulators of stomatal movements in plants, particularly during biotic or abiotic stress imposition in plants.

Interestingly, ROS signals are intricately associated with the high-level CO_2 triggered movements of the stomatal apparatus in plants. The rate at which different materials can pass through the stomatal apparatus (stomatal conductance) is negatively regulated by the levels of CO_2 in the surroundings. Moreover, a rise in CO_2 levels can result in a reduction in the apertures of the stomatal apparatus and also the stomatal number per unit area of the leaf, thereby decreasing the stomatal conductance progressively. Decreased conductance of stomata results in a fall in the rate of evapotranspiration, which in turn mediates the conservation of water and generates a cooling effect in the plants during stress, due to reduction in water loss via leaves (Long and Ort 2010; Keenan et al. 2013). However, this reduction in stomatal conductance as a protective strategy against different stressors might result in sub-optimal yields (Engineer et al. 2016). After the entry of CO_2 inside the cells, this gaseous molecule is sensed by certain specific plasma membrane receptors. Several propositions indicate that β-carbonic anhydrase in plants carries out the function of the receptors that can sense CO_2 (Frommer 2010) and also plays an important role in the movements of stomata in response to alterations in the CO_2 levels in Arabidopsis. Carbonic anhydrases also mediate the conversion of CO_2 into HCO_3^-. Treatment of plants with higher doses of HCO_3^- leads to the production of ROS and also induces the plasma membrane-associated NADPH oxidase, which can also mediate stomatal closure, indicating the crucial role of ROS in the CO_2 induced closure of the stomatal apparatus. Moreover, the guard cells displayed a sharp decrease in the levels of ROS at elevated concentrations of CO_2. This can be explained by the fact that high CO_2 levels reduce oxygenase activity of RuBisCo, thereby leading to a subsequent reduction in glycolate oxidase activity (associated with the process of photorespiration), which in turn results in a considerable decline in ROS generation (Fahnenstich et al. 2008). Thus, NADPH oxidase-mediated production of ROS efficiently controls the high CO_2 regulated closure of stomatal apertures in plants. In addition, a series of cell wall-associated peroxidases also contribute to the process of high CO_2-induced movements of stomata. However, the signaling cascades involving ROS in CO_2 modulated stomatal movements can constitute other signaling members, but the detailed scientific information has not yet been gathered, as it depends on the unraveling of other molecules involved in the cascades. Deciphering of such information and subsequent translation of this knowledge into important crop plants can aid in the generation of stress tolerance and also lead to enhancement in crop yield.

14.4 Conclusion and Future Perspectives

Over the past few years, the role of gasotransmitters in association with plant environmental stress response has been considered a hot issue and potential research has been conducted in this area. Present reports indicate that plants can synthesize such gaseous molecules during adverse environmental conditions and thereby resulting in the mediation of plant tolerance against a myriad of detrimental environmental cues. The gasotransmitters enhance stress tolerance in plants via mitigation of oxidative damages, reduction of peroxidation of membrane lipids, maintenance of ionic and metabolic homeostasis, and also by regulation of anti-oxidative enzymatic activities in plants. Many growing studies indicate that gasotransmitters are produced within the plant system under conditions of abiotic and biotic stress, but future research is necessary, particularly focusing on the molecular insights involving the biosynthesis of these gaseous molecules. In addition, the possible interactions of these gasotransmitters with other cellular mediators and the subsequent transcriptional, translational and post-translational alterations taking place as a part of the signaling mechanism also demand a clearer understanding. Although a series of advances have been observed in the field of gasotransmitter research, investigation of the direct molecular target of these gaseous molecules and the downstream signaling processes still remains to be partially elucidated in plants. Several new approaches, like in situ real-time quantifications of these gaseous molecules and their rate of production in different cellular organelles, can provide further information associated with plant research in this regard. These data will aid in a more comprehensive understanding of the gasotransmitter biology within different members of the plant kingdom.

Acknowledgements Financial assistance from Council of Scientific and Industrial Research (CSIR), Government of India, through the research grant [38(1387)/14/EMR-II], Science and Engineering Research Board, Government of India through the grant [EMR/2016/004799] and Department of Higher Education, Science and Technology and Biotechnology, Government of West Bengal, through the grant [264(Sanc.)/ST/P/S&T/1G-80/2017] to Prof. Aryadeep Roychoudhury is gratefully acknowledged.

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Chapter 15
Carbon Monoxide (CO) and Its Association with Other Gasotransmitters in Root Development, Growth and Signaling

Piyush Mathur, Rewaj Subba, and Soumya Mukherjee

Abstract In higher plants, the root formation is accomplished through cell signaling that regulates meristem differentiation. Off late, carbon monoxide (CO) has been discovered to be an important gaseous regulator of cellular components and it controls various metabolic pathways in animal and plant system. Root architecture is regulated by precise signaling-mediated by CO, nitric oxide (NO), and hydrogen sulphide (H_2S). Like in the animal systems, CO also plays regulatory roles in various biological processes in plants such as seed germination, stomatal closure and root development. Intracellular CO is majorly produced by the activity of heme oxygenase (HO) isoforms present in various plant tissues. Apart from the enzymatic pathway, CO is also known to be non-enzymatically produced from routes of lipid peroxidation and ureide metabolism pathways. CO resembles structural and chemical similarities to NO (diatomic gas). It is interesting to know that pieces of evidence in the animal system have revealed the presence of associative crosstalk between NO synthase (NOS) and CO-HO components. CO in plant cells is known to provide a similar response to that of NO where dose-dependent effects have been reported. CO-NO crosstalk has some evidence from investigations in various plant systems, but not much information is available on CO-H_2S interactions during root development. Although persuasive at present, evidence shows that H_2S can alter the activity of HO and thus participates in NO signaling cascades. The present chapter reviews the inter-relations among CO, NO, and H_2S in mediating root development and signaling in higher plants.

Keywords Carbon monoxide · Hydrogen sulphide · Nitric oxide · Root · Rhizosphere

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T. Aftab, F. J. Corpas (eds.), *Gasotransmitters Signaling in Plants under Challenging Environment*, Plant in Challenging Environments 5,
https://doi.org/10.1007/978-3-031-43029-9_15

15.1 Introduction: Root Apex Cognition, Shaping of Root Architecture, and Rhizosphere Signaling

The rhizosphere is a distinct interface of plant-soil communication that is influenced by various factors. In this context, it is critical to evaluate the rhizosphere's microbial flora, which controls plant development and nutrient acquisition in plants. Soil organic matter (SOM) and humus production, among the several abiotic variables controlling the biology of the rhizosphere, play a crucial role in regulating nutrient uptake in roots. The soil-root interface exchanges a wide range of metabolites. Cell wall thickening is absent at the root tip's developing apex. Root hairs abound in the absorptive zone of roots. This zone is active in the exchange of nutrients from the soil solution. The amounts of nitric oxide (NO) formation in this root area are believed to be influenced by nitrogen availability in the soil.

Furthermore, rhizosphere nutrition levels have a strong influence on root morphology and architecture (Forde and Lorenzo 2001; Forde 2002). Root development is critical for optimum plant growth in normal and challenging environments (Villordon et al. 2014). Root formation is accomplished signaling events associated with meristem differentiation. The ground meristem's quiescent centre contains stem cells that eventually develop various regions of the root (Motte et al. 2019). Various signaling networks are responsible for changes in root morphology and fine-tuning of root development (Meng et al. 2019). For instance, under selenite stress, NO and cytokinin accumulation in Arabidopsis wild-type and nitrate reductase-deficient mutants (*nia1nia2*) are low in the root meristem (Lehotai et al. 2016). During Arabidopsis root growth, ethylene receptors (ETR1, ETR2, ERS1, and ERS2) transduce the phosphorylation signal to downstream pathways associated with auxin and cytokinin interaction (Liu et al. 2017). During root growth, auxin signaling involves regulation of auxin and cytokinin metabolism. Genetic approaches can be used to comprehend the mechanism of these hormones' complicated interplay during root growth. Biochemical, molecular, and pharmacological studies over the last few decades have revealed that the gasotransmitters NO, CO, and H_2S (hydrogen sulphide) are involved in growth, morphogenesis and plant signaling in distinct ways (Xie et al. 2008; Kolbert et al. 2019).

There are just about 30 papers on the combined involvement of NO, CO, and H_2S crosstalk in root growth. Deciphering gene network for root development, stress amelioration (NO, CO, and H_2S donors) antioxidative defense and plant tolerance are some of the recently discovered trends from diverse publications. Although reports on NO in relation to root physiology is on the rise (622 reports from 2000 to 2020), there are far fewer for CO (33) and H_2S (129) mediated root signaling in the PubMed database. The reports obtained till date show a larger increase in reports of H_2S and CO in the context of root development.

Regulation of root architecture related to CO, NO, and H_2S metabolism during physiological and stressful situations is linked to the zonal differentiation of roots (Mukherjee and Corpas 2020). ROS accumulation, root lignification, and tyrosine nitration of numerous regulatory proteins are all linked to the NO signaling in roots.

Anoxia-induced aerenchyma development appears to be aided by indole acetic acid (IAA)-NO interaction. In roots, CO signaling affects glutathione metabolism and interacts with IAA, NO, and jasmonic acid (JA) activity. H_2S preferentially regulates ion homeostasis, electrolyte leakage, and root development regulation pathways involving reactive oxygen species (ROS), mitogen activated protein kinase (MAPK), and auxin (Mukherjee and Corpas 2020). H_2S inhibits the production of ethylene, which further limits root development. Modulation of biosynthesis and triggering is involved in crosstalk among the three gasotransmitters (NO, CO, and H_2S).

15.2 Historical Perspectives of CO as a Signaling Molecule in Plants

The past few decades ago, in the early twentieth century, CO was considered toxic. It is a diatomic gas with low molecular weight and shows a ubiquitous distribution in nature. Off late, it has been discovered to be an important gaseous regulator of cellular components and it controls a wide range of biological activity in plants (Xie et al. 2008). CO is produced in biological systems primarily as the oxidation product of heme-methene bridge, which is catalyzed by heme oxygenase enzymes (HOs, EC 1.14.14.18) (Bilban et al. 2008). In animal systems, CO functions as a neurotransmitter (Boehning et al. 2003), a platelet aggregation inhibitor (Brüne and Ullrich 1987), and also represses acute hypertension (Motterlini et al. 1998).

Like in the animal system, CO regulates various biological processes in plants such as seedling growth (Dekker and Hargrove 2002), root development, and stomatal closure (Cui et al. 2015). CO brings about stress alleviation in plants subjected to oxidative damage (Meng et al. 2011). Interestingly, CO also interacts with other biomolecules (NO, H_2S, and phytohormones) during plant growth and development, in addition to acting as a signaling molecule. Here we review the role of CO and its interaction with NO and H_2S in regulating root development and signaling in plants.

15.3 Biosynthetic Regulation of CO in Plants

Various pieces of evidence provide clues to the biosynthetic routes of CO in plant tissues (Shekhawat and Verma 2010; Mahawar and Shekhawat 2018). Intracellular CO is majorly produced through metabolic reactions mediated by HO isoforms present in various plant tissues. HOs catalyze (O_2 and NADPH-dependent) the conversion of heme compound which is oxidized to biliverdin (BV) and the reaction is accompanied by the formation of CO and also releases free iron (Fe^{2+}) (Bilban et al. 2008). BV is converted by the activity of biliverdin reductase which liberates bilirubin (BR) in plant cells. Investigation in the animal system has reported three HO isozymes HO1, HO2, and HO3 ranging from 32–36 kDa in size. Evidence reveals that HO1 is largely modulated by environmental cues while the other two isoforms show constitutive expression with relatively lower activity. Muramoto et al. (2002) have reported that recombinant-heme oxygenase (AtHO1) localized in the plastid is responsible for the *in vitro* formation of CO from heme as a substrate for HO. HO1 and HO2 sub-family contains *HO* genes reported in various plants (Shekhawat and Verma 2010). However, HO2 subfamily enzymes do not exhibit CO liberating activity in plant cells. Plants like *Arabidopsis*, soybean or rice have been investigated for the presence of HO transcripts (Liu et al. 2007; Han et al. 2008).

Apart from the enzymatic pathway, CO is also known to be non-enzymatically produced from routes of lipid peroxidation and ureide metabolism pathways. CO is generated from reactions of splitting of heme-methylene bridges which occurs in presence of H_2O_2 and ascorbic acid (Zilli et al. 2014). Plant roots are responsive to altered levels of CO generation and associated signaling routes during abiotic stress, pathogen attack and photoperiods (Cui et al. 2015; Chen et al. 2017). According to Wang and Liao (2016) endogenous levels of auxin, NO, and JA are known to regulate CO generation in plants.

15.4 Brief Role of CO in Plant Growth and Development

Investigations provide evidence that CO regulates cellular signaling in plants. CO, for example, delayed gibberellins (GA)-induced programmed cell death (PCD) in wheat aleurone cells by increasing the expression of major antioxidative enzymes like ascorbate peroxidase (APX) and catalase (CAT) thus leading to a subsequent reduction in H_2O_2 content (Wu et al. 2010). Several studies have shown that CO exhibits concentration-dependent effects on seed germination. Modest amounts of exogenous CO (0.1 or 1%) enhanced seed germination of foxtail (*Setaria faberi*). However, germination reduced with higher percent of CO due to inhibition of mitochondrial respiration (Dekker and Hargrove 2002). The physiological process of seed germination in *Oryza sativa* was enhanced by both CO donor heme and CO aqueous in a dose-dependent manner by activating amylase activity and enhancing the generation of energy resources (Liu et al. 2007). CO was also found to play a role in radicle emergence in wheat (Liu et al. 2010) and *Brassica* sp. (Liu et al. 2010; Amooaghaie et al. 2015).

Stomatal movement is an important regulator of plant water status, leaf vapor pressure, and transpiration that can be regulated by a variety of environmental or hormonal responses. Under drought and humidity stress, abscisic acid (ABA) is a critical factor to control stomatal conductance (Grondin et al. 2015). After discovering that ABA treatment increased CO content catalysed by the reaction of HO, researchers looked into the link of CO mediated stomatal movement. Further findings revealed that hematin and CO could enhance CO release and stomatal closure in relation with time and concentration (Cao et al. 2007a). CO has stomatal movement effects like NO and H_2O_2 (She and Song 2008; Song 2008).

15.5 CO Orchestrates Root Development and Signaling in a Normal and Challenging Environment

15.5.1 Lateral and Adventitious Rooting Mediated by CO

Lateral root (LR) is formed during the pericycle of the parent root when mature cells are driven to dedifferentiate and proliferate to produce an LR primordium, which eventually leads to LR emergence. LR is critical for the formation of the plant root network, necessary for growth and establishment (Guo et al. 2008). The development of LR has been demonstrated to be induced by CO. Hematin promotes the overall LR proliferation in rapeseed seedlings in a dose-dependent manner, but the favourable effects were completely reversed by the scavenger haemoglobin (Hb) or inhibitor zinc protoporphyrin-IX (ZnPPIX) (Cao et al. 2007a). Exogenous CO increased the expression and quantity of heme oxygenase-1 (LeHO-1) proteins, which accelerated the growth of LR in tomato (Guo et al. 2008).

Adventitious root (AR) formation is a vital phase in vegetative propagation that includes re-establishing meristem tissue in explants (Liao et al. 2012). Multiple endogenous and exogenous variables influence AR production due to the action of IAA (Xuan et al. 2008). CO showed favourable effects on AR development in mung bean seedlings. CO enhanced AR proliferation in low-IAA containing seedlings of cucumber. According to Xuan et al. (2008), during AR, specific genes (*CSDNAJ-1* and *CSCDPK1/5*) are expressed. AR formation by methane-rich water (MRW) was also shown to be inhibited by ZnPPIX (Cui et al. 2015). Furthermore, CO may promote AR formation in IAA-depleted seedlings by upregulating NO production (Xuan et al. 2012). Endogenous HO-1 has also been implicated in the production of AR in cucumber explants caused by hydrogen-rich water (HRW) (Lin et al. 2014).

15.5.2 CO and NO-Mediated Pathways Involve HO Signaling in Roots

CO resembles structural and chemical similarities to NO, which is also a diatomic gas. The two gaseous molecules reveal similar molecular masses (30.01 and 28.01), and also in physic-chemical properties like water solubility and bond lengths (Hartsfield 2002). Although the two gases have some properties in common, differences exist in their potential for redox reaction and affinity to various metals.

NO can form stable NO-metal complexes by forming nitrosonium ion produced by the virtue of its free electron (Hartsfield 2002). However, CO does not possess any free electrons (Hartsfield 2002). Although NO has a higher affinity to heme proteins, it is also known to facilitate CO in binding to heme proteins. On the other way around, in animal systems CO regulates endogenous NO distribution in the cells and, therefore, participates in amplifying the NO-mediated signaling responses (Piantadosi 2002). It is interesting to know that evidence in the animal system has revealed the presence of associative crosstalk between NO-NOS and CO-HO components.

Thus, presumptive evidence in both animal and plant systems reveals that NO-CO crosstalk is an important component of NO signaling in cells. CO in plant cells is known to provide a similar response to that of NO where dose-dependent effects have been reported. The effects of CO have been reported in various instances like regulation of stomatal movement (Song et al. 2008) and ion homeostasis (Xie et al. 2008). An interaction between CO and NO alleviates stress-induced radicle emergence in *Triticum* sp. (Liu et al. 2010). Similarly, NO activity controls expression of HO transcripts in *Glycine max* (Santa-Cruz et al. 2010). A commonly used CO donor (hemin) instigates NO signaling as a downward response to auxin regulated AR formation (Xuan et al. 2012). Similarly, a CO-mediated increase in NO accumulation has been observed in the roots of salt-stressed wheat seedlings (Xie et al. 2008). CO signaling, therefore, operates through NO/cGMP-dependent routes in plant roots subjected to challenging environments. Instances of nutrient deprivation like iron deficiency have been known to be mitigated by the combined effect of CO and NO in Arabidopsis roots (Kong et al. 2010). CO exerts growth promoting effects associated with lateral and adventitious rooting. LR emergence is a precise signaling event during the formation of pericycle which in turn produces the LR primordia. Furthermore, phenotypic plasticity in roots is associated with the regulation of LR initiation and its proliferation (Guo et al. 2008). Reports suggest that CO in aqueous and hemin-mediated forms can induce LR and AR formation. Pharmacological studies with CO scavengers (Hb and ZnPPIX) can reverse the effects of CO on root growth (Cao et al. 2007b).

The enzymatic pathway of CO generation in plants is accomplished mainly by the activity of HO. Expression of tomato heme oxygenase-1 (LeHO-1) has been associated with CO-mediated lateral root formation and proliferation of root hair (Guo et al. 2008). CO-mediated regulation of AR formation is orchestrated by the activity of various other biomolecules and gasotransmitters namely NO and H_2S. Reports suggest that cucumber plants under low IAA show AR formation triggered by CO which is also accompanied by increased expression of genes, namely *DNAJ-1* and *CDPK1/5* (Xuan et al. 2008). CO-induced AR formation is possible by the addition of methane-rich water to plants. Pharmacological analysis by use of specific CO-inhibitors like ZnPPIX indicates that CO participates in signaling during LR emergence and its growth (Cui et al. 2015). Methane water-induced CO regulates adventitious rooting mediated by the signaling of HO/CO and Ca^{2+} pathways. Furthermore, reports suggest that auxin signaling and cell cycle regulation appear to be crucial components in the molecular pathway of CO-dependent AR formation. Earlier evidence suggests the role of HO1/CO system in root development in higher plants where temporal regulation of HO1 expression is crucial for rooting response (Xuan et al. 2008; Cui et al. 2015). Contrastingly, CO can alter the responses of *Arabidopsis nia1/2/noa1* mutants during salinity stress, thus modulating the antioxidative defense mechanisms (Xie et al. 2013).

Reports indicate the role of CO-regulated AR formation (methane-rich water) and Ca^{2+}-CaM signaling. CO promotes endogenous NO production during AR formation in seedlings under low IAA levels (Xuan et al. 2012). Similarly, hydrogen water-induced adventitious root formation includes association of high activity of HO-1 isoform. Both NO and CO signaling appears to exert mutualistic and positive effects during adventitious rooting which is associated with a surge in HO activity. Chen et al. (2017) have reported H_2-CO interaction in mitigating drought conditions and promoting AR development. It is, however, important to know that the concentration of CO donors (hematin and aqueous CO) promoted AR formation, but appeared inhibitory at the higher concentrations. The effect of H_2/CO interaction on AR formation was altered by treatment with Hb or ZnPPIX. Stress ameliorating roles of CO has been affirmed in the roots of *Medicago sativa* where improvement in glutathione (GSH) has been observed in Cd-stressed plants (Han et al. 2008). In this context, exogenous CO exerts regulatory effects on enzymes of the GSH metabolism pathway and subsequently modulates the GSH:GSSG (reduced:oxidized glutathione) ratio. A positive correlation has been obtained between CO-mediated Cd stress amelioration and an increase in *HO 1* transcripts. Mung bean hypocotyls also exhibit promoting effects of CO on AR formation (Xu et al. 2006). Similarly, alfalfa plants show osmotic tolerance associated with H_2-mediated AR initiation and an increase in HO 1 (Jin et al. 2016). H_2 and CO in combination promote adventitious rooting (Lin et al. 2014). Improvement in root proliferation by application of CO has been observed during Hg toxicity in *Brassica juncea* plants (Meng et al. 2011). Similarly, CO is involved in the modulation of root growth and signaling in tobacco plants subjected to heat stress (Cheng et al. 2018).

The association of NO in the CO signaling pathway in plants mostly seems to be functioning as a downstream component (Xie et al. 2008). During salinity stress in roots of wheat seedlings, NO signal is associated with regulation of ion homeostasis. Application of both exogenous CO and NO donors (aqueous CO, sodium nitroprusside (SNP), and diethylenetriamine-nitric oxide (DETANO)) provides a similar response to that of CO signaling. Thus, wheat seedling roots subjected to salt stress (150 mM NaCl) show precise interaction of CO and NO in mediating resilience in moderately tolerant varieties (Xie et al. 2008). Thus, in this context, endogenous CO production in the roots corroborates with an increase in HO activity. Furthermore, exogenous application of CO induced a higher accumulation of NO in the root apical meristems of roots. Thus, our current understanding of CO-NO crosstalk can be summarized as events of root signaling, antioxidative defense, primordia generation, and phenotypic plasticity in primary, lateral, and adventitious roots under normal and challenging environments.

15.6 Does H_2S-NO Crosstalk Mimic CO Signaling in Roots?

H_2S in plants appears to be involved in signaling and crosstalk with the other two gasotransmitters, namely NO and CO. H_2S metabolism in plant cells shows spatial-temporal regulation and is related to sulfide signaling in the cytosol and organelles like chloroplast and mitochondria (García et al. 2015). Pieces of evidence suggest that in certain cases H_2S functions similarly to NO (Kolluru et al. 2015). H_2S signaling can modulate the activity of proteins by carrying out protein persulfidation which in turn modulates metabolism (Aroca et al. 2015). Persulfides are low molecular weight signaling molecules that regulate cysteine and sulfide homeostasis in plants (Kimura et al. 2017). Unlike CO, NO and H_2S are represented by the families of reactive nitrogen and sulphur species (RNS and RSS, respectively).

H_2S controls primary, lateral, and adventitious roots in a variety of plants in normal and stressed situations. Hypoxia prevents the amount of the root and shoot system, resulting in a reduction in agricultural yield. H_2S biosynthesis is known to be induced in cells during hypoxia (Cheng et al. 2013). NaHS (a H_2S donor) supplementation relieved Al toxicity-induced suppression of root length in *Hordeum vulgare* L., with a subsequent reduction in Al levels in seedlings thus suggesting redox homeostasis (Chen et al. 2013). Similar benefits have been discovered in the case of Zn poisoning (Liu et al. 2016). NaHS treatment to strawberry roots increased H_2S production in leaves (Christou et al. 2013). In *Medicago sativa* (Wang et al. 2012) and *Arabidopsis*, NaHS restored ion homeostasis (Li et al. 2014). The control of Na^+ transport, membrane potential, and H_2O_2 activity are parts of the H_2S signaling system. In heat-stressed strawberry roots, H_2S treatment induced gene expression of heat shock proteins and aquaporins (Christou et al. 2014). H_2S treatment in roots transcriptionally integrates a dynamic signaling network of heat shock protection pathways. Interestingly, H_2S reduced tip damage in roots of pea plants by decreasing ethylene synthesis in hypoxia-stressed roots (Cheng et al. 2013). During heat stress, maize roots exposed to H_2S showed a reduction in electrolytic leakage (Li et al. 2013). During adventitious rooting, H_2S regulated IAA and NO-signaling as a upstream regulator (Zhang et al. 2009). H_2S-facilitated adventitious rooting and its association with CO requires HO-1 activity (Lin et al. 2012). Hydroponic culture of *Fragaria ananassa* cv. Camarosa roots were treated with NaHS for 48 h and demonstrated tolerance to salinity. Various stress priming molecules in plant aerial organs were used to investigate the stress priming effects of H_2S (Christou et al. 2013). By reducing the K^+ outward-rectifying channel genes in alfalfa roots, NaHS therapy ideally maintained K^+/Na^+ equilibrium (Lai et al. 2014). In salt-stressed barley, a similar mechanism operates. NaHS controlled Na^+ build-up in salt-stressed barley seedling roots by upregulating H^+-ATPase, H^+-ATPase subunit, and the vacuolar Na^+/H^+ antiporter (Chen et al. 2015). H_2S also induced restriction of primary root proliferation, accompanied by the ROS-NO pathway (Zhang et al. 2017). H_2S

toxicity in Arabidopsis roots results in the production of ROS and NO, which in turn activates MAPK6. In *Arabidopsis*, high levels of NaHS reduced primary root length via a NO-dependent mechanism. The test's malleability was determined by using the NOS inhibitor NG-nitro-L-Arg-methyl ester (L-NAME) or the NO scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO) to ameliorate H_2S-facilitated PR growth inhibition.

Although CO-NO interaction has some pieces of evidence, not much information is available for CO-H_2S signaling during root development. Figure 15.1 shows summarized evidence of the roles of NO and H_2S in mediating CO signaling in roots. Although persuasive at present, evidence shows that H_2S can alter the activity of heme oxygenase and thus participates in NO signaling cascades. On the other way around, CO-H_2S signaling can function as a canonical pathway to NO-mediated effects in plant root development and signaling. However, concrete evidence is still required to decipher the precise role of CO and H_2S in regulating NO signaling during root development.

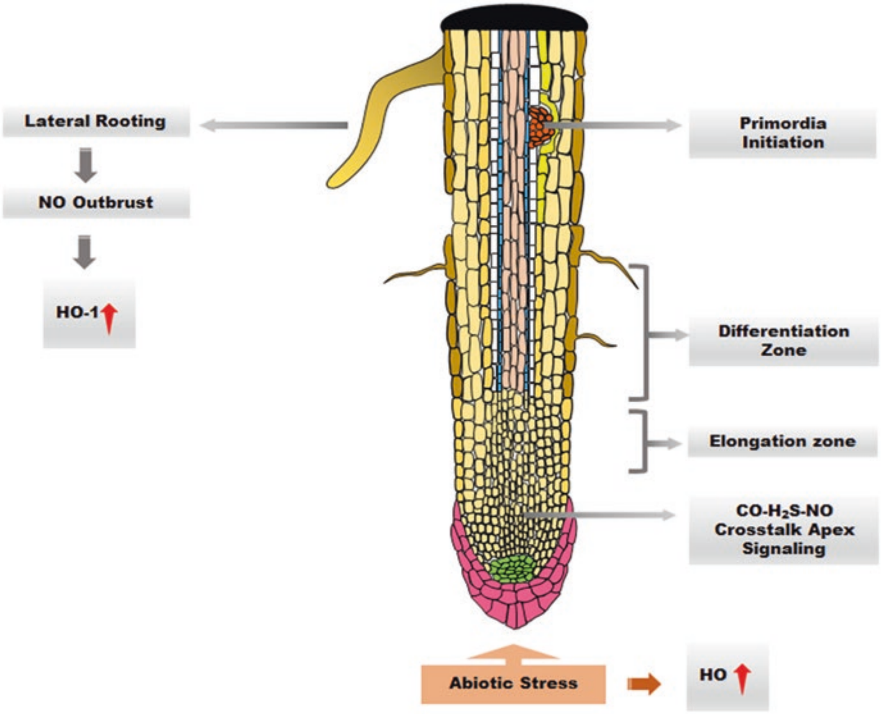

Fig. 15.1 The associative roles of NO and H_2S in mediating CO-induced regulation of root differentiation, growth and signalling. *HO* heme oxygenase

15.7 Rhizospheric CO Generation and Root Phenotyping: Future Perspectives

More investigations are necessary to explain the importance of wetlands in regulating CO levels in the root zones. Algal development in marshy, well-lit areas appear to be good source of CO generation in the rhizosphere (King 2000). Degradation of organic matter by bacteria may potentially contribute to accumulation of CO levels in soils. Rhizosphere CO is expected to be devoured by a variety of bacteria, including aerobic CO oxidizers, methane/ammonia oxidizers, and anaerobes including acetogenins, methanogens, and sulfate reducers (Ragsdale 2004; King 2006). Modulation of CO levels in saline marshy soils suggests that lithotrophic and heterotrophic bacteria produce and oxidize CO at the same time (King 2006). CO has been reported to be consumed by soybean and corn roots during incubation at 100 ppm concentrations. When the roots were incubated with CO concentrations in the air, they also emitted CO (King and Crosby 2002). Cultivated soils absorb CO at a rate of 3–6 mg CO m^{-2} per day (King and Crosby 2002).

Root-soil interfaces at forest and grazing environments have different CO consumption capacities. The microbial makeup of the soil *in situ* has an impact on rates of CO exchange (King and Crosby 2002). CO exchange in plant roots is influenced by organic content in soil, and CO-oxidizing bacteria. Wetland CO emissions are regulated by rhizosphere CO consumption by macrophyte roots, according to a kinetic study (Rich and King 1998). Furthermore, for different plant species, the amount and diversity of CO-oxidizing bacteria in the rhizosphere vary. Soil humic compounds play a big role in rhizosphere CO generation (Rich and King 1998). CO interaction among soil, microorganisms, and plant roots is elucidated by bacteria which are capable of oxidizing CO in the rhizosphere (Rich and King 1998). The oxidation of rhizosphere carbon is largely regulated by root respiration, which increases at higher temperatures. Plant roots have been seen to consume CO regardless of nitrate or NO levels. High CO uptake was observed in root-soil interface with high organic content, suggesting that plant succession plays a key role in CO dynamics (King and Weber 2008). The assembly of CO-oxidizer bacteria is influenced by the environment. Some of the most prevalent CO-oxidizers in the rhizosphere are *Carboxydothermus* sp., as well as members of the Rhizobiaceae and Burkholderia (King and Weber 2007).

Thus, it is important to undertake future investigations on the role of rhizospheric CO in the regulation of root growth, phenotypic plasticity, and nutrient acquisition. Marshy wetlands and mangrove soils also show seasonal variations in NO and H_2S emissions. Thus, it would be interesting to decipher the integrative role of these gaseous molecules in the regulation of humus composition, rhizospheric microflora, and their subsequent impact on root growth and signaling. As a result, biochemical and molecular research is needed to unravel the complicated mechanism of NO, CO, and H_2S interaction in the rhizosphere, which is thought to play a role in soil fertility, plant growth, and yield of the crop. Rhizobiology will gain a new dimension concerning NO, CO, and H_2S crosstalk which may appear to be advantageous in crop management, especially in arid areas of cultivation.

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