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REVIEW PAPER

Plant gasotransmitters: light molecules interplaying with heavy metals

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Abstract The past decades have witnessed the discovery of several gaseous signaling molecules in plants, including nitric oxide (NO), carbon monoxide (CO), hydrogen sulfide (H_2S), and methane (CH_4). These gasotransmitters (GTs) are endogenously synthesized in plant cells and participate in a variety of developmental processes and stress responses. Heavy metals (HMs) are one of the most widespread environmental cues that cause hazardous effects in plants and raise wide safety concerns. The involvement of GTs in the plant responses to HM has been demonstrated; however, our understanding of their exact roles and mechanisms in this area remains very fragmented. In this review, we provide an overview of the recent advances in the biosynthesis and regulation of each of the four GTs, their roles and mechanisms in plant HM uptake, accumulation, and detoxification, and the crosstalk between various GTs related to HM. Some reports on the negative roles of GTs on plant HM responses are also discussed. Certain important future directions for more in-depth studies are proposed based on the current understanding. Overall, this review provides a collection of rare information that helps to elucidate the regulation and functions of GTs in plant HM responses. This subject is of significant importance in the strategies of the agricultural efforts to reduce the risks associated with HMs by manipulating GTs.

Keywords Gasotransmitter · Nitric oxide · Carbon monoxide · Hydrogen sulfide · Methane · Heavy metal

Abbreviations

AACT	Al-activated citrate transporter
ABC	ATP-binding cassette
ALMT1	Al-activated malate transporter 1
APX	Ascorbate peroxidase
AR	Adventitious root
AsA	Ascorbic acid
CA	Carbonic anhydrase
CAS	Cyanoalanine synthase
CAT	Catalase
CDPKs	Calcium-dependent protein kinases
cGMP	Cyclic guanosine monophosphate
CH_4	Methane
CO_2	Carbon dioxide
CO	Carbon monoxide
cPTIO	2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxy-3-oxide
CS	Cysteine synthase
D-DES/DCD	D-cysteine desulfhydrase
DHAR	Dehydroascorbate reductase
FRO2	Ferric reduction oxidase
GA	Gibberellin

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GPX	Glutathione peroxidase
GR	Glutathione reductase
GSH	Glutathione
GSNO	*S*-nitrosoglutathione
GSNOR	*S*-nitrosoglutathione reductase
GST	Glutathione-*S*-transferase
GT	Gasotransmitter
H_2S	Hydrogen sulfide
HT	Hypotaurine
HMA2/4	Heavy metal ATPase 2/4
HM	Heavy metal
HO	Heme oxygenase
H_2O_2	Hydrogen peroxide
HY1	Heme oxygenase1, HO-1
IRT1/2	Iron-regulated transporter 1/2
L-DES/ LCD	L-cysteine desulfhydrase
L-NAME	L-NG-nitro arginine methyl ester
LR	Lateral root
MDA	Malondialdehyde
MDH1/2	Malate dehydrogenase 1/2
MDHAR	Monodehydroascorbate reductase
MPK6	Mitogen-activated protein kinase 6
MT	Metallothionein
NADPH	Nicotinamide-adenine dinucleotide phosphate
NaHS	Sodium hydrosulfide hydrate
NO	Nitric oxide
NOS	Nitric oxide synthesis
NRAMP	Natural resistance-associated macrophage protein
NR	Nitrate reductase
OAS	O-acetyl-L-serine
PCD	Programmed cell death
PC	Phytochelatin
PME	Pectin methyl esterase
POD	Peroxidase
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SAT1	Serine acetyltransferase 1
SiR	Sulfite reductase
SNP	Sodium nitroprusside
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive substances
UV	Ultraviolet radiation
ZIP	Zinc-regulated transporter

1 Introduction

The term 'heavy metal' (HM) generally refers to metals with a density higher than 4–5 g cm^{-3}, such as cadmium (Cd), copper (Cu), zinc (Zn), nickel (Ni), cobalt (Co), chromium (Cr), lead (Pb), and mercury (Hg) (Arao et al. 2010). Due to the wide use of chemical fertilizers and sewage sludge in agriculture, inadequate irrigation, deployment of mining industry, and poor industrial waste disposal, agricultural lands in many areas of the world are contaminated by HMs (Mahar et al. 2016; Toth et al. 2016). Some of the HMs, including Co, Cu, iron (Fe), manganese (Mn), molybdenum (Mo), Ni, and Zn, are necessary for the normal growth of plants; however, HM concentrations must be strictly maintained below the threshold values to avoid toxicity. Other HMs, namely, Cd, Hg, and Pb, are nonessential and highly toxic to plants (Rascio and Izzo 2011). The cellular mechanisms of HM toxicity to plants include oxidative stress inducing free radicals and/or functional disruption of key enzymes due to the replacement of essential metals; HM stress typically causes attenuated plant growth and lower yield (Guala et al. 2010). In addition to harming plant growth, HMs accumulated in plant tissues can enter the food chain and threaten human health due to bioaccumulation.

To cope with the harmful HM concentrations in the soil, the activation and coordination of a complex signaling network in plants is required. Players of this network include reactive oxygen species (ROS), calcium–calmodulin system, phosphorylation cascades, hormones, and other emerging signal transmitters, such as gasotransmitters (GTs) (Dalcorso et al. 2010; Lamattina and García-Mata 2016). GTs are a class of signal molecules that meet the following criteria: (i) they are small gas molecules; (ii) they can freely cross biological membranes independent of cognate membrane receptors; (iii) they are endogenously generated by specific enzymes; (iv) their functions can be mimicked by exogenous application of donors; and (v) they have specific cellular and molecular targets, but their effects do not necessarily rely on secondary messengers (Wang 2002; García-Mata and Lamattina 2013). Four GTs, namely, nitric oxide (NO), carbon monoxide (CO), hydrogen sulfide (H_2S), and methane (CH_4), have been recently reported to be regulated by HM stimuli, and exogenous application of their donors alters cellular HM accumulation, redox status, ion homeostasis and photosynthesis (Han et al. 2008; Gill et al. 2013; Han et al. 2014; Kaur et al. 2015; Mostofa et al. 2015; Cui et al. 2017; Samma et al. 2017; Yu et al. 2019). Numerous review articles have described the biology of plant abiotic stress related to HM; however, there are no comprehensive reviews on the GT-HM relationship in plants. To address this gap in knowledge, we summarize the recent research progress on the biosynthesis, regulation, biochemical function and signal transduction of GTs in responses to HM stress in plants. Specifically, we provide a systematic view on the mechanisms that underpin GTs-regulated HM stress tolerance and point out their unique features. Furthermore, the crosstalk between different GTs and their roles in the interaction with HM are analyzed and discussed. Therefore, our review provides insight into the interplay between plant HM tolerance and GTs and proposes important future directions for additional in-depth studies to understand the functions of GTs in HM stress tolerance.

2 Functions of gasotransmitters in response to heavy metals in plants

2.1 Nitric oxide

2.1.1 General information, biochemical characteristics and biosynthesis

NO is naturally released as a toxic compound from industrial waste, vehicle exhaust and cigarette smoke. NO has been recognized as a GT mediating the vasodilatory effect of a number of factors in animal cells since the 1980s (Furchgott and Zawadzki 1980; Garthwaite et al. 1988; Schmidt and Walter 1994). The signaling functions of NO in plants were defined almost two decades later. In higher plants, NO can be produced by the reductive pathways or the oxidative pathways (Table 1, Cooney et al. 1994; Rockel 2002; Tun et al. 2006; Corpas et al. 2009). Cytosol-localized Nitrate reductase (NR), plasma membrane-bound nitrite-NO-reductase (NiNOR), and xanthine oxidoreductase (XOR) that is associated with peroxisomes are the key enzymes involved in the reductive pathways (Rockel 2002; Corpas et al. 2008; Neill et al. 2008). In addition, cytochrome-c-oxidase involved in the electron-transport chain of mitochondria also can reduce nitrite to produce NO (Farnese et al. 2016). The polyamine, L-arginine and hydroxylamine have been considered as the substrates for the synthesis of NO in the oxidative routes (Tun et al. 2006; Corpas et al. 2009; Rumer et al. 2009). It is assumed that polyamine oxidase and NO synthase (NOS) play roles in these oxidative transformations (Corpas et al. 2009; Farnese et al. 2016). However, the mechanisms underlying these oxidation reactions remain largely unclear (Shivaraj et al. 2020). Nonenzymatic systems for NO synthesis have also been reported, which include the ascorbic acid-, carotenoid- and nitrification-denitrification cycle-mediated pathways (Cooney et al. 1994; Wojtaszek 2000; Tun et al. 2006).

2.1.2 Regulation

Endogenous NO levels in plants can rapidly respond to many hormonal and stress stimuli implying fundamental functions of this GT in plants (Gupta et al. 2011; Shi et al. 2012; Gill et al. 2013). Pharmacological studies using an inhibitor of mammalian NO synthase (NOS) [L-NG-nitro arginine methyl ester (L-NAME)], NR inhibitor (tungsten), NO donors [S-nitrosoglutathione (GSNO), sodium nitroprusside (SNP) and *S*-nitroso-N-acetyl-D-penicillamine (SNAP)], and NO scavenger [2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxy-3-oxide (cPTIO)] (Shi et al. 2012; Cerana and Malerba 2015; Yang et al. 2016; Kaya et al. 2020), together with several lines of genetic evidence, have further supported the role of NO as an important signaling element in diverse physiological processes including HM response. However, the regulation of *in vivo* NO content by HMs appears to be concentration and species dependent (Table 2; Fig. 1, Bartha et al. 2005; Rodríguez-Serrano et al. 2009; Leterrier et al. 2012; Cerana and Malerba 2015).

In wheat (*Triticum aestivum*) roots, Pb treatment at a concentration of 250 μM dramatically decreased the endogenous NO level, and exposure to 50 μM Pb slightly decreased the root NO level (Kaur et al. 2015). Treatment of *Pogonatherum crinitum* root cells with Pb at a concentration of 100 μM immediately induced the NR-dependent NO burst (Yu et al. 2012). NR-mediated early NO production was also observed in the shoots of hulless barley (*Hordeum vulgare*) upon treatment with a high concentration of Cu (450 μM) (Hu et al. 2015). In soybean (*Glycine max*) roots, Cd-induced generation of NO was also accompanied with

Table 1 Substrates and enzymes involved in biosynthesis of plant gasotransmitters

GT	Substrates	Enzymes of synthesis	Location	References
NO	Nitrite	Nitrate reductase	Cyt	Rockel (2002)
	Nitrite	Nitrite-NO-reductase	Pla	Neill et al. (2008)
	Nitrite	Xanthine oxidoreductase	Per	Corpas et al. (2008)
	Nitrite	Cytochrome-c-oxidoreductase	Mit	Farnese et al. (2016)
	Polyamines	Polyamine oxidase**?**	**?**	Tun et al. (2006)
	L-arginine	NOS-type enzyme	Chl, Mit, Per	Corpas et al. (2009), Farnese et al. (2016)
	Hydroxylamine	**?**	**?**	Rumer et al. (2009)
CO	Heme	Heme oxygenase	Chl	Xuan et al. (2008a)
H_2S	L-cysteine	L-cysteine desulfhydrase	Pla, Mit	Álvarez et al. (2010), Romero et al. (2013)
	D-cysteine	D-cysteine desulfhydrase	Cyt	Riemenschneider et al. (2005)
	L-cysteine	Cyanoalanine synthase	Mit	Akopyan et al. (1975)
	L-cysteine and acetate	Cysteine synthase	Chl, Mit, Cyt	Wirtz and Hell (2006)
	Carbonyl sulfide	Carbonic anhydrase	Chl, Cyt	Notni et al. (2007), Rudenko et al. (2015)
	Sulfite	Sulfite reductase	Chl	Gotor et al. (2019)
CH_4	Pectin**?**	**?**	Mit**?**	Li et al. (2019)

GT gasotransmitter, *NO* nitric oxide, *CO* carbon monoxide, *H_2S* hydrogen sulfide, *CH_4* methane, *Cyt* cytosol, *Pla* plasmalemma, *Per* peroxisomes, *Mit* mitochondria. Question mark: unconfirmed

the induction of NR activity (Perez-Chaca et al. 2014). In Arabidopsis (*Arabidopsis thaliana*), Cd-induced NO synthesis was suggested to be catalyzed by an as yet unidentified NOS-like enzyme (De Michele et al. 2009).While Cd treatment induces NO in the majority of investigated plant species, including Arabidopsis, rice (*Oryza sativa*), *Barassica juncea*, wheat, bermudagrass (*Cynodon dactylon*), and soybean (Besson-Bard et al. 2009; De Michele et al. 2009; Verma et al. 2013; Perez-Chaca et al. 2014; Shi et al. 2014; Yang et al. 2016; Kaya et al. 2020), inhibition of NO generation was reported in pea (*Pisum sativum*) in both root and leaf tissues after exposure to 50 µM Cd for 14 days (Rodríguez-Serrano et al. 2006, 2009). In addition, Akinyemi et al. (2017) reported a significant decrease of NO level in maize (*Zea mays*) plants accompanied by lowered content of total phenolics and glutathione (GSH) after 5 ppm Cd treatment. Al treatment at the concentration of 100 µM could inhibit NOS-mediated endogenous NO generation in *Hibiscus moscheutos* (Tian et al. 2007), while a release of NR-dependent NO was detected in *Phaseolus vulgaris* root after exposure to 50 µM Al for 24 h (Wang et al. 2010a). Excessive NO accumulation induced by Zn in *Solanum nigrum* roots was almost completely inhibited by a NOS inhibitor named L-NAME (Xu et al. 2010b). Taken together, these results indicate a complex regulation and function of NO in HM tolerance in plants.

2.1.3 Cytotoxic and cytoprotective roles

NO is a signal molecule and a member of the reactive nitrogen species (RNS); however, whether NO plays a cytotoxic or cytoprotective role under HM stress is controversial (Fig. 2a). On the one hand, excessive accumulation of NO or NO-derived RNS, including peroxynitrite ($ONOO^-$), dinitrogen trioxide (N_2O_3) and GSNO, may induce nitrosative stress that can damage DNA, lipids, proteins, and carbohydrates leading to impaired cellular functions (Corpas et al. 2007; Liu et al. 2018). For example, NO contributes to Zn toxicity in the primary root tips of *Solanum nigrum* by increasing ROS accumulation and promoting programmed cell death (PCD) (Xu et al. 2010b). NO aggravates Cd-induced PCD by promoting mitogen-activated protein kinase 6 (MPK6)-mediated caspase-like activation, modulating the concentration and function of phytochelatin (PC) through protein *S*-nitrosylation or regulation of iron deprivation response

Table 2 Regulation of endogenous gasotransmitters by heavy metals in plants

Heavy metal	Plant species	Tissue	Collection time	Concentration (μM)	Gas level	Regulatory mechanism	References
Cd	*Arapidopsis thaliana*	Hypocotyl cell	Within 72 h	50, 100, 150	NO↑	Mediated by NOS-like enzyme	De Michele et al. (2009)
	Arapidopsis thaliana	Root, leaf	7 h for root, 96 h for leaf	50, 200	NO↑	Cd-induced iron deficiency	Besson-Bard et al. (2009)
	Barassica juncea	Root	96 h	5, 10, 25, 50, 75, 100	NO↑	Not mentioned	Verma et al. (2013)
	Glycine max	Root	6, 24, 72, 144 h	40	NO↑	Upregulation of NR activity	Perez-Chaca et al. (2014)
	Pisum sativum	Root	24 h	50	NO↓	Mainly regulation of NOS activity	Rodríguez-Serrano et al. (2006)
	Cynodon dactylon	Whole plant	12, 24, 48 h	100, 250, 500, 750, 1000, 2000, 5000	NO↑	Not mentioned	Shi et al. (2014)
	Oryza sativa	Root	6 h	10	NO↑	Not mentioned	Yang et al. (2016)
	Triticum aestivum	Leaf	4 weeks	100	NO↑	Not mentioned	Kaya et al. (2020)
	Medicago sativa	Root	3 d	100, 200	CO↑	Upregulation of *HO-1*	Han et al. (2008)
	Medicago sativa	Root	6 h	1, 10, 100, 200, 500	CO?	GSH depletion induced-*HO-1* expression	Cui et al. (2011)
	Arabidopsis thaliana	Whole plant	120 h	100	H_2S↑	Induction of LCD activity that raised by CDPKs	Qiao et al. (2015)
	Brassica napus	Root	7 d	20	H_2S↓	Inhibition of LCD activity	Yu et al. (2019)
	Brassica rapa	Root	72 h	10	H_2S↑	Upregulation activities of *LCD* and *DCD*	Hu et al. (2018)
	Salix matsudana	Leaf, root	60 d	5,10, 30	H_2S↑	Not mentioned	Yang et al. (2018)
	Oryza sativa	Leaf	3 d	250, 500	H_2S↑	Not mentioned	Mostofa et al. (2015)
	Medicago sativa	Root	24, 48, 72 h	100	CH_4↑	Not mentioned	Gu et al. (2018)
Cu	*Hordeum vulgare*	Shoot	6, 12, 24, 48, 72, 96 h	450	NO↑	Dependance on NR activity	Hu et al. (2015)
	Medicago sativa	Generating Seed	72 h	1500	CH_4↑	Not mentioned	Samma et al. (2017)
Al	*Hibiscus moscheutos*	Root	20 min	100	NO↓	Inhibition of NOS activity	Tian et al. (2007)
	Phaseolus vulgaris	Root	24 h	50	NO↑	Dependence on NR activity	Wang et al. (2010a)
Al	*Triticum aestivum*	Generating seed	Within 48 h	30,000	H_2S↑	Not mentioned	Zhang et al. (2010b)
	Medicago sativa	Root	12 h	150	CH_4↑	Not mentioned	Cui et al. (2017)

Table 2 continued

Heavy metal	Plant species	Tissue	Collection time	Concentration (μM)	Gas level	Regulatory mechanism	References
Pb	*Pogonatherum crinitum*	Root cell	40 min	100	NO↑	Upregulation of NR activity	Yu et al. (2012)
	Triticum aestivum	Root	2, 4, 6, 8 h	50, 250	NO↓	Not mentioned	Kaur et al. (2015)
	Capsicum annuum	Leaf	2 weeks	100	H_2S↑	Not mentioned	Kaya (2020)
Zn	*Solanum nigrum*	Root	Within 10 d	200, 400	NO↑	NOS-like enzyme**?**	Xu et al. (2010b)
	Solanum nigrum	Leaf, root	5 d	400	H_2S↑	Enhancement of LCD activity	Liu et al. (2016)
Fe	*Lotus japonicus*	Nodule	3 h	300	NO↑	Not mentioned	Chungopast et al. (2017)
Hg	Brassica napus	Root	12 h	10, 20, 40	CO**?**	Upregulation of *HO-1*	Shen et al. (2011)
Ni	*Oryza sativa*	Root, shoot, leaf	14 d	200	H_2S↑	Not mentioned	Rizwan et al. (2019)
Cr	*Setaria italica*	Leaf	24 h	5000	H_2S↑	Upregulation expression levels of *LCD, DCD1/2, DES*	Fang et al. (2016)

in Arabidopsis (Besson-Bard et al. 2009; De Michele et al. 2009; Ye et al. 2013). Arabidopsis mutants with lower NO content in the roots were more resistant to Cd stress than WT plants due to better maintenance of the antioxidant systems (Terron-Camero et al. 2020). In pea plants, NO promoted Cd stress by inhibiting the expression and activity of *S*-nitrosoglutathione reductase (GSNOR) (Barroso et al. 2006). Collectively, these data demonstrate cytotoxic roles of the excessive NO triggered by heavy metal stress in plant cells.

On the other hand, NO can exert cellular protective functions in some cases. NO can directly or indirectly reduce ROS levels in plant cells (Saxena and Shekhawat 2013; Kaur et al. 2015). This effect is due to the antioxidant properties of NO, which can scavenge ROS, such as superoxide anion (O_2^-), to form $ONOO^-$, which is less toxic than peroxides. SNP (100 μM) supplementation counteracted Pb-induced oxidative stress by modulating malondialdehyde, conjugated dienes, hydroxyl ions, and superoxide anion but not the antioxidant enzyme activities in wheat roots thereby suggesting its role as an antioxidant (Kaur et al. 2015). Additionally, NO can indirectly reduce ROS through activation of the antioxidant systems (Saxena and Shekhawat 2013). NO supplementation enhanced the activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) in tomato under Cu stress (Wang et al. 2010b) and increased the levels of polyamine and proline in *Medicago truncatula* under Cd stress (Filippou et al. 2013). Verma et al. (2013) showed that NO alleviated Cd-induced cytotoxicity by scavenging ROS through regulation of antioxidant responses in *Brassica juncea*. GSH is known to play a role in metal chelation and sequestration and stimulates the antioxidant system by regulating the redox signals under HM stress (Hasan et al. 2016). In *Medicago truncatula*, NO induced expression of the genes regulating GSH (Xu et al. 2011). Metallothioneins (MTs) are low molecular weight cysteine-rich metal-binding proteins that detoxify plant cells under HM stress. Exogenous NO can increase the content of MTs and enhance the resistance of tomato to Cu stress. In antisense-MT transgenic tomato plants, exogenous NO failed to significantly enhance plant resistance to Cu stress indicating that MTs play a role in NO-dependent alleviation of HM toxicity (Wang et al. 2010b).

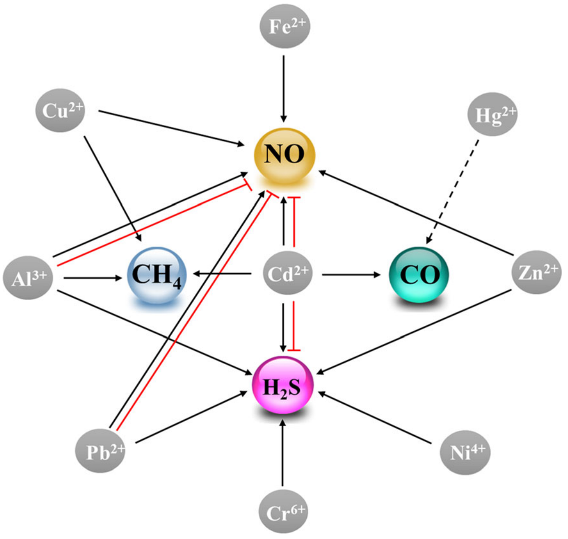


Fig. 1 Illustration of the stimulatory or inhibitory effects of different heavy metals on endogenous gastransmitters release in plants. In general, the generation of CO and CH_4 was stimulated by heavy metal treatments, and the accumulation of NO and H_2S could be triggered or inhibited by different heavy metal stimuli. In details: despite the general stimulatory effect on all four gastransmitters, Cd treatment at specific conditions inhibited the generation of NO and H_2S; Pb could induce H_2S synthesis and either inhibit or activate NO production, depending on treatment scenarios; Al could trigger H_2S and CH_4 synthesis, and it exerted a dual function on NO; Cu enhanced the synthesis of NO and CH_4; Fe could activate NO burst; Zn had a positive role on synthesis of NO and H_2S; Cr and Ni were proved to trigger H_2S generation; Hg was observed to induce gene expression of the CO synthesis enzyme, but whether it triggered CO release needs experimental confirmation. Arrowhead: activation; blunt end: inactivation

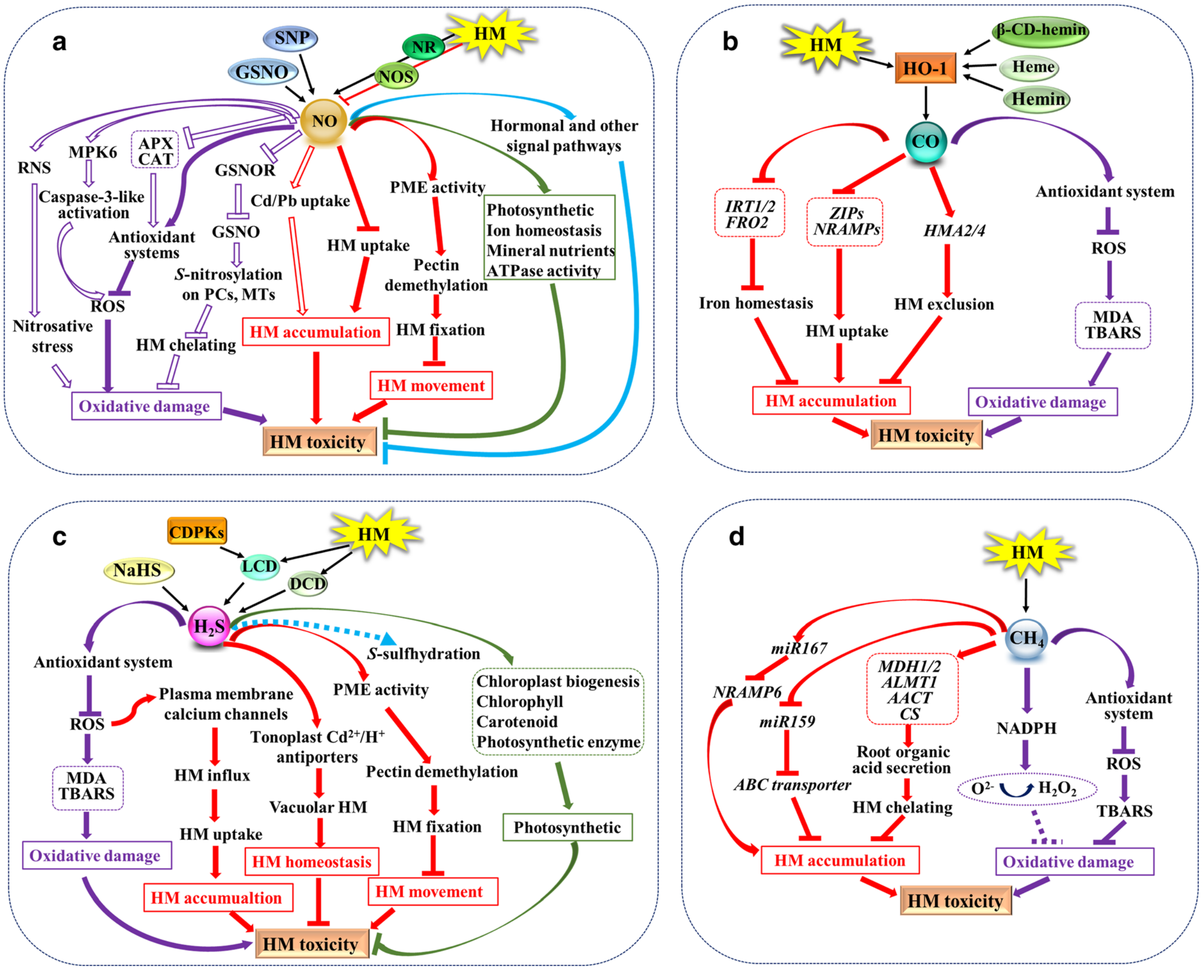
2.1.4 *Regulation of metal uptake and accumulation*

A burst in NO is involved in Pb uptake and Cd accumulation (Besson-Bard et al. 2009; Yu et al. 2012); however, exogenous NO application can reduce the absorption of Cd by alfalfa (Xu et al. 2010a), Al by *Cassia tora*. (Wang and Yang 2005), argentum (Ag) by pea (Tripathi et al. 2017) and Zn accumulation in wheat and peas (Abdel-Kader 2007). The cell wall is the first barrier blocking HM entrance into protoplast and an important site of HM storage in plants; hence, deposition of the cell wall is considered a crucial mechanism of HM tolerance (Xiong et al. 2009). Treatment with 0.1 mM SNP significantly increased pectin and hemicellulose contents but decreased cellulose content in the cell walls of rice root, suggesting a mechanism of Cd reduction in soluble fraction of leaves by promoting Cd retention in root cell walls (Xiong et al. 2009). Wheat plants treated with the NO scavenger cPTIO had decreased activity of pectin methyl esterase (PME) in the roots resulting in an increase in pectin methylation in the cell wall and a reduction in the affinity of Al binding to pectin. Consequently, cPTIO boosted root growth and reduced Al accumulation in the roots (Sun et al. 2015).

2.1.5 *Interaction of nitric oxide with hormonal signals*

NO can interact with the hormonal signals in plants. This interaction can occur at different points of hormone actions, such as biosynthesis, degradation, conjugation, distribution or signaling, at the transcriptional or posttranslational level (Freschi 2013). For example, Cd stress inhibited the growth of the primary root and induced the formation of the lateral root (LR) and adventitious root (AR). Supplementation with the SNP donor GSNO increased the number of LR and AR under Cd stress, and use of cPTIO reversed this effect (Xu et al. 2011). The authors proposed that NO acts as a messenger partly by interacting with auxin to influence LR and AR development (Xu et al. 2011). NO interaction with plant hormones may play a role in Cd-mediated inhibition of root meristem growth (Yuan and Huang 2016). Cd stabilized the indole-3-acetic acid inducible 17 (AXR3/IAA17) protein to suppress auxin signaling in Cd-mediated root meristem growth inhibition, and the role of NO was confirmed by using the NO scavenger cPTIO or NOS inhibitor L-NAME (Yuan and Huang 2016). Salicylic acid (SA) and NO were coordinately involved in protective reactions of wheat under HM stress (Gilvanova et al. 2012). NO signaling was shown to participate in gibberellin (GA)-alleviated Cd toxicity in Arabidopsis (Zhu et al. 2012).

Moreover, NO can help to maintain cellular ion homeostasis, balance the mineral nutrients, reestablish ATPase activity, and hold the photosynthetic efficiency of plants (Gong et al. 2017; Kabala et al. 2019). The mechanisms of NO-dependent alleviation of HM toxicity also include modulation of protein kinase activities and elevation of cytosolic Ca^{2+} concentration through regulating of Ca^{2+} channels/transporters. The mobilization of other secondary messengers involved in the signaling cascade of gene expression regulation, such as is the case for *cyclic guanosine*



monophosphate (*cGMP*) and *cyclic adenosine diphosphate ribose* (*cADPR*), was reported (Besson-Bard et al. 2009; Shi et al. 2014; Amooaghaie et al. 2015). The crosstalk between NO and other GTs, including CO and H_2S, is discussed in detail in the third section of this review (3. Crosstalk of GTs in response to HMs).

2.2 Carbon monoxide

2.2.1 General information, biochemical characteristics and biosynthesis

CO is a colorless and odorless gaseous molecule that is ubiquitous in nature. CO is known as a "silent killer" due to high toxicity to humans and animals. The physiological functions of CO depend on its ability to bind to heme proteins. Similar to animals, the main source of endogenous CO production in plants is heme oxygenase (HO), which catalyzes degradation of heme to produce free iron, biliverdin Ixα, and CO (Table 1, Kikuchi et al. 2005; Ryter et al. 2006; Xuan et al. 2008a). In Arabidopsis, the HO family comprises four members that fall into two subfamilies as opposed to three subfamilies in animals (Dulak and Jozkowicz 2003; Bauer et al. 2008). The HO1 subfamily includes HO-1 (also known as HY1), HO-3, and HO-4, and the HO2 subfamily consists of only HO-2 (Emborg et al. 2006). In plants, the generation of CO is closely linked to the HO-1 enzymatic source (Xuan et al. 2008a; He aand He 2014). CO release in plants was reported as early as 1959 (Wilks 1959). So far, various roles of CO in plant physiology and stress tolerance have been revealed by using CO fumigation, aqueous CO

◀**Fig. 2** Generalized model of gastransmitter signaling in response to heavy metal stresses in plants. **a** Cytotoxic and protective roles of NO in response to HMs. HMs trigger or inhibit *in vivo* NO release through up- or down-regulation of NO synthesis enzymes including NOS and NR. GSNO and SNP supplement can enhance the accumulation of NO under HM conditions. NO contributes to HM toxicity via two pathways. First, NO can induce nitrosative stress, activate MPK6-mediated Capase-3-like activation of ROS generation, reduce antioxidant capability through inhibition of APX and CAT activities, or enhance GSNOR-inhibited *S*-nitrosylation on PCs and MTs followed by a decrease of chelated HM level to enhance oxidative damage. Second, NO can promote uptake and further metal accumulation such as Cd and Pb. Beneficial effects can also be triggered by NO. NO can block HM accumulation through inhibiting HM uptake and root-to-shoot movement mediated by PEM-activated pectin demethylation. Photosynthetic, iron homeostasis, mineral nutrients and ATPase activity can be enhanced by NO signal in response to HM stress. In addition, NO can crosstalk with hormonal and other signals to alleviate HM toxicity. **b** The protective role of CO in response to HM. CO release triggered by HM or through supplementation of its donors including β-CD-hemin, heme and hemin alleviates HM toxicity through regulating HM accumulation and redox homeostasis. In relation to inhibition of HM accumulation, CO improves iron homeostasis by down-regulating *IRT1*, *IRT2* and *FRO2*, blocks HM uptake by down-regulating *ZIPs* and *NRMPs* and enhances HM exclusion by up-regulating *HMA2/4*. **c** The protective role of H_2S in response to HM. HM-regulated H_2S generation is mediated by DCD activity and CDPKs-triggered LCD activity. H_2S can activate antioxidant system to scavenge excessive ROS, leading to alleviated oxidative damage by HM. The inhibition of ROS by H_2S also lowers HM uptake and accumulation as the plasma membrane calcium channels controlling HM influx is blocked. H_2S can also promote HMs sequestration by enhancing their movement from cytosol to vacuolar, trigger PME-mediated HMs fixation to reduce their shootward movement, and improve photosynthesis. **d** The protective role of CH_4 in response to HM. CH_4 can scavenge ROS by activating antioxidant system or NADPH-dependent H_2O_2 signal to alleviate HM-induced oxidative damage. CH_4 regulates expression of *miRNA* genes, whereby affect their mRNA targets to inhibit HM accumulation. Besides, CH_4 can enhance organic acid secretion in roots by up-regulating expression of genes including *MDH1/2*, *ALMT1*, *AACT* and *CS*, leading to HM chelation. Solid line: protective pathway activated by HM. Hollow line: cytotoxic pathway triggered by HM. Dotted line: pathways need confirmation. Arrowhead: activation; blunt end: inhibition

solution, the artificial CO donors hematin and hemin, and combination of these agents with genetic approaches, e.g. transgenic overexpression of HO-1 or knockdown mutants (Han et al. 2008, 2014; Shen et al. 2011; Wei et al. 2011). However, CO has been only recently recognized as the second GT by the order of discovery after nitric oxide (Sukmansky and Reutov 2016).

2.2.2 *Regulation*

The determination of CO emission and increase in the activity of the rate-limiting enzyme HO-1 in plants upon HM treatment suggests the involvement of CO signaling in the HM stress response (Table 2; Fig. 1). Treatment with 100 μM or 200 μM Cd increased CO release in the root tissues of alfalfa as a result of upregulation of *HO-1* expression (Han et al. 2008). GSH depletion is considered a part of the mechanisms involved in the transcriptional regulation (Cui et al. 2011). Furthermore, enhanced HO-1 activity combined with an increase in the relative expression level of *HO-1* was characterized in Hg stress (Wei et al. 2011). Pretreatment of alfalfa plants with 50% CO-saturated aqueous solution for 6 h followed by Cd exposure resulted in an increase in CO and alleviation of Cd-induced oxidative damage (Han et al. 2008). Similarly, plants treated with exogenous CO and subsequently exposed to $HgCl_2$ accumulated lower amount of Hg and displayed mitigated root inhibition (Meng et al. 2011). The induction of the HO-1 transcript in the root tissue of alfalfa by hematin and β-CD-hemin protected the plants from HM-induced oxidative damage. Overexpression of *BnHO-1* in *Brassica napus* enhanced plant tolerance to Hg toxicity, indicating that HO-1-mediated CO signaling plays a cytoprotective role under HM stress (Shen et al. 2011). Moreover, HO-1-mediated CO release may function downstream of SA to alleviate Cd-induced oxidative stress in the root tissues of alfalfa seedlings (Cui et al. 2012). Collectively, the CO signal triggered by HM can protect the plants from the negative effect caused by HM.

2.2.3 *Roles in suppressing heavy metal accumulation*

The transcription and activity of HO-1 have been extensively shown to be markedly upregulated by hemin and are inducible by its own substrate, heme (Maines 1988; Xuan et al. 2008a; Hyvelin et al. 2010). Zhu et al. (2019) suggested that heme-induced amelioration of Cd toxicity in Chinese cabbage seedlings may be associated with inhibition of Cd uptake. In alfalfa seedlings, the induction of *HO-1* by β-CD-hemin and hemin pretreatment reduced Cd toxicity partly by lowering Cd accumulation (Fu et al. 2011). A similar observation was reported in *BnHO1*-overexpressing transgenic rapeseed (*Brassica napus*) plants, which accumulated less Hg per unit of biomass compared to that in wild type plants (Shen et al. 2011). The inhibitory effect of CO on HM accumulation was confirmed in algae because overexpression of *HO-1* in *Chlamydomonas reinhardtii* decreased Hg accumulation (Wei et al. 2011). Pharmacological experiments indicated that protection of the plants by CO against other HMs, including Al, Zn, Pb, and Cr, is related to suppressed metal accumulation in the tissues (Cui et al. 2013; Chen et al. 2017a, 2018).

The mechanism of CO inhibition of HM accumulation in plants remains poorly understood (Fig. 2b). It is known that Cd^{2+} uptake by the roots is mediated by low-specificity metal transporters or channels for Fe^{2+}, Zn^{2+}, and Ca^{2+} (Clemens 2006). Previous data showed that Cd^{2+} competed with Fe^{2+} in the process of absorption by inducing the transcripts of *iron-regulated transporter 1* (*IRT1*) gene and activating a Fe-starvation signal (Vert et al. 2002). Therefore, homeostasis of essential metals, especially iron, can contribute to Cd uptake and transportation. In Arabidopsis, overexpression of *HY1* (encodes HO-1) could down-regulate the genes related to Fe uptake including *ferric reduction oxidase 2* (*FRO2*) and *IRT1/2*, thus contributing to Fe homeostasis and Cd^{2+} exclusion (Han et al. 2014). The result suggests that CO regulates nonessential HM uptake partly by controlling iron homeostasis. Additionally, downregulation of the genes related to HM uptake, such as members of the *zinc-regulated transporter* (*ZIP*) family and *natural resistance-associated macrophage protein* (*NRAMP*) family, may be another mechanism of CO -dependent reduction in HM accumulation (Chen et al. 2018; Zhu et al. 2019).

2.2.4 *Functions in cytoprotection*

In addition to suppression of metal accumulation, cytoprotection is an important mechanism involved in CO-mediated HM stress tolerance (Fig. 2b). First, CO can regulate the activities of antioxidant enzymes, including glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), POD and SOD, leading to the scavenging of overproduced ROS induced by HM (Han et al. 2007; Shen et al. 2011). Treatment of HM-stressed rice plants with hemin, a HO-1 inducer, dramatically stimulated SOD, ascorbate peroxidase (APX) and GR activities and increased the content of ascorbic acid (AsA) and GSH, as opposed to the non-treated control (Chen et al. 2017a). Second, endogenous release of CO or upregulation of HO-1 can alleviate HM-induced oxidative damage by reestablishing GSH homeostasis via regulation of the enzymes responsible for GSH metabolism (Han et al. 2008; Fu et al. 2011). Third, nonenzymatic antioxidants, such as proline, have been shown to be involved in CO-dependent HM detoxification (Meng et al. 2011). Moreover, the HO-1/CO system can protect plants from HM-induced oxidative injury by improving accumulation of pigments including chlorophyll and carotenoid. Chen et al. (2017a) reported that application of hemin improved the accumulation of chlorophylla/b in the leaves of rice plants subjected to Zn, Pb and Cr stress. In Chinese cabbage, hemin-induced elevation in the chlorophyll and carotenoid content and photosynthetic enhancement were related to alleviation of Cd toxicity (Zhu et al. 2019).

2.3 Hydrogen sulfide

2.3.1 *General information, biochemical characteristics and biosynthesis*

H_2S, with its typical odor of rotten eggs, has long been considered a phytotoxic substance (Jiang et al. 2016). The endogenous production and signaling role of H_2S as a secondary messenger was established back in 1996 (Abe and Kimura 1996), and it was accepted as a GT a few year later (Wang 2002).

In higher plants, H_2S release can occur mostly through the enzymatic pathways (Table 1), and a part of H_2S is generated by the nonenzymatic pathways (Aroca et al. 2018). In the enzymatic pathways, L-cysteine desulfhydrase (L-DES/LCD) and D-cysteine desulfhydrase (D-DES/DCD) use L-cysteine and D-cysteine, respectively, as a substrate to generate H_2S (Riemenschneider et al. 2005; Álvarez et al. 2010; Gotor et al. 2010). Cyanoalanine synthase (CAS) converts L-cysteine to cyanide that in turn leads to H_2S synthesis (Akopyan et al. 1975), and cysteine synthase (CS) catalyzes the reversible conversion of L-cysteine and acetate to O-acetyl-L-serine (OAS) and H_2S (Wirtz and Hell 2006). Carbonic anhydrase (CA) uses carbonyl sulfide taken up through stomata to generate

H_2S and carbon dioxide (CO_2) (Notni et al. 2007; Rudenko et al. 2015). Sulfite reductase (SiR) catalyzes the reduction of sulfite to H_2S in plant cells in the presence of ferredoxin (Da-Silva and Modolo 2018). Considerable evidence has proven the involvement of H_2S in multiple physiological, developmental and metabolic processes (Xuan et al. 2020), including the modulation of stomatal movement (Jin et al. 2013), tissue maturation and senescence (Zhang et al. 2011), lateral root formation (Fang et al. 2014), and tolerance to various environmental stimuli (Ma et al. 2016; Zhou et al. 2018). In the following several paragraphs, the latest progress on the relationship between H_2S and HM stimuli is summarized.

2.3.2 *Gene expression and enzymatic activity regulation*

Endogenous H_2S levels in plants are inducible by various types of HM (Table 2; Fig. 1), including Cd (in bermudagrass, Shi et al. 2014; *Brassica rapa*, Hu et al. 2018; *Salix matsudana*, Yang et al. 2018 and rice, Mostofa et al. 2015), Zn (in *Solanum nigrum*, Liu et al. 2016), Cr (in *Setaria italica*, Fang et al. 2016) and Ni (in rice, Rizwan et al. 2019), Al (in barley, Chen et al. 2013), and Pb (in *Capsicum annuum*, Kaya 2020). However, a decrease of *in vivo* H_2S in *Brassica napus* was observed after exposure of the plants to 20 μM $CdCl_2$ for 7 days (Yu et al. 2019). Pretreatment of bermudagrass plants with sodium hydrosulfide hydrate (NaHS), an H_2S donor, led to a strong induction of endogenous H_2S upon Cd treatment compared to that in the non-pretreated controls indicating a positive feedback relationship (Shi et al. 2013). Cd-stimulated endogenous H_2S level was found to be accompanied by elevated mRNA expression levels and enhanced enzymatic activities of both LCD and DCD in the roots of $CdCl_2$-treated *Brassica rapa* (Hu et al. 2018). In Arabidopsis, Cd induction of LCD activity was further revealed to be mediated by calcium-dependent protein kinases (CDPKs) (Qiao et al. 2015). $ZnCl_2$ treatment at the concentration of 400 μM, which is another type of HM stress, caused a similar enhancement of LCD activity and H_2S content in the leaf and root tissues of *Solanum nigrum*, a known Cd/Zn hyperaccumulator (Liu et al. 2016). These results collectively suggest that LCD and DCD are important enzymes involved in H_2S biosynthesis in response to HM stress.

2.3.3 *Cytoprotection*

The induction of H_2S *in vivo* by HM is thought to play a cytoprotective role based on pharmacological experiments (Fig. 2c). Exogenous application of NaHS, an H_2S donor, alleviated the growth inhibition induced by Cd stress in alfalfa, whereas the use of H_2S synthesis inhibitors eliminated NaHS-induced Cd resistance (Cui et al. 2014). The mitigating effect of NaHS on Cd stress was also observed in other plants, including alfalfa, rape (*Brassica napus*), rice, and cucumber (*Cucumis sativus*) (Zhang et al. 2015; Fang et al. 2019; Kabala et al. 2019; Yu et al. 2019). In addition to reducing Cd toxicity, the application of NaHS can alleviate damage caused by Cr, Cu, Zn, Al, Ni, and Hg in a number of plant species partly by activating cellular protective pathways (Zhang et al. 2008, 2010a, b; Liu et al. 2016; Chen et al. 2017b; Rizwan et al. 2019). Maintenance and activation of the antioxidant system are considered one of the major pathways of cytoprotective function of H_2S. H_2S effectively blocked elevation of ROS upon Cd treatment by activation of protective enzymes (SOD, CAT, POD and APX) and GSH signaling cassettes to remove ROS in the roots of *Brassica rapa* (Zhang et al. 2015; Hu et al. 2018). In addition to the roots, application of NaHS activated a series of ROS-detoxifying enzymes (SOD, APX, CAT, MDHAR, dehydroascorbate reductase (DHAR), GR and glutathione peroxidase (GPX) and increased the levels of the nonenzymatic antioxidants AsA and GSH in the leaves of rice plants (Mostofa et al. 2015). In Arabidopsis, Cd-triggered H_2S production increased cysteine level by upregulating the cysteine biosynthesis genes *serine acetyltransferase 1* (*SAT1*) and *cysteine synthase 1* (*OASA1*), which subsequently led to an increase in the expression of the MT and PC genes that are known ROS scavengers and HM chelators (Cobbett 2002; Jia et al. 2016). Additionally, proline content adjustment has been indicated as an important aspect of the maintenance of ROS homeostasis by H_2S. In Cd-treated foxtail millet seedlings, inhibition of endogenous H_2S blocked Cd-induced endogenous proline and aggravated cellular lipid peroxidation (Tian et al. 2016).

2.3.4 Photosynthetic enhancement

H_2S was shown to modulate photosynthesis by promoting chloroplast biogenesis, photosynthetic enzyme expression, thiol redox modification and the activity of ribulose-1,5-bisphosphate carboxylase (Fig. 2c, Garcia-Mata and Lamattina 2010; Chen et al. 2011). In agreement with these findings, supplementation with H_2S of Cd-stressed plants was found to alleviate the trend of chlorophyll and carotenoid decrease and to rescue the decrease in photosynthetic parameters, such as net photosynthetic rate, stomatal conductance, internal CO_2 concentration, and transpiration rate (Ali et al. 2014; Mostofa et al. 2015). Therefore, enhancement of photosynthetic activity is considered to be an additional mechanism of H_2S-triggered protective effects in response to HM. However, certain interesting questions remain. For instance, how does H_2S regulate stomatal closure to affect its conductance? It was reported that H_2S signaling induced stomatal closure under drought by impacting the activities of the inward K^+ ion and anion channels (Papanatsiou et al. 2015; Wang et al. 2016; Jin et al. 2017; Du et al. 2018), whether the same mechanism holds true under HM stress is not known and may be an interesting subject of further studies.

2.3.5 Roles in regulating heavy metal uptake, movement and accumulation

Additionally, H_2S plays a role in regulating HM uptake, movement and accumulation in plants (Fig. 2c, Mostofa et al. 2015; Fang et al. 2019). Pretreatment with NaHS significantly inhibited accumulation of Al and Cr in *Hordeum vulgare* (Ail et al. 2013; Chen et al. 2013), Cd in *Brassica napus*, rice, Arabidopsis and *Salix matsudana* (Ali et al. 2014; Mostofa et al. 2015; Yang et al. 2018), and Zn in *Solanum nigrum* (Liu et al. 2016). However, Sun et al. (2013) found that mitigation of Cd-induced oxidative damage by H_2S in *Populus euphratica* cells was not only due to decreased Cd influx through hydrogen peroxide (H_2O_2)-activated plasma membrane calcium channels but also to increased partitioning of Cd to the vacuole through activation of tonoplast Cd^{2+}/H^+ antiporters. In *Brassica napus*, NaHS application markedly reduced Cd content in the stems and leaves and increased Cd retention in the root cell walls (Yu et al. 2019). Therefore, H_2S plays a role in the root-shoot Cd translocation in plants, which is an important factor to consider with regard to the food safety and bioremediation of crops. However, our understanding of the mechanism of H_2S regulation of HM intake to the roots and the subsequent translocation is very limited.

2.3.6 S-sulfhydration

S-sulfhydration is a posttranslational modification of proteins that converts cysteinyl thiolates (Cys-S(-)) to persulfides (Cys-S-S(-)) and is a specific mechanism of action of H_2S (Aroca et al. 2015). *S*-sulfhydration can either activate, as described for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), parkin E3 ligase and respiratory burst oxidase homologue D (RBOHD) (Mustafa et al. 2009; Vandivder et al. 2013) or inactivate enzyme activities, as described for protein tyrosine phosphatases 1B (PTP1B) (Krishnan et al. 2011). *S*-sulfhydration has also been shown to modify protein-protein interactions, e.g. in the case of Kelch-like ECH-associated protein 1 (KEAP1), which acts as a negative regulator of nuclear factor (erythroid-derived 2)-like 2 (NRF2), a master regulator of the antioxidant response in mice (Yang et al. 2013). In plants, H_2S-dependent *S*-sulfhydration has been involved in responses to heat, low temperature, ultraviolet radiation (UV), salinity, and osmotic stress (Hancock 2018; Gotor et al. 2019). Cysteines in proteins are the primary sites of *S*-sulfhydration (Aroca et al. 2015), and the GSSH content can be an indicator of the protein *S*-sulfhydration level (Qiao et al. 2015). Since GSSH content under HM stress is affected by H_2S, *S*-sulfhydration is likely to occur in cysteine-rich proteins that are accumulated under HM stress, such as PCs and MTs.

2.4 Methane

2.4.1 Property and source

CH_4 is the second most prevalent greenhouse gas on earth with the characteristics of being colorless, odorless, nontoxic, volatile and slightly soluble in water (Keppler et al. 2009; Li et al. 2019). CH_4 was recognized as a GT only recently after NO, CO, and H_2S (Wang 2014). Biological production of CH_4 is predominantly related to anoxic environments and the activity of anaerobic prokaryotes (Archaea) (Conrad 2009; Kirschke et al. 2013); however, endogenous release of CH_4 has also been observed in eukaryotes even in the absence of microbes and in the presence of oxygen (Keppler et al. 2006; Wang et al. 2011; Lenhart et al. 2012; Althoff et al. 2014). Enzymes involved in endogenous production of CH_4 in plants are largely unknown (Table 1, Li et al. 2019). Numerous studies in plants have confirmed direct endogenous generation of CH_4 under both normal growth and stress conditions, including cutting injuries, UV-B radiation, hypoxia, high temperature, water stress, low light, and salinity (Keppler et al. 2006; McLeod et al. 2008; Vigano et al. 2008; Bruggemann et al. 2009; Qaderi and Reid 2009; Wang et al. 2011; Abdulmajeed et al. 2017; Martel and Qaderi 2017).

2.4.2 Biological roles in plant heavy metal responses

A number of studies have revealed the role of CH_4 in regulation of plant physiology. For example, CH_4 acted as an upstream signaling molecule of NO to control lateral and adventitious rooting, redox homeostasis and starch metabolism (Qi et al. 2017; Zhang et al. 2018; Jin et al. 2020). Several recent lines of evidence have established a relationship between CH_4 and HM response in plants (Table 2; Fig. 1, Samma et al. 2017; Cui et al. 2017; Gu et al. 2018). Aerobic nonmicrobial CH_4 emission was determined in Cu-stressed alfalfa seeds, and the function of the emission can be mimicked by 0.39 mM CH_4 in fresh saturation solution (Samma et al. 2017). Exogenous CH_4 application mitigated Cu-inhibited seed germination and seedling growth and alleviated oxidative stress by decreasing lipid peroxidation and increasing plasma membrane integrity (Samma et al. 2017). The protective role of CH_4 against Al stress was also established in alfalfa seedlings as demonstrated by the remission of Al-induced inhibition of root elongation, nutrient disorder, and relative electrolyte leakage (Cui et al. 2017). The release of CH_4 in alfalfa root tissues was further confirmed under Cd stress, and pretreatment with exogenous CH_4 mitigated Cd-induced inhibition of seedling growth (Gu et al. 2018). Taken together, these results suggest a generally advantageous effect of CH_4 on HM tolerance in plants. Thus, regulation of cellular redox homeostasis and inhibition of metal accumulation have been linked to the mechanisms of the protective effects of CH_4 in HM toxicity (Fig. 2d).

2.4.3 Roles in maintaining redox homeostasis

CH_4 ameliorates HM-induced redox imbalance by regulating antioxidant enzymes, proline metabolism and GSH homeostasis. Reduction in SOD, APX and CAT activities and/or their gene expression levels under Cd, Al, and Cu stress was significantly suppressed by CH_4 pretreatment (Cui et al. 2017; Samma et al. 2017; Gu et al. 2018). POD activity induced by Cu, Al, and Cd stress was further enhanced by CH_4 (Cui et al. 2017; Samma et al. 2017; Gu et al. 2018). CH_4 pretreatment blocked the oxidation of the GSH pool in alfalfa induced by Cd stress, which was accompanied by an increase in the expression levels of *γ-glutamylcysteinyl synthetase* (*ECS*), *homoglutathione synthetase* (*hGS*), *GR1/2*, and *glutathione-S-transferase* (*GST*); all these enzymes are related to the GSH synthesis and/or metabolism (Gu et al. 2018).

Despite the fact that overproduction of ROS under HM stress can be blocked by CH_4, ROS are considered inducers of nonmicrobial CH_4 emission. Conclusive evidence has shown that aerobic nonmicrobial CH_4 production is limited by ROS removal and enhanced by the enzymes inhibiting this removal (Messenger et al. 2010). Moreover, certain types of ROS, e.g. nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase-dependent H_2O_2 production, can be triggered by CH_4 to induce LR formation in tomato seedlings (Zhao et al. 2019). These lines of evidence indicate complex interactions between CH_4 and ROS, and the biological significance of these interactions during HM stress remains to be explored in detail.

2.4.4 Roles in suppressing heavy metal accumulation

CH_4 was shown to impede Cd accumulation in the roots of alfalfa seedlings by regulating HM transporters. Specific microRNAs, namely, *miR159* and *miR167* that participated in the modulation of the ATP-binding cassette (ABC) and NRAMP6 transporters, were down- and up-regulated by CH_4, respectively (Gu et al. 2018). In CH_4-pretreated alfalfa seedlings under Al stress, a reduction in Al intake was accompanied by upregulation of genes involved in organic acid metabolism and transport, i.e. *citrate synthase*, *malate dehydrogenase 1/2* (*MDH1/2*), *Al-*

activated malate transporter 1 (*ALMT1*), and *Al-activated citrate transporter* (*AACT*) were upregulated suggesting that CH_4 prevents HM accumulation likely in an organic acid-dependent fashion (Cui et al. 2017).

3 Crosstalk of gasotransmitters in response to heavy metals

In addition to individual GTs that are indispensable for plant growth, development and environmental responses, the interactions of GTs have also been observed in the regulation of various physiological processes. For example, the HO-1/CO system was proposed to be a component or a signaling partner of the H_2S-induced cytoprotection against PCD in wheat aleurone layers treated by GA (Xie et al. 2014). Cucumber HO-1 is also involved in NaHS-induced AR formation (Lin et al. 2012). CH_4 functions upstream of NO in both LR and AR formation (Qi et al. 2017; Jin et al. 2020). In terms of root development, CO and H_2S may be the second messengers downstream of CH_4 (Cui et al. 2015; Kou et al. 2018; Mei et al. 2019). Similar results were demonstrated under the stress conditions coupled with redox imbalance thus confirming that CO, NO, and H_2S are interlinked via the molecular signaling mechanisms involved in the CH_4-induced stress tolerance in plant tissues (Zhu et al. 2016; Han et al. 2017; Samma et al. 2017; Zhang et al. 2018). However, to the best of our knowledge, there is no evidence showing crosstalk between CO and H_2S or CH_4 and other GTs in plant tolerance against HMs. The relationship between NO and CO under HM stress is discussed in the next section. The crosstalk between NO and H_2S in HM detoxification is also discussed (Fig. 3).

3.1 Crosstalk between nitric oxide and carbon monoxide

It is becoming clear that NO and CO can modulate the actions of each other (Li et al. 2011; Xuan et al. 2012). For example, in *Vicia* guard cells, CO generation mediated by HO-1 causes stomatal closure in a NO- and cGMP-dependent manner (Cao et al. 2007). HO-1 is the enzymatic source of CO involved in the modulation of cucumber AR formation (Xuan et al. 2008b) and LR formation related with auxin and NO signal in tomato (Guo et al. 2008) via NO signaling. In response to osmotic stress, CO alleviated inhibition of seed germination and induction of lipid peroxidation, and this effect required participation of NO (Liu et al. 2010). Moreover, CO induced salt tolerance through maintenance of ion homeostasis and upregulation of antioxidant defense in wheat seedlings, and the effect was also mediated by NO (Xie et al. 2008). These results suggest that NO functions downstream of CO in physiological processes in the plants. This conclusion is also true under HM stress conditions; the difference is that NO is not induced but is inhibited by the HO/CO system. In detail, *HY1* null mutant of Arabidopsis was able to induce NO overproduction and Cd accumulation. Overexpression of *HY1* enhanced Cd exclusion via modulation of the expression levels of *FRO2*, *IRT1* and *heavy metal ATPase 2/4* (*HMA2/4*), suggesting that *HY1* is beneficial to plants under Cd stress by decreasing NO production and improving Fe homeostasis in the root tissues thus contributing to a decrease in Cd accumulation and phytotoxicity (Han et al. 2014). However, in an attempt to protect soybean leaves against oxidative damage caused by Cd, Noriega et al. (2007)

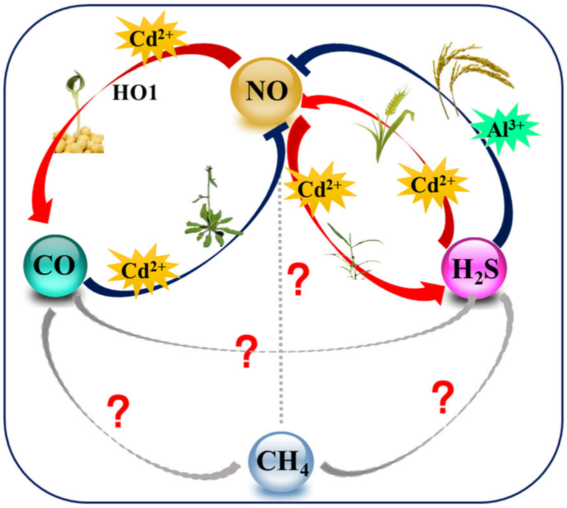


Fig. 3 Crosstalk of gastransmitters under heavy metal stresses. NO generation was inhibited by CO under Cd stress in Arabidopsis. However, CO has proved to be triggered by NO under Cd stress in soybean. Under Cd stress, NO was upstream of H_2S signal in bermudagrass but downstream in wheat. Under Al stress, H_2S inhibited NO synthesis in rice root. There is no evidence yet showing crosstalk between CO and H_2S or CH_4 and other GTs in plant tolerance against HMs. Arrowhead: activation; blunt end: inactivation. Question mark: undetected

demonstrated that supplementation with 100 µM SNP, which is an NO donor, can upregulate the expression of the *HO-1* gene, whereas cPTIO, a specific NO scavenger, blocked the NO-induced upregulation of *HO-1* and NO-mediated protective effects. This result suggests that in response to Cd stress, NO acts upstream of CO. Currently, the mechanism of NO-CO interaction in plants is unclear; however, it is known that NO binds to Fe^{2+} of the heme group of human heme oxygenase HO-1 *in vitro*. Exploration of the putative feedback mechanism that controls the biological activity of NO and CO in plants is an interesting subject for future studies.

3.2 Crosstalk between nitric oxide and hydrogen sulfide

Since both NO and H_2S are involved in plant responses to HM and share the same mechanisms of action, including antioxidant system maintenance, inhibition of HM accumulation, and interplay with Ca^{2+}, it is intuitive to ask whether and how NO and H_2S interact to coordinate their functions in these processes. H_2S treatment reduced the negative charge in the root cell wall by decreasing the activity of PMEs, blocked Al-binding sites in the root cell wall, increased the secretion of citrate to reduce Al biding, enhanced antioxidant enzyme activities, and compartmentalized Al into the vacuole (Zhu et al. 2018). Zhu et al. (2018) found that applications of H_2S and the NO donor SNP reduced and increased Al content, respectively, in the roots of Al-treated rice plants. The authors also observed a decrease in the NO content in rice roots after H_2S treatment thereby implying that a decrease in the endogenous NO levels is involved in the H_2S-induced alleviation of Al toxicity. In alfalfa seedlings, NaHS induced Cd tolerance phenotypes, including mitigated lipid peroxidation, arrested growth inhibition, and reduced Cd accumulation, and these effects were reversed by NO scavenger cPTIO (Li et al. 2012). In the roots of Cd-stressed wheat plants, NaHS and SNP application induced endogenous production of H_2S and NO. Interestingly, the effect of NaHS was reversed by H_2S scavenger hypotaurine (HT) and partly inhibited by cPTIO, whereas the effect of SNP was only sensitive to cPTIO (Kaya et al. 2020). These results suggest that NO acts as a downstream component of the H_2S signaling pathway in Cd stress responses. Nevertheless, in Cd-stressed bermudagrass, induction of H_2S by SNP was reversed by cPTIO and H_2S scavenger/inhibitors, while NaHS-induced H_2S generation was specifically blocked by HT indicating that NO plays a role upstream of H_2S in Cd responses (Shi et al. 2014). Collectively, these experiments suggest a complex interplay of the two GTs that may depend on the plant species, tissue type and HM stress condition. Extensive future studies are thus needed to clarify this process.

4 Conclusions and future perspectives

Studies on GT regulation of important traits and environmental responses have become a research hotspot in the plant science. In the specific area of HM-related stress biology, accumulating evidence has proven the induction or inhibition of endogenous GTs in plants by different HM stimuli (Fig. 1; Table 2).- The vast majority of the published work indicate that GTs enhance plant tolerance to HM stress, except for certain studies on NO that described negative roles, such as an increase in HM uptake and accumulation and promotion of HM-induced oxidative stress (Besson-Bard et al. 2009; Xu et al. 2010b; Yu et al. 2012; Terron-Camero et al. 2020). Identified mechanisms of GT regulation of HM stress include suppression of HM accumulation, regulation of antioxidant enzymes, restoration of redox and GSH homeostasis, maintenance of ion homeostasis, and promotion of photosynthesis (Fig. 2). From an agricultural perspective, a better understanding of how GTs regulate HM translocation in various parts of a plant is of particular interest because it may provide a foundation for breeding safer crops (low HM accumulation in edible parts) without compromising the ability of bioremediation (high HM accumulation in nonedible parts). In reality, GTs do not independently but coordinately work with others to modulate HM response (Fig. 3). The understanding of the biological interplay between GTs and other molecules is improving. For example, H_2S was found to be necessary for abscisic acid (ABA)-stimulated NO accumulation and stomatal closure (Scuffi et al. 2014). The stimulatory effect of SNP on Ca^{2+} accumulation and *calmodulin 1* (*CaM1*) expression was abolished by inhibiting H_2S synthesis suggesting a "mediator" role of H_2S between the NO and Ca^{2+} signaling pathways. However, currently published studies are far from creating a comprehensive picture of how these GTs modulate the protective and signaling pathways by interacting with phytohormones, second messengers and each other. More efforts are required to identify the global network mechanisms of the effects of GT on plant responses to HM.

To achieve more in-depth mechanistic understandings of GTs, some very interesting questions are worth to be explored in the near future: What are the direct target proteins of these GTs in the HM response? What types of chemical reactions/modifications occur during these processes? Is the information about GT crosstalk gained in other growth conditions/treatment scenarios transferable to the HM stress conditions? Although a large number of studies showed that GTs may be either induced or inhibited by HM stimulus, future studies on the metabolic pathways of these GTs should focus on the molecular details of their production pathways under HM stress. The intricate mechanisms associated with their responses to HM stimuli remain a subject of great interest. So far, proteomic studies have revealed at least three types of posttranslational modifications of the target proteins by NO: metal nitration, tyrosine nitration, and *S*-nitrosation (Astier and Lindermayr 2012). H_2S may directly modify protein thiol groups via *S*-sulfhydration (Qiao et al. 2015). However, direct protein targets of these two GTs under HM stress are largely unknown. Answers to these questions will provide a clearer picture.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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