

The glutathione-dependent alarm triggers signalling responses involved in plant acclimation to cadmium

Peer-reviewed author version

IVEN, Verena; VANBUEL, Isabeau; HENDRIX, Sophie & CUYPERS, Ann (2023)

The glutathione-dependent alarm triggers signalling responses involved in plant acclimation to cadmium. In: JOURNAL OF EXPERIMENTAL BOTANY, 74 (11) , p. 3300-3312.

DOI: 10.1093/jxb/erad081

Handle: <http://hdl.handle.net/1942/39998>

1 **THE GLUTATHIONE-DEPENDENT ALARM TRIGGERS SIGNALLING**
2 **RESPONSES INVOLVED IN PLANT ACCLIMATION TO CADMIUM**

3 Verena Iven^{†,1}, Isabeau Vanbuel^{†,1}, Sophie Hendrix¹, Ann Cuypers^{*,1}

4

5 ¹ Environmental Biology, Centre for Environmental Sciences, Hasselt University,
6 Diepenbeek, Belgium

7

8 [†] Equal contribution

9 ^{*} Correspondence: ann.cuypers@uhasselt.be

10

11 verena.iven@uhasselt.be

12 isabeau.vanbuel@uhasselt.be

13 sophie.hendrix@uhasselt.be

14 ann.cuypers@uhasselt.be

15

16

17 **Date of submission:** 01/02/2023

18 **Number of tables:** 3

19 **Number of figures:** 2

20 **Word count:** 5468

21 **Running title:** Glutathione in cadmium stress responses

22

23 **Highlight**

24 This review focuses on the role of glutathione in the trade-off between antioxidative defence
25 and metal chelation, subsequent signal transduction and acclimation in plant responses to
26 cadmium stress.

27 **Abstract**

28 Cadmium (Cd) uptake from polluted soils inhibits plant growth and disturbs physiological
29 processes, at least partly due to disturbances in the cellular redox environment. Although the
30 sulfur-containing antioxidant glutathione is important in maintaining redox homeostasis, its
31 role as an antioxidant can be overruled by its involvement in Cd chelation as a phytochelatin
32 precursor. Following Cd exposure, plants rapidly invest in phytochelatin production, thereby
33 disturbing the redox environment by transiently depleting glutathione concentrations.
34 Consequently, a network of signalling responses is initiated, in which the phytohormone
35 ethylene is an important player involved in the recovery of glutathione levels. Furthermore,
36 these responses are intricately connected to organellar stress signalling and autophagy and
37 contribute to cell fate determination. In general, this may pave the way for acclimation (e.g.
38 restoration of glutathione levels and organellar homeostasis) and plant tolerance in case of
39 mild stress conditions. This review addresses connections between these players and discusses
40 the possible involvement of the gasotransmitter hydrogen sulfide in plant acclimation to Cd
41 exposure.

42 **Keywords**

43 Autophagy, cadmium, endoplasmic reticulum stress, ethylene, glutathione, hydrogen sulfide,
44 mitochondrial retrograde regulation, phytochelatin, reactive oxygen species, sulfur

45 **1 Glutathione is pivotal in cadmium responses**

46 Cadmium (Cd) is a metal naturally present in soils in low concentrations, although it
47 accumulates above background levels in many areas worldwide. While this accumulation can
48 be reached by natural phenomena, the primary sources of environmental Cd contamination are
49 human activities, including smelting and mining activities, the use of phosphate fertilisers and
50 sewage irrigation in agriculture (Haider *et al.*, 2021; Zulfiqar *et al.*, 2022). Non-essential
51 metals, such as Cd, can even be phytotoxic at low doses (Lin and Aarts, 2012). As Cd is non-
52 biodegradable and has a high bioavailability in the soil, it is taken up by plants and introduced
53 into the food chain, thereby endangering human and environmental health (Clemens *et al.*,
54 2013).

55 Cadmium is mainly present in the soil as a divalent cation and can be taken up by transporters
56 designed for essential divalent metals, such as iron (Fe), zinc (Zn) and manganese (Mn) (Zhu
57 *et al.*, 2021; El Rasafi *et al.*, 2022). Once absorbed by plant roots, Cd enters the apoplastic and
58 symplastic pathways to the xylem and phloem, eventually reaching stems, leaves, fruits and
59 seeds (Sterckeman and Thomine, 2020; Zhu *et al.*, 2021). Subsequently, it can disturb
60 physiological processes, for example by affecting electron transport in mitochondria and
61 chloroplasts and inhibit plant growth and development (Haider *et al.*, 2021; Zulfiqar *et al.*,
62 2022). Such effects often result from a disrupted cellular homeostasis that can be caused by
63 the indirect Cd-induced increase in reactive oxygen species (ROS) (El Rasafi *et al.*, 2022).
64 This occurs shortly after Cd exposure by a depletion in glutathione (GSH) levels in the roots,
65 which results from a fast induction of phytochelatin (PC) biosynthesis (Figure 1) (Jozefczak *et*
66 *al.*, 2014; Deckers *et al.*, 2020). Glutathione is an antioxidant tripeptide consisting of
67 glutamine, cysteine and glycine, and is synthesised in two steps catalysed by glutamate-
68 cysteine ligase (GSH1) in plastids and GSH synthetase (GSH2) in plastids and the cytosol.
69 Glutathione has multiple roles in maintaining the redox balance. For example, it can reduce

70 hydrogen peroxide (H_2O_2) directly or via participation in the ascorbate (AsA)-GSH cycle
71 (Jozefczak *et al.*, 2012; Hasanuzzaman *et al.*, 2019). Additionally, GSH is the precursor of
72 PCs and is thereby involved in chelating Cd molecules as a consequence of the high affinity
73 of Cd towards thiol groups. Phytochelatins are metal-chelating molecules with a (γ -Glu-
74 Cys)_n-Gly (n=2-11) structure synthesised from GSH by phytochelatin synthase (PCS) upon
75 post-translational activation by Cd or other metals (Cobbett, 2000; Jozefczak *et al.*, 2012;
76 Sharma *et al.*, 2016). Once formed within the cytosol, PC-Cd complexes are transported into
77 the vacuole (via two ATP-BINDING CASSETTE (ABCC) transporters, AtABCC1 and
78 AtABCC2, in *Arabidopsis thaliana*) (Park *et al.*, 2012; Sharma *et al.*, 2016). Previous
79 research suggested that the initial GSH depletion, as a consequence of Cd-induced PC
80 production in roots, functions as a redox-dependent alarm triggering different downstream
81 responses (Figure 1) (Deckers *et al.*, 2020).

82 In addition to altering the redox environment, Cd exposure disrupts the homeostasis of
83 phytohormones such as jasmonic acid, salicylic acid, auxin and ethylene. These
84 phytohormones are involved in plant responses to Cd stress (De Smet *et al.*, 2015; El Rasafi *et al.*,
85 2022) and are also intertwined (Saini *et al.*, 2021). Nevertheless, this review will focus on
86 ethylene, given its profound changes and signalling role during Cd exposure and its close
87 connection to GSH metabolism, as it is synthesised from the sulfur-containing amino acid
88 methionine (Keunen *et al.*, 2016). Altered ethylene levels and their interplay with the Cd-
89 induced increases in ROS levels may ultimately result in retrograde organellar signalling and
90 autophagy, which can be involved in stress acclimation (Figure 1) (Depaepe *et al.*, 2021). The
91 following parts of this review focus on the downstream signalling processes induced by the
92 GSH-dependent alarm and their contribution to plant signalling and acclimation upon Cd
93 stress, for instance to restore GSH levels, during which hydrogen sulfide (H_2S) potentially
94 plays a fine-tuning role (Figure 1).

95 **2 The role of the cadmium-induced glutathione-dependent alarm in**
96 **mounting an ethylene response**

97 While crucial during development (Keunen *et al.*, 2016), ethylene is also an important player
98 in the intricate stress response network that allows acclimation or cell death depending on the
99 stress severity that is perceived by the organism (Depaepe *et al.*, 2021). Furthermore, this
100 phytohormone is connected to the antioxidant GSH at multiple levels. For instance, both
101 depend on cysteine for their biosynthesis, although ethylene itself is not a sulfur-containing
102 compound (Keunen *et al.*, 2016). Moreover, in the AsA-GSH cycle, GSH is involved in the
103 reduction of dehydroascorbate to AsA (Zechmann, 2014), which also serves as a co-substrate
104 for the enzyme catalysing the final step of ethylene biosynthesis (Pattyn *et al.*, 2021).
105 Furthermore, Deckers *et al.* (2020) proposed that a transient GSH-dependent change in the
106 redox environment in roots serves as an alarm to elicit ethylene biosynthesis and signalling
107 that contribute to the plant response to Cd (Figure 2). In this context, it was demonstrated that
108 it is the early Cd-induced decrease in root GSH concentrations, rather than an altered GSH
109 redox state, that influences the redox environment, as indicated by an increased H₂O₂/GSH
110 ratio in *A. thaliana* roots during acute and mild Cd stress (Deckers *et al.*, 2020). Cadmium-
111 induced ROS were proposed to be sensed by oxidative signal-inducible 1 (OXI1) (Schellingen
112 *et al.*, 2015b), a protein kinase that is transcriptionally induced and activated by H₂O₂ in *A.*
113 *thaliana* (Rentel *et al.*, 2004) and is also rapidly and transiently upregulated in *A. thaliana*
114 roots following Cd exposure (Deckers *et al.*, 2020). Its contribution to the activation of
115 mitogen-activated protein kinases (MPK) 3 and 6 (Rentel *et al.*, 2004) may link Cd-induced
116 ROS to increased ethylene biosynthesis (Schellingen *et al.*, 2015b), as these kinases increase
117 1-aminocyclopropane-1-carboxylic acid (ACC) synthase 2 (ACS2) and ACS6 at the transcript
118 (via the transcription factor WRKY DNA-binding protein 33, WRKY33) and protein levels
119 (Figure 2) (Li *et al.*, 2012). These ACS enzymes catalyse the conversion of S-

120 adenosylmethionine (SAM) to ACC, which is subsequently converted to ethylene by ACC
121 oxidase (ACO) (Keunen *et al.*, 2016; Pattyn *et al.*, 2021). Additionally, it has been
122 demonstrated that ACS2 and ACS6 are important contributors to Cd-induced ethylene
123 emission (Schellingen *et al.*, 2014). Consistent with the proposed MAPK-ACS pathway, a
124 Cd-mediated induction of MPK3 and/or MPK6 activity was reported and appears to be
125 reciprocally linked to ROS (Liu *et al.*, 2010; Jin *et al.*, 2013). Furthermore, both an
126 upregulation of *MPK3* and *MPK6* and an increase in ACC levels were observed in *A. thaliana*
127 roots after only a few hours of Cd exposure. These observations hint at a fast enhancement of
128 ACS protein stability in roots (Deckers *et al.*, 2020). Moreover, the connection between GSH
129 and ethylene is further supported by the observation that simultaneous administration of GSH
130 and Cd at the root level of *Zea mays* seedlings limited the Cd-induced emission of ethylene
131 (Li *et al.*, 2017), while GSH-deficient *cadmium-sensitive 2 (cad2)* *A. thaliana* plants showed
132 increased root ACC levels (Deckers *et al.*, 2021) and higher ethylene emission (Verena Iven,
133 unpublished results) compared to wild-type (WT) plants after Cd exposure.

134 Subsequently, a further increase in ACC levels as well as ACS2 and ACS6 transcripts after
135 prolonged Cd exposure was hypothesised to indicate a slow transcriptional stimulation of the
136 MAPK-ACS pathway. Notably, it is also at these later stages of the mild stress response that
137 the upregulation of several oxidative stress marker genes and increases in H₂O₂ levels were
138 reported in *A. thaliana* leaves and roots (Deckers *et al.*, 2020). This change in the redox
139 environment may be partly elicited by ethylene (Figure 2), as ethylene biosynthesis *acs2-1*
140 *acs6-1* (Schellingen *et al.*, 2015a) and ethylene signalling *ethylene-insensitive 2-1 (ein2-1)*
141 mutant *A. thaliana* plants generally showed a weaker Cd-mediated induction of several
142 transcripts encoding superoxide (O₂^{•-})-generating respiratory burst oxidase homologues
143 (RBOHs, i.e. NADPH oxidases) and oxidative stress markers in comparison to WT plants
144 (Schellingen *et al.*, 2015b). Such findings in the model organism *A. thaliana* are in line with

145 those in the crop plant *Solanum lycopersicum*, where H₂O₂ levels induced by long-term Cd
146 treatment were lower in leaves of an ethylene-insensitive cultivar, despite their higher Cd
147 concentration (Alves *et al.*, 2017). Furthermore, lower NADPH oxidase and higher
148 superoxide dismutase (SOD) activities as well as lower O₂^{•-} levels were respectively observed
149 in roots and root tips of *A. thaliana* seedlings simultaneously treated with Cd and ACC rather
150 than with Cd alone. The authors hence proposed that ethylene regulates O₂^{•-} levels in the root
151 tip to adjust root architecture during Cd stress (Abozeid *et al.*, 2017). Accordingly, root hair
152 length, ROS, and the induction of the ethylene reporter construct EIN3 binding site
153 (*EBS*):*GUS* shared a similar pattern across various Cd concentrations in *A. thaliana* seedlings,
154 being stimulated by Cd up to a certain concentration (Bahmani *et al.*, 2022). Taken together,
155 these results suggest that Cd-induced ethylene production, triggered by a GSH-dependent
156 alarm, alters plant stress responses by, for example, influencing the redox environment. Such
157 ethylene-mediated redox changes may subsequently be amplified by further stimulation of
158 ethylene production through the MAPK-ACS pathway, of which the most profound
159 transcriptional induction during Cd stress in leaves indeed generally coincided with H₂O₂
160 increases (Deckers *et al.*, 2020) and was largely EIN2-dependent (Schellingen *et al.*, 2015b).
161 It should be noted, however, that this MAPK pathway responds to ROS that likely originate
162 from additional sources besides the aforementioned NADPH oxidases, including organelles
163 (Liu and He, 2017; Jalmi *et al.*, 2018).

164 According to these obtained results and proposed models, the rapid Cd-induced GSH
165 depletion contributes to early ethylene production and signalling that, in turn, closes the loop
166 by influencing the redox environment and stimulating its own production. As this puts
167 forward an initialising role for PC production in stimulating Cd-induced ethylene production,
168 it might seem contradictory that roots of PC-deficient *cad1-3 A. thaliana* also showed
169 stronger *ACS2*, *ACS6* and ethylene response factor 1 (*ERF1*) inductions, ACS activity and

170 ACC concentrations compared to WT plants following short-term Cd exposure (Chen *et al.*,
171 2020). This is in accordance with the enhanced ethylene emission found in this mutant
172 compared to WT plants following Cd exposure (Verena Iven, unpublished results). While this
173 mutant might more strongly rely on GSH for Cd chelation, inducing the perception of GSH
174 depletion, it was proposed by Chen *et al.* (2020) that the stimulation of ethylene biosynthesis
175 results from strong Cd toxicity elicited by a lack of PCs. Indeed, preliminary data show that
176 these mutants suffer from a higher extent of oxidative stress following Cd exposure (Verena
177 Iven, unpublished results), which may cause a severe burst of ethylene rather than a controlled
178 increase as a result of a GSH-dependent alarm. This hypothesis further highlights the delicate
179 balance between controlled signalling and damage depending on stress intensity.

180 **3 Restoring glutathione levels during acclimation relies on the cadmium-** 181 **induced glutathione-dependent alarm and subsequent ethylene** 182 **signalling**

183 Cadmium exposure in plants stimulates sulfur assimilation (Mendoza-Cózatl *et al.*, 2005;
184 Khan *et al.*, 2016a). The increased expression of sulfate transporter 1;2 (*SULTRI;2*) in Cd-
185 stressed *A. thaliana* was proposed to rely on the depletion of cysteine, the GSH precursor γ -
186 glutamylcysteine (γ -EC) and GSH as well as the shift to a more oxidising environment (Jobe
187 *et al.*, 2012). This suggests that the previously discussed GSH-dependent alarm, as well as
188 subsequent changes in the redox environment, may contribute to Cd-induced sulfur
189 assimilation. Accordingly, prolonged Cd exposure is generally associated with enhanced GSH
190 biosynthesis (Table 1, Figure 1). Nevertheless, caution is advised when comparing the results
191 of studies using different experimental set-ups (e.g. different exposure conditions), as this can
192 affect GSH levels (Koffler *et al.*, 2014).

193 In contrast to roots showing an initial GSH depletion, leaf GSH levels increased in *A. thaliana*
194 upon mild Cd stress (Jozefczak *et al.*, 2014). As the upregulation of *ERF1* in leaves of Cd-

195 exposed *A. thaliana* preceded the elevation of ACC concentrations in this organ, it was
196 proposed that ACC or ethylene originating from roots following the GSH-dependent alarm
197 during mild Cd exposure activates ethylene signalling in leaves to allow timely induction of
198 GSH biosynthesis (Deckers *et al.*, 2020). This hypothesis is in accordance with the
199 observation that scions of a WT *S. lycopersicum* cultivar contained less sulfur when they were
200 grafted to Cd-exposed rootstocks of an ethylene-insensitive cultivar rather than a WT cultivar
201 and further exposed to Cd for 30 days (Alves *et al.*, 2017). In addition, increases in GSH
202 levels or GSH metabolism-related gene expression after Cd exposure were lower or absent in
203 root tips of *Lycium chinense* treated with the ACC biosynthesis inhibitor 2-
204 aminoethoxyvinylglycine (Guan *et al.*, 2015a) and in leaves of *A. thaliana ethylene response*
205 *1-1 (etr1-1)*, *ein2-1* and *ein3-1* ethylene signalling mutants (Schellingen *et al.*, 2015b).
206 Furthermore, it was shown that long-term Cd-induced sulfur assimilation and elevation of
207 cysteine and GSH levels in *Brassica juncea* leaves further increased when plants were
208 additionally treated with ethylene-releasing ethephon (Khan *et al.*, 2016b). Taken together,
209 these results suggest that Cd-induced ethylene production positively affects GSH production
210 (Figure 2).

211 **4 Cadmium-induced organellar responses triggered by the glutathione-** 212 **dependent alarm and subsequent signalling**

213 Glutathione is present in different subcellular compartments (Zechmann, 2014), where it can
214 be influenced by Cd stress (Kolb *et al.*, 2010; Koffler *et al.*, 2014). In *A. thaliana* mesophyll
215 cells, Cd exposure was found to affect GSH localised in chloroplasts (Koffler *et al.*, 2014),
216 which are also the sites where the first step of GSH biosynthesis predominantly occurs
217 (Zechmann, 2014) and where *de novo* methionine production from cysteine for ethylene
218 biosynthesis takes place (Bittner *et al.*, 2022). Furthermore, chloroplasts are known to be
219 affected by Cd at the level of their photosystems (Parmar *et al.*, 2013). In response to

220 environmental stressors, these organelles can employ various retrograde signalling arms that
221 are activated, among others, via redox signals (Cejudo *et al.*, 2021; Jan *et al.*, 2022). One such
222 example can be the redox regulation of the Calvin-Benson cycle, which is responsible for the
223 production of triose phosphate (Cejudo *et al.*, 2021). This metabolite was reported to activate
224 MPK6 and subsequently induce transcription of ethylene signalling genes following
225 chloroplast-to-cytosol transport via the triose phosphate/phosphate translocator (TPT) in *A.*
226 *thaliana* as a response to high light stress (Vogel *et al.*, 2014). Accordingly, Cd exposure was
227 shown to alter the chloroplast redox environment (Table 2) and decrease triose phosphate
228 utilisation in hybrid *Pennisetum* leaves (Song *et al.*, 2019). Moreover, it was found to
229 upregulate the expression of *TPT*, which was further enhanced by additional ethephon
230 treatment, in leaves of *Catharanthus roseus* (Chen *et al.*, 2017). While Chen *et al.* (2017)
231 propose that this last observation is linked to the transport of increased catharanthine levels, a
232 potential connection between triose phosphate-dependent retrograde signalling and Cd stress
233 seems plausible and deserves further attention. To the best of our knowledge, it remains
234 elusive whether and how the chloroplast retrograde pathways function during Cd stress.
235 However, it is important to note that chloroplasts play an important role in the Cd response as
236 they are inevitably involved in restoring GSH levels during acclimation.

237 Furthermore, Cd also causes mitochondrial stress. For instance, clustering has been observed
238 between transcriptional responses to the mitochondrial complex III inhibitor antimycin A and
239 Cd (Yu *et al.*, 2001) and mitochondrial homeostasis pathways were recently reported to
240 represent an important part of the Cd stress response (Liu *et al.*, 2023). In addition, Cd has
241 previously been shown to alter the mitochondrial redox environment (Table 2) (Keunen *et al.*,
242 2011). To continue electron flow from the tricarboxylic acid (TCA) cycle in mitochondria
243 without excessive ROS production by electron leakage from the electron transport chain
244 (ETC), plants redirect electrons through an alternative pathway relying on alternative oxidase

245 (AOX) (Keunen *et al.*, 2011). The induction of *AOX1a* is part of a mitochondrial retrograde
246 regulation (MRR) response. Together with other mitochondrial dysfunction stimulon (MDS)
247 genes (e.g. *OXII*), it can be regulated by the NAC (no apical meristem/*Arabidopsis*
248 transcription activation factor/cup-shaped cotyledon) transcription factor ANAC017 following
249 its cleavage from the endoplasmic reticulum (ER) upon mitochondrial stress (Figure 2) (De
250 Clercq *et al.*, 2013; Ng *et al.*, 2013). It should be noted that ANAC017 was found to also play
251 a role in the retrograde response of chloroplasts upon methyl viologen treatment, indicating
252 that retrograde signalling by ANAC017 can be triggered by both organelles (Van Aken *et al.*,
253 2016). In addition, the potential involvement of MPK6 and/or phytohormones, including
254 ethylene, in mitochondrial stress signalling has become evident (Wang and Auwerx, 2017;
255 Kacprzak *et al.*, 2020) and a model was put forward where ANAC017 serves as an upstream
256 mediator of ethylene biosynthesis (He *et al.*, 2022). Notably, Cd-induced stress responses
257 share several characteristics with mitochondrial stress signalling. As such, a mitochondrial
258 stress signal stimulating MAPK was observed in Cd-exposed *Oryza sativa*, where inhibition
259 of mitochondrial permeability transition pore opening lowered Cd-induced MPK activity
260 (Yeh *et al.*, 2007). Moreover, Cd-induced *AOX1a* (Keunen *et al.*, 2015) and *OXII*
261 (Schellingen *et al.*, 2015b) upregulation in *A. thaliana* leaves was generally less pronounced
262 in *acs2-1 acs6-1* and/or *ein2-1* mutants compared to WT plants. These results suggest that Cd
263 stress leads to MRR, which may be (partially) mediated by ethylene that depends on the GSH-
264 dependent alarm (Figure 2).

265 Apart from the abovementioned organelles, the ER is also an important target of Cd toxicity.
266 Furthermore, it has interesting redox-related properties as it needs to maintain an oxidising
267 environment for oxidative protein folding, which also produces H₂O₂. Interestingly, the ER
268 does not contain glutathione reductase (GR), making the fate of GSSG in this compartment
269 unclear (Meyer *et al.*, 2021; Cao *et al.*, 2022). As such, the ER is an interesting organelle to

270 focus on during GSH-depleting Cd stress. Increased expression of ER stress markers was
271 found in *A. thaliana* seedlings (Xi *et al.*, 2016) and a *Nicotiana tabacum* cell line (Xu *et al.*,
272 2013) shortly after Cd exposure, suggesting that Cd induces ER stress in plants (Figure 2).
273 This occurs as a reaction to the accumulation of unfolded proteins in the ER, which can be
274 triggered by various stressors and activates the unfolded protein response (UPR). The UPR
275 consists of multiple arms of which the inositol requiring enzyme 1 (IRE1)-dependent splicing
276 of basic leucine zipper 60 (*bZIP60*) mRNA and the proteolytic cleavage of bZIP17 and
277 bZIP28 are the best-characterised in plants. Consequent processing of these bZIPs allows their
278 translocation to the nucleus, where they induce target gene expression to restore proteostasis
279 (Afrin *et al.*, 2020; Cao *et al.*, 2022; Simoni *et al.*, 2022). Although the exact mechanism of
280 UPR induction by Cd requires further characterisation, a role for SAL1 has been uncovered,
281 as a *sall* *A. thaliana* mutant showed less or no upregulation of ER stress response markers
282 compared to WT plants during Cd exposure (Xi *et al.*, 2016). For the generation of sulfate-
283 containing molecules during secondary sulfur metabolism, SAL1 degrades 3'-
284 phosphoadenosine 5'-phosphate (PAP), a by-product resulting from sulfate group donation to
285 target compounds, in chloroplasts and mitochondria (Chan *et al.*, 2019). The activity of this
286 enzyme is repressed by oxidation, allowing PAP accumulation to serve as a chloroplast
287 retrograde signal during oxidative stress (Van Aken and Pogson, 2017; Chan *et al.*, 2019).
288 Consequently, this pathway induces a set of target genes that partly overlaps with that of
289 ANAC017 (Van Aken and Pogson, 2017). This additionally suggests that organellar
290 retrograde signalling is involved in regulating the Cd-induced UPR, which is also in line with
291 the observations that mitochondrial or chloroplast ETC inhibitors influence the expression of
292 several ER stress markers (Ozgun *et al.*, 2015; Fuchs *et al.*, 2022) and that bZIP60 is a target
293 of ANAC017 (He *et al.*, 2022). Furthermore, evidence points towards a reciprocal interaction
294 between ROS and ER stress (Depaepe *et al.*, 2021; Cao *et al.*, 2022) and ethylene is also

295 involved in several ER stress outcomes (Depaepe *et al.*, 2021) and may be linked to UPR
296 induction (Malerba *et al.*, 2010; Czékus *et al.*, 2022). Such connections would hence be an
297 interesting topic for further study of Cd-induced stress responses.

298 Another outcome of ER stress is autophagy, as recently reviewed (Simoni *et al.*, 2022).
299 During macroautophagy, a double-membranous vesicle (i.e. an autophagosome) surrounds
300 cytoplasmic material and transports it to the vacuole for degradation and subsequent recycling
301 of released nutrients. Although important for growth and developmental processes, it is also
302 induced during stress responses to target damaged material (e.g. proteins and organelles) and
303 generally enhances plant stress tolerance (Signorelli *et al.*, 2019; Su *et al.*, 2020). Autophagic
304 bodies or their remnants have been observed in vacuoles of Cd-treated plants (Figure 4), such
305 as *Glycine max* (Gzyl *et al.*, 2017) and *Triticum aestivum* (Yue *et al.*, 2018), as well as in an
306 *N. tabacum* cell line (Kutik *et al.*, 2014). Moreover, short-term treatment of *A. thaliana* with
307 Cd led to increased transcript and protein levels of autophagy-related 8 (ATG8) (Calero-
308 Muñoz *et al.*, 2019). This ATG protein is involved in autophagosome formation and target
309 isolation and can, due to its presence on autophagosomal membranes following lipidation (Su
310 *et al.*, 2020), serve as an autophagy marker (Klionsky *et al.*, 2021). Although autophagosome
311 formation is a complex process involving a network of other autophagy proteins and post-
312 translational modifications (Su *et al.*, 2020), Calero-Muñoz *et al.* (2019) also observed an
313 autophagosomal membrane containing ATG8 surrounding peroxisomes in leaves of Cd-
314 stressed *A. thaliana*, indicating that Cd exposure triggers selective autophagy of peroxisomes
315 (i.e. pexophagy). While it was suggested that this process may occur due to peroxisomal
316 membrane protein oxidation as a result of Cd-induced ROS, this requires further investigation
317 (Calero-Muñoz *et al.*, 2019). Nevertheless, a reciprocal connection between ROS and
318 autophagy has frequently been observed and reviewed (Signorelli *et al.*, 2019; Su *et al.*,
319 2020). Hence, it is plausible that a link between redox alterations and autophagy also exists

320 during Cd-induced stress responses. Indeed, autophagy was shown to be involved in
321 controlling peroxisome numbers and might hence influence Cd-induced peroxisomal ROS
322 formation in *A. thaliana* leaves (Calero-Muñoz *et al.*, 2019). Furthermore, a reciprocal
323 connection exists between ethylene and autophagy (Su *et al.*, 2020) and other components of
324 the MAPK-ACS pathway (such as WRKY33 (Lai *et al.*, 2011) and ACC (Zhu *et al.*, 2018))
325 were found to positively modulate autophagy during stress or enhance ATG8-target
326 interaction (in case of MPK3 (Brillada *et al.*, 2021)). Additionally, other players may connect
327 autophagy and the Cd stress response. Treatment of ER-stressed *Chlamydomonas reinhardtii*
328 with GSH mitigated enhanced ATG8 accumulation. Here, GSH appears to play a specific
329 role, as supplementation with the antioxidative metabolite AsA did not affect the ER stress-
330 induced increased ATG8 abundance. Nevertheless, it remains to be determined whether this
331 occurs through redox changes or via an alternative effect of GSH on the ER (Pérez-Martín *et*
332 *al.*, 2014) and how this translates to autophagy in plants. Moreover, autophagy induction
333 during drought stress in leaves of ACC-pretreated *S. lycopersicum* was generally stronger in
334 *AOX1a*-overexpressing lines and lower in *AOX1a*-RNA interference lines compared to WT
335 plants. Overall, these effects were less pronounced when ROS levels in these mutants were
336 altered, suggesting that AOX affects autophagy by keeping ROS levels within an appropriate
337 range (Zhu *et al.*, 2018). Accordingly, overexpression of *AOX1a*-regulating *ANAC017* was
338 reported to increase the expression of several ATG genes (Meng *et al.*, 2019). While these
339 results further highlight the delicate balance between (organellar) ROS signalling and
340 autophagy, they again suggest that similar mechanisms may contribute to Cd-induced
341 autophagy and deserve further attention, as ACC/ethylene, GSH and *AOX1a* are important
342 players in Cd-induced stress responses (Figure 2).

343 Although ROS, ethylene and the retrograde signalling processes may contribute to plant
344 acclimation to Cd stress, it should be acknowledged that acclimation to stressors depends on

345 additional factors, such as the stress severity (Depaepe *et al.*, 2021). For instance, AOX1a was
346 found to contribute to limiting the upregulation of several oxidative stress marker genes and
347 increasing GSH levels in leaves of *A. thaliana* following exposure to 5 but not 10 μM Cd,
348 suggesting that more severe Cd stress overwhelms its protective function (Keunen *et al.*,
349 2015). Likewise, the UPR is tied to multiple outcomes with the first goal being the repair of
350 ER function, although this restorative function can switch to cell death following
351 uncontrollable ER stress (Afrin *et al.*, 2020; Simoni *et al.*, 2022). Overexpression of the ER
352 chaperone Binding immunoglobulin Protein (*BiP*) reduced Cd-induced cell death in an *N.*
353 *tabacum* cell line (Xu *et al.*, 2013) and *G. max* plants (Quadros *et al.*, 2022), and *LcBiP*
354 overexpression in Cd-exposed *N. tabacum* led to higher GSH levels in its leaves (Guan *et al.*,
355 2015b). Furthermore, there are indications that the UPR, in response to the ER stressor
356 tunicamycin, contributes to raising GSH levels in *A. thaliana* (Ozgur *et al.*, 2014) and *C.*
357 *reinhardtii* (Yamaoka *et al.*, 2018). Such observations suggest that the UPR contributes to
358 elevating GSH levels and to acclimation. Nevertheless, root growth of the *bzip28 bzip60 A.*
359 *thaliana* double mutant was less inhibited by Cd treatment compared to that of WT plants (Xi
360 *et al.*, 2016), again indicating that the outcome of the Cd-induced UPR differs depending on
361 the conditions. Furthermore, autophagy may tip the scale towards cell death if the balance
362 between the extent of autophagy and the maintenance of osmotic homeostasis is disrupted
363 (Signorelli *et al.*, 2019). In this process, called mega-autophagy, cell death often occurs as a
364 result of vacuolar hydrolase release into the cytoplasm (Signorelli *et al.*, 2019; Su *et al.*,
365 2020). Taken together, these results highlight the plasticity of organellar signalling during Cd
366 exposure.

367 **5 Is hydrogen sulfide involved in finetuning acclimation to cadmium**
368 **stress?**

369 Hydrogen sulfide is a small gasotransmitter with a simple molecular structure, which can
370 easily travel within and between cells. Although it is a signalling molecule, H₂S can become
371 phytotoxic at elevated intracellular concentrations due to restricted cell respiration as a result
372 of the inhibition of mitochondrial respiratory complex IV (Aroca *et al.*, 2021; Aroca and
373 Gotor, 2022). Its production in plants mainly occurs via three processes. First, in the sulfate
374 assimilation pathway, the enzymes sulfite reductase (SiR) in the chloroplast and O-
375 acetylserine-(thiol)lyase (OAS-TL) in the cytosol, chloroplasts and mitochondria produce H₂S
376 (Aroca *et al.*, 2018; Vojtovič *et al.*, 2021). Secondly, β-cyanoalanine synthase C1 (CAS-C1)
377 produces H₂S along with β-cyanoalanine in the mitochondria (Aroca *et al.*, 2018; Liu *et al.*,
378 2021). Finally, cysteine degradation by cysteine desulfhydrases, the most prominent being L-
379 cysteine desulfhydrase 1 (DES1), is responsible for the cytosolic production of H₂S along
380 with ammonia and pyruvate (Aroca *et al.*, 2018). Recently, the involvement of 3-
381 mercaptopyruvate sulfurtransferases (MSTs) in H₂S production was proposed (Moseler *et al.*,
382 2021). Nonetheless, their exact roles in this process are just being elucidated in plants and
383 hence require further investigation.

384 Once H₂S is produced within the cell, it can exert its effects by protein persulfidation. During
385 this post-translational modification, the thiol groups of reactive cysteine residues within target
386 proteins are converted to persulfide (-SSH) groups. Persulfide groups display a stronger
387 nucleophilic character compared to the original thiol groups, rendering the modified cysteines
388 more reactive. However, how H₂S specifically modifies certain targets remains elusive, as the
389 direct thiol-H₂S reaction is thermodynamically unfavourable. Upon persulfidation, proteins
390 display alterations in their enzymatic activities, structures and subcellular localization (Aroca
391 *et al.*, 2018; He *et al.*, 2018). Using the tag-switch method, Aroca *et al.* (2017) identified that

392 at least 5% of the entire proteome is persulfidated in *A. thaliana* leaves under physiological
393 conditions. These persulfidated proteins are involved in a broad range of processes related to,
394 among others, protein synthesis, post-translational modifications and degradation (Aroca *et*
395 *al.*, 2017).

396 Upon Cd exposure, the expression of L-cysteine desulphydrase (*LCD*) and *DESI* is increased
397 in *A. thaliana* roots, resulting in higher H₂S levels (Jia *et al.*, 2016). Additionally, mutants
398 with decreased LCD or D-cysteine desulphydrase (*DCD*) activity are more sensitive to Cd
399 stress, whereas overexpressors are less sensitive (Qiao *et al.*, 2015; Zhang *et al.*, 2020). In
400 order to assess the role of H₂S during Cd exposure, exogenous application of the H₂S donor
401 sodium hydrosulfide (NaHS) is frequently used. Supplementing plants with NaHS increased
402 their Cd tolerance as a result of a decreased Cd uptake, reduced Cd translocation from roots to
403 shoots and/or enhanced Cd retention within cell walls, as demonstrated in multiple plant
404 species (Table 3). Additionally, H₂S can mitigate Cd-induced oxidative stress through its
405 positive effects on antioxidant enzyme activities and GSH levels (Table 3), hereby
406 demonstrating that it is additionally involved in regulating the redox environment and related
407 signalling during Cd stress (Figure 2). In conclusion, H₂S appears to contribute to restoring
408 and maintaining GSH levels and restoring the redox environment following the initial Cd-
409 induced GSH depletion resulting from PC synthesis.

410 Apart from influencing thiol levels, H₂S may additionally influence Cd-induced ethylene
411 production and signalling. For example, postharvest *S. lycopersicum* fruits displayed reduced
412 *ACS* and *ACO* gene expression upon combined NaHS and ethephon application compared to
413 treatment with ethephon alone (Hu *et al.*, 2019). Additionally, NaHS supplementation resulted
414 in reduced *ACS* and *ACO* activities, a lower ACC content and eventually reduced ethylene
415 production in postharvest *Prunus persica* fruits (Zhu *et al.*, 2019). This suggests that H₂S
416 inhibits ethylene biosynthesis at the transcript and protein levels. Effects of H₂S on ethylene

417 during vegetative growth were also addressed by exposing *S. lycopersicum* seedlings to
418 NaHS. The results showed that NaHS decreased ethylene concentrations, probably as a
419 consequence of increased SIACO1 and SIACO2 persulfidation and negative regulation of
420 ACO activity (Jia *et al.*, 2018). To the best of our knowledge, no information on the
421 interactions between H₂S and ethylene during Cd stress is available. However, a model was
422 proposed by Sehar *et al.* (2022) in which heat stress induces oxidative stress, consequently
423 increasing sulfur assimilation, which results in increased H₂S and ethylene biosynthesis. The
424 increased H₂S and ethylene concentrations subsequently regulate antioxidants to scavenge
425 heat-induced ROS and thereby induce tolerance. Additionally, H₂S-induced ethylene
426 inhibition and additional crosstalk was suggested to be essential in heat tolerance (Sehar *et al.*,
427 2022). Furthermore, an interplay between H₂S and ethylene was recently demonstrated to
428 mitigate hexavalent chromium [Cr(VI)] stress in two *Vigna* species. Notably, the authors
429 demonstrated that H₂S acts downstream of ethylene as NaHS supplementation reduced the
430 Cr(VI)-induced increase in O₂^{•-} and H₂O₂ production resulting from inhibited ethylene
431 biosynthesis, whereas ethylene supplementation could not improve effects of inhibited H₂S
432 biosynthesis (Husain *et al.*, 2021). Consequently, it is possible that similar processes are
433 involved in the response to Cd stress. We propose that during Cd exposure, H₂S functions
434 downstream of ethylene to limit ethylene biosynthesis, avoiding long-term persistence of
435 increased ethylene levels which might not be beneficial during acclimation (Figure 1).

436 Aside from its regulatory effects on GSH and ethylene metabolism, H₂S affects other cellular
437 responses that can be important during plant acclimation to Cd stress, such as mitochondrial
438 homeostasis (Table 3) and autophagy. For instance, NaHS exposure induced the expression of
439 *AOX1a*, *AOX1b* and *AOX1c* in non-stressed *O. sativa* cell suspension cultures (Xiao *et al.*,
440 2010) and alleviated Cd-induced mitochondrial stress and cell death in *Cucumis sativus* root
441 tips (Luo *et al.*, 2021). Moreover, H₂S was reviewed to affect autophagy through protein

442 persulfidation (Aroca and Gotor, 2022). More specifically, it was shown that 17 *A. thaliana*
443 proteins with a key role in autophagy can be persulfidated (Jurado-Flores *et al.*, 2021).
444 Accordingly, in the *des1-1 A. thaliana* mutant, which has reduced endogenous H₂S levels,
445 autophagy induction appeared to be enhanced compared to WT plants under control
446 conditions (Álvarez *et al.*, 2012). Consequently, it can be concluded that H₂S negatively
447 regulates autophagy (Álvarez *et al.*, 2012; Aroca and Gotor, 2022). Although this might seem
448 contradictory to its potential protective function, we hypothesise that this regulation finetunes
449 autophagy during Cd stress to timely restore autophagic activity to baseline conditions and
450 prevent cell death as a consequence of uncontrolled autophagy.

451 **6 Conclusion and perspectives**

452 As sulfur is a pivotal component of the antioxidant GSH and metal-chelating PCs, it plays a
453 crucial role during the Cd response. As such, an early Cd-induced change in the redox
454 environment is initiated by PC-induced GSH depletion and leads to subsequent signalling
455 events (e.g. related to the phytohormone ethylene). The altered redox environment and
456 ethylene signalling appear interdependent and can also be associated with the induction of
457 organellar stress signalling (e.g. MRR and UPR) and autophagy. Although Cd-induced stress
458 responses have been extensively investigated, these processes are often addressed
459 individually, focusing on one specific cellular compartment, on whole plants or a certain plant
460 organ, as well as on a certain stage of exposure (e.g. the initial defence response or long-term
461 acclimation). As such, their interconnections are often overlooked and require further
462 investigation in multiple organelles and tissues and at different time points following Cd
463 exposure. Furthermore, it is highly relevant to further unravel the role of H₂S in Cd-induced
464 increases in GSH and PC levels. Additionally, we hypothesise that this molecule also
465 finetunes ethylene signalling, mitochondrial homeostasis and autophagy during acclimation in
466 Cd-exposed plants. Identification of persulfidated proteins in Cd-exposed plants will

467 significantly advance our understanding of the involvement of H₂S in plant responses to Cd
468 stress.

469

470

471 **Author contributions**

472 All authors contributed to the conceptualization of the review. VI and IV wrote
473 the original manuscript. AC and SH revised the outline and the final manuscript.

474

475 **Declaration of interests**

476 The authors have no conflicts to declare

477

478 **Funding**

479 This research was funded by Special Research Fund UHasselt (BOF) through a
480 PhD grant fellowship for Verena Iven (BOF18DOC02) and Isabeau Vanbuel
481 (BOF20DOC08). Additional funding came from the FWO project (G0C7518N)
482 and iBOF project (IBOF/23/070) to Ann Cuypers and Sophie Hendrix.

483

Table 1 Selected studies demonstrating a general increase in glutathione (GSH) biosynthesis upon prolonged exposure of various plant species to cadmium (Cd).

Plant	[Cd]	Exposure duration	Parameter	Reference
<i>Arabidopsis thaliana</i>	5 μ M CdSO ₄	1 - 8 d	transcripts GSH biosynthesis genes GSH levels	Hendrix <i>et al.</i> (2020)
<i>Brassica juncea</i>	50 μ M CdCl ₂	30 d	GSH levels	Khan <i>et al.</i> (2016b)
<i>Hordeum vulgare</i>	5 μ M CdCl ₂	1 - 25 d	GSH levels	Chen <i>et al.</i> (2010)
<i>Lycium chinense</i>	100 μ M CdCl ₂	3 - 24 h	transcripts GSH biosynthesis genes GSH levels	Guan <i>et al.</i> (2015a)
<i>Oryza sativa</i>	50 μ M CdCl ₂	0 - 7 d	GSH levels	Zhang <i>et al.</i> (2013)
<i>Populus x canescens</i>	75 μ M CdCl ₂	27 d	GSH levels	Ding <i>et al.</i> (2017)
<i>Triticum aestivum</i>	50 μ M CdCl ₂	10 d	transcripts GSH biosynthesis genes GSH levels	Li <i>et al.</i> (2021)

Table 2 Selected studies demonstrating a changed redox environment in mitochondria and/or chloroplasts of various plant species exposed to cadmium (Cd).

Plant	[Cd]	Exposure duration	Response	Reference
<i>Arabidopsis thaliana</i>	50, 100 μM CdSO ₄	12 - 96 h 7, 14 d	time-dependent changes in mitochondrial and chloroplastic GSH levels	Koffler <i>et al.</i> (2014)
	5 mM CdCl ₂	30 min	↑ mitochondrial roGFP2-oxidation	Schwarzländer <i>et al.</i> (2009)
	20 μM CdCl ₂	1, 2 h	↑ mitochondrial and chloroplastic ROS production in protoplasts	Bi <i>et al.</i> (2009)
<i>Cucumis sativus</i>	200 μM CdCl ₂	48 h	↑ mitochondrial H ₂ O ₂ level	Luo <i>et al.</i> (2021)
<i>Hordeum vulgare</i>	30 μM CdCl ₂	30 min	↑ O ₂ ^{•-} and H ₂ O ₂ production that is affected by mitochondrial complex III inhibition	Zelinová <i>et al.</i> (2019)
	10 - 60 μM CdCl ₂	30 min	↑ O ₂ ^{•-} and H ₂ O ₂ production that is affected by mitochondrial complex I inhibition	Tamás <i>et al.</i> (2016)
<i>Nicotiana tabacum</i> bright yellow-2 cells	3 mM CdCl ₂	1 h	↑ O ₂ ^{•-} production that is affected by mitochondrial complexes I-II and complex III inhibition	Garnier <i>et al.</i> (2006)
<i>Nicotiana tabacum</i>	2.24 mg.kg ⁻¹ CdCl ₂	10 d	altered transcription and enzymatic activity of redox regulators, including chloroplastic PRXs	Yang <i>et al.</i> (2021)
<i>Solanum tuberosum</i>	10, 30 μM CdCl ₂	5 min (for O ₂ ^{•-}) 30 min (for H ₂ O ₂)	↑ O ₂ ^{•-} and H ₂ O ₂ production in isolated mitochondria <i>in vitro</i>	Heyno <i>et al.</i> (2008)

Table 3 Selected studies demonstrating increased cadmium (Cd) tolerance upon sodium hydrosulfide (NaHS) supplementation as a consequence of increased chelation and antioxidative defence strategies in various plant species.

Plant	[Cd]	[NaHS]	Exposure duration	Response	Reference
<i>Arabidopsis thaliana</i>	50 μ M CdCl ₂	50 μ M	6 h	↑ GSH content	Jia <i>et al.</i> (2016)
<i>Brassica juncea</i>	50 μ M CdCl ₂	100 μ M	30 d	↑ antioxidant enzyme activity ↑ GSH and AsA content	Kaur <i>et al.</i> (2022)
<i>Brassica napus</i>	20 μ M CdCl ₂	50 μ M - pretreatment	1 week	↑ Cd retention in cell walls ↓ Cd translocation	Yu <i>et al.</i> (2019)
<i>Cynodon dactylon</i>	750 μ M CdCl ₂	500 μ M	1 d	↑ antioxidant enzyme activity ↑ GSH content	Shi <i>et al.</i> (2014)
<i>Hordeum vulgare</i>	5, 25 μ M CdCl ₂	200 μ M	25 d	↓ Cd translocation ↑ antioxidant enzyme activity	Fu <i>et al.</i> (2019)
<i>Isatis indigotica</i>	4.5 - 22.5 μ M CdCl ₂	50-200 μ M	14 d	↑ Cd retention in cell walls ↓ Cd translocation ↑ PC content ↓ Cd influx	Jia <i>et al.</i> (2020)
<i>Populus euphratica</i> cell culture	100 μ M CdCl ₂	50 μ M - pretreatment	24-72 h	↑ antioxidant enzyme activity ↑ vacuolar Cd ↓ Cd influx	Sun <i>et al.</i> (2013)
<i>Salix matsudana</i>	5 - 30 μ M CdCl ₂	0.3 mM	60 d	↑ antioxidant enzyme activity ↑ Cd retention in cell walls ↑ GSH content	Yang <i>et al.</i> (2018)
<i>Trigonella foenum- graecum</i>	1 - 2 mM CdCl ₂	100 - 200 μ M	2 weeks	↑ antioxidant enzyme activity ↑ phenolics and flavonoids	Javad <i>et al.</i> (2022)
<i>Zingiber officinale</i>	7.5 mg.l ⁻¹ CdCl ₂	0.8 mM	2, 4, 6, 8, 10 d	↑ antioxidant enzyme activity ↑ PCS transcription	Chen <i>et al.</i> (2022)

References

- Abozeid A, Ying ZJ, Lin YC, Liu J, Zhang ZH, Tang ZH.** 2017. Ethylene Improves Root System Development under Cadmium Stress by Modulating Superoxide Anion Concentration in *Arabidopsis thaliana*. *Frontiers in Plant Science* 8, 1-15.
- Afrin T, Diwan D, Sahawneh K, Pajerowska-Mukhtar K.** 2020. Multilevel regulation of endoplasmic reticulum stress responses in plants: where old roads and new paths meet. *Journal of Experimental Botany* 71 (5), 1659-1667.
- Álvarez C, García I, Moreno I, Pérez-Pérez ME, Crespo JL, Romero LC, Gotor C.** 2012. Cysteine-generated sulfide in the cytosol negatively regulates autophagy and modulates the transcriptional profile in *Arabidopsis*. *The Plant Cell* 24 (11), 4621-4634.
- Alves LR, Monteiro CC, Carvalho RF, Ribeiro PC, Tezotto T, Azevedo RA, Gratao PL.** 2017. Cadmium stress related to root-to-shoot communication depends on ethylene and auxin in tomato plants. *Environmental and Experimental Botany* 134, 102-115.
- Aroca A, Benito JM, Gotor C, Romero LC.** 2017. Persulfidation proteome reveals the regulation of protein function by hydrogen sulfide in diverse biological processes in *Arabidopsis*. *Journal of Experimental Botany* 68 (17), 4915-4927.
- Aroca A, Gotor C.** 2022. Hydrogen Sulfide: A Key Role in Autophagy Regulation from Plants to Mammals. *Antioxidants* 11 (2), 327.
- Aroca A, Gotor C, Romero LC.** 2018. Hydrogen Sulfide Signaling in Plants: Emerging Roles of Protein Persulfidation. *Frontiers in Plant Science* 9, 1369.
- Aroca A, Zhang J, Xie Y, Romero LC, Gotor C.** 2021. Hydrogen sulfide signaling in plant adaptations to adverse conditions: molecular mechanisms. *Journal of Experimental Botany* 72 (16), 5893-5904.
- Bahmani R, Kim D, Modareszadeh M, Hwang S.** 2022. Cadmium enhances root hair elongation through reactive oxygen species in *Arabidopsis*. *Environmental and Experimental Botany* 196, 104813.
- Bi YH, Chen WL, Zhang WN, Zhou Q, Yun LJ, Xing D.** 2009. Production of reactive oxygen species, impairment of photosynthetic function and dynamic changes in mitochondria are early events in cadmium-induced cell death in *Arabidopsis thaliana*. *Biology of the Cell* 101 (11), 629-643.
- Bittner A, Cieřła A, Gruden K, Lukan T, Mahmud S, Teige M, Vothknecht UC, Wurzinger B.** 2022. Organelles and phytohormones: a network of interactions in plant stress responses. *Journal of Experimental Botany* 73 (21), 7165-7181.
- Brillada C, Teh OK, Ditengou FA, et al.** 2021. Exocyst subunit Exo70B2 is linked to immune signaling and autophagy. *The Plant Cell* 33 (2), 404-419.
- Calero-Muñoz N, Exposito-Rodríguez M, Collado-Arenal AM, et al.** 2019. Cadmium induces reactive oxygen species-dependent autophagy in *Arabidopsis* leaves. *Plant Cell and Environment* 42 (9), 2696-2714.

- Cao J, Wang C, Hao N, Fujiwara T, Wu T.** 2022. Endoplasmic Reticulum Stress and Reactive Oxygen Species in Plants. *Antioxidants* 11 (7), 1240.
- Cejudo FJ, González M-C, Pérez-Ruiz JM.** 2021. Redox regulation of chloroplast metabolism. *Plant Physiology* 186 (1), 9-21.
- Chan KX, Phua SY, Van Breusegem F.** 2019. Secondary sulfur metabolism in cellular signalling and oxidative stress responses. *Journal of Experimental Botany* 70 (16), 4237-4250.
- Chen F, Wang F, Wu F, Mao W, Zhang G, Zhou M.** 2010. Modulation of exogenous glutathione in antioxidant defense system against Cd stress in the two barley genotypes differing in Cd tolerance. *Plant Physiol Biochem* 48 (8), 663-672.
- Chen J, Wang X, Zhang WW, Zhang SQ, Zhao FJ.** 2020. Protein phosphatase 2A alleviates cadmium toxicity by modulating ethylene production in *Arabidopsis thaliana*. *Plant Cell and Environment* 43 (4), 1008-1022.
- Chen Q, Wu K, Tang Z, Guo Q, Guo X, Wang H.** 2017. Exogenous ethylene enhanced the cadmium resistance and changed the alkaloid biosynthesis in *Catharanthus roseus* seedlings. *Acta Physiologiae Plantarum* 39 (12), 267.
- Chen ZJ, Liu CY, Cao BL, Xu K.** 2022. A hydrogen sulfide application can alleviate the toxic effects of cadmium on ginger (*Zingiber officinale* Roscoe). *Environmental Science and Pollution Research* 29, 68422-68431.
- Clemens S, Aarts MGM, Thomine S, Verbruggen N.** 2013. Plant science: the key to preventing slow cadmium poisoning. *Trends in Plant Science* 18 (2), 92-99.
- Cobbett CS.** 2000. Phytochelatins and their roles in heavy metal detoxification. *Plant Physiology* 123 (3), 825-832.
- Czékus Z, Szalai G, Tari I, Khan MIR, Poór P.** 2022. Role of ethylene in ER stress and the unfolded protein response in tomato (*Solanum lycopersicum* L.) plants. *Plant Physiology and Biochemistry* 181, 1-11.
- De Clercq I, Vermeirssen V, Van Aken O, et al.** 2013. The membrane-bound NAC transcription factor ANAC013 functions in mitochondrial retrograde regulation of the oxidative stress response in *Arabidopsis*. *The Plant Cell* 25 (9), 3472-3490.
- De Smet S, Cuypers A, Vangronsveld J, Remans T.** 2015. Gene Networks Involved in Hormonal Control of Root Development in *Arabidopsis thaliana*: A Framework for Studying Its Disturbance by Metal Stress. *International Journal of Molecular Sciences* 16 (8), 19195-19224.
- Deckers J, Hendrix S, Prinsen E, Vangronsveld J, Cuypers A.** 2020. Identifying the Pressure Points of Acute Cadmium Stress Prior to Acclimation in *Arabidopsis thaliana*. *International Journal of Molecular Sciences* 21 (17), 6232.
- Deckers J, Hendrix S, Prinsen E, Vangronsveld J, Cuypers A.** 2021. Glutathione Is Required for the Early Alert Response and Subsequent Acclimation in Cadmium-Exposed *Arabidopsis thaliana* Plants. *Antioxidants* 11 (1), 6.

Depaepe T, Hendrix S, Janse van Rensburg HC, Van den Ende W, Cuypers A, Van Der Straeten D. 2021. At the Crossroads of Survival and Death: The Reactive Oxygen Species-Ethylene-Sugar Triad and the Unfolded Protein Response. *Trends in Plant Science* 26 (4), 338-351.

Ding S, Ma C, Shi W, Liu W, Lu Y, Liu Q, Luo Z-B. 2017. Exogenous glutathione enhances cadmium accumulation and alleviates its toxicity in *Populus × canescens*. *Tree Physiology* 37 (12), 1697-1712.

El Rasafi T, Ouakarroum A, Haddioui A, Song H, Kwon EE, Bolan N, Tack FMG, Sebastian A, Prasad MNV, Rinklebe J. 2022. Cadmium stress in plants: A critical review of the effects, mechanisms, and tolerance strategies. *Critical Reviews in Environmental Science and Technology* 52 (5), 675-726.

Fu M-M, Dawood M, Wang N-H, Wu F. 2019. Exogenous hydrogen sulfide reduces cadmium uptake and alleviates cadmium toxicity in barley. *Plant Growth Regulation* 89, 227-237.

Fuchs P, Bohle F, Lichtenauer S, et al. 2022. Reductive stress triggers ANAC017-mediated retrograde signaling to safeguard the endoplasmic reticulum by boosting mitochondrial respiratory capacity. *The Plant Cell* 34 (4), 1375-1395.

Garnier L, Simon-Plas F, Thuleau P, Agnel JP, Blein JP, Ranjeva R, Montillet JL. 2006. Cadmium affects tobacco cells by a series of three waves of reactive oxygen species that contribute to cytotoxicity. *Plant Cell and Environment* 29 (10), 1956-1969.

Guan CF, Ji J, Wu DY, Li XZ, Jin C, Guan WZ, Wang G. 2015a. The glutathione synthesis may be regulated by cadmium-induced endogenous ethylene in *Lycium chinense*, and overexpression of an ethylene responsive transcription factor gene enhances tolerance to cadmium stress in tobacco. *Molecular Breeding* 35 (5), 123.

Guan CF, Jin C, Ji J, Wang G, Li X. 2015b. *LcBiP*, a endoplasmic reticulum chaperone binding protein gene from *Lycium chinense*, confers cadmium tolerance in transgenic tobacco. *Biotechnology Progress* 31 (2), 358-368.

Gzyl J, Chmielowska-Bak J, Przymusinski R. 2017. Gamma-tubulin distribution and ultrastructural changes in root cells of soybean (*Glycine max* L.) seedlings under cadmium stress. *Environmental and Experimental Botany* 143, 82-90.

Haider FU, Liqun C, Coulter JA, Cheema SA, Wu J, Zhang R, Wenjun M, Farooq M. 2021. Cadmium toxicity in plants: Impacts and remediation strategies. *Ecotoxicology and Environmental Safety* 211, 111887.

Hasanuzzaman M, Bhuyan M, Anee TI, Parvin K, Nahar K, Mahmud JA, Fujita M. 2019. Regulation of Ascorbate-Glutathione Pathway in Mitigating Oxidative Damage in Plants under Abiotic Stress. *Antioxidants* 8 (9), 384.

He C, Liew LC, Yin L, Lewsey MG, Whelan J, Berkowitz O. 2022. The retrograde signalling regulator ANAC017 recruits the MKK9-MPK3/6, ethylene, and auxin signalling pathways to balance mitochondrial dysfunction with growth. *The Plant Cell* 34 (9), 3460-3481.

He H, Li Y, He L-F. 2018. The central role of hydrogen sulfide in plant responses to toxic metal stress. *Ecotoxicology and Environmental Safety* 157, 403-408.

Hendrix S, Iven V, Eekhout T, Huybrechts M, Pecqueur I, Horemans N, Keunen E, De Veylder L, Vangronsveld J, Cuypers A. 2020. Suppressor of Gamma Response 1 Modulates the DNA Damage Response and Oxidative Stress Response in Leaves of Cadmium-Exposed *Arabidopsis thaliana*. *Frontiers in Plant Science* 11, 366.

Heyno E, Klose C, Krieger-Liszkay A. 2008. Origin of cadmium-induced reactive oxygen species production: mitochondrial electron transfer versus plasma membrane NADPH oxidase. *New Phytologist* 179 (3), 687-699.

Hu KD, Zhang XY, Wang SS, et al. 2019. Hydrogen Sulfide Inhibits Fruit Softening by Regulating Ethylene Synthesis and Signaling Pathway in Tomato (*Solanum lycopersicum*). *HORTSCIENCE* 54 (10), 1824-1830.

Husain T, Suhel M, Prasad SM, Singh VP. 2021. Ethylene needs endogenous hydrogen sulfide for alleviating hexavalent chromium stress in *Vigna mungo* L. and *Vigna radiata* L. *Environmental Pollution* 290, 117968.

Jalmi SK, Bhagat PK, Verma D, Noryang S, Tayyeba S, Singh K, Sharma D, Sinha AK. 2018. Traversing the Links between Heavy Metal Stress and Plant Signaling. *Frontiers in Plant Science* 9.

Jan M, Liu Z, Rochaix J-D, Sun X. 2022. Retrograde and anterograde signaling in the crosstalk between chloroplast and nucleus. *Frontiers in Plant Science* 13.

Javad S, Shah AA, Ramzan M, et al. 2022. Hydrogen sulphide alleviates cadmium stress in *Trigonella foenum-graecum* by modulating antioxidant enzymes and polyamine content. *Plant Biology* 24 (4), 618-626.

Jia H, Chen S, Liu D, et al. 2018. Ethylene-Induced Hydrogen Sulfide Negatively Regulates Ethylene Biosynthesis by Persulfidation of ACO in Tomato Under Osmotic Stress. *Frontiers in Plant Science* 9, 1517.

Jia H, Wang X, Shi C, Guo J, Ma P, Ren X, Wei T, Liu H, Li J. 2020. Hydrogen sulfide decreases Cd translocation from root to shoot through increasing Cd accumulation in cell wall and decreasing Cd²⁺ influx in *Isatis indigotica*. *Plant Physiology Biochemistry* 155, 605-612.

Jia HL, Wang XF, Dou YH, Liu D, Si WT, Fang H, Zhao C, Chen SL, Xi JJ, Li JS. 2016. Hydrogen sulfide - cysteine cycle system enhances cadmium tolerance through alleviating cadmium-induced oxidative stress and ion toxicity in *Arabidopsis* roots. *Scientific Reports* 6, 39702.

Jin C-W, Mao Q-Q, Luo B-F, Lin X-Y, Du S-T. 2013. Mutation of *mpk6* enhances cadmium tolerance in *Arabidopsis* plants by alleviating oxidative stress. *Plant and Soil* 371 (1), 387-396.

Jobe TO, Sung DY, Akmakjian G, Pham A, Komives EA, Mendoza-Cozatl DG, Schroeder JI. 2012. Feedback inhibition by thiols outranks glutathione depletion: a luciferase-based screen reveals glutathione-deficient γ -ECS and glutathione synthetase mutants impaired in cadmium-induced sulfate assimilation. *The Plant Journal* 70 (5), 783-795.

Jozefczak M, Keunen E, Schat H, Blik M, Hernandez LE, Carleer R, Remans T, Bohler S, Vangronsveld J, Cuypers A. 2014. Differential response of *Arabidopsis* leaves and roots to cadmium: Glutathione-related chelating capacity vs antioxidant capacity. *Plant Physiology and Biochemistry* 83, 1-9.

Jozefczak M, Remans T, Vangronsveld J, Cuypers A. 2012. Glutathione Is a Key Player in Metal-Induced Oxidative Stress Defenses. *International Journal of Molecular Sciences* 13 (3), 3145-3175, 10.3390/ijms13033145.

Jurado-Flores A, Romero LC, Gotor C. 2021. Label-Free Quantitative Proteomic Analysis of Nitrogen Starvation in *Arabidopsis* Root Reveals New Aspects of H₂S Signaling by Protein Persulfidation. *Antioxidants (Basel)* 10 (4), 508.

Kacprzak SM, Dahlqvist A, Van Aken O. 2020. The transcription factor ANAC017 is a key regulator of mitochondrial proteotoxic stress responses in plants. *Philosophical Transactions of the Royal Society of London* 375 (1801), 20190411.

Kaur H, Hussain SJ, Al-Huqail AA, Siddiqui MH, Al-Huqail AA, Khan MIR. 2022. Hydrogen sulphide and salicylic acid regulate antioxidant pathway and nutrient balance in mustard plants under cadmium stress. *Plant Biology* 24 (4), 660-669.

Keunen E, Remans T, Bohler S, Vangronsveld J, Cuypers A. 2011. Metal-Induced Oxidative Stress and Plant Mitochondria. *International Journal of Molecular Sciences* 12 (10), 6894-6918.

Keunen E, Schellingen K, Van Der Straeten D, Remans T, Colpaert J, Vangronsveld J, Cuypers A. 2015. ALTERNATIVE OXIDASE1a modulates the oxidative challenge during moderate Cd exposure in *Arabidopsis thaliana* leaves. *Journal of Experimental Botany* 66 (10), 2967-2977.

Keunen E, Schellingen K, Vangronsveld J, Cuypers A. 2016. Ethylene and Metal Stress: Small Molecule, Big Impact. *Frontiers in Plant Science* 7, 23.

Khan MIR, Iqbal N, Masood A, Mobin M, Anjum NA, Khan NA. 2016a. Modulation and significance of nitrogen and sulfur metabolism in cadmium challenged plants. *Plant Growth Regulation* 78 (1), 1-11.

Khan NA, Asgher M, Per TS, Masood A, Fatma M, Khan MIR. 2016b. Ethylene Potentiates Sulfur-Mediated Reversal of Cadmium Inhibited Photosynthetic Responses in Mustard. *Frontiers in Plant Science* 7, 15.

Klionsky DJ, Abdel-Aziz AK, Abdelfatah S, et al. 2021. Guidelines for the use and interpretation of assays for monitoring autophagy (4th edition). *Autophagy* 17 (1), 1-382.

Koffler BE, Polanschütz L, Zechmann B. 2014. Higher sensitivity of *pad2-1* and *vtc2-1* mutants to cadmium is related to lower subcellular glutathione rather than ascorbate contents. *Protoplasma* 251 (4), 755-769.

Kolb D, Müller M, Zellnig G, Zechmann B. 2010. Cadmium induced changes in subcellular glutathione contents within glandular trichomes of *Cucurbita pepo* L. *Protoplasma* 243 (1-4), 87-94.

- Kutik J, Kuthanova A, Smertenko A, Fischer L, Opatrny Z.** 2014. Cadmium-induced cell death in BY-2 cell culture starts with vacuolization of cytoplasm and terminates with necrosis. *Physiologia Plantarum* 151 (4), 423-433.
- Lai ZB, Wang F, Zheng ZY, Fan BF, Chen ZX.** 2011. A critical role of autophagy in plant resistance to necrotrophic fungal pathogens. *The Plant Journal* 66 (6), 953-968.
- Li G-Z, Chen S-J, Li N-Y, Wang Y-Y, Kang G-Z.** 2021. Exogenous Glutathione Alleviates Cadmium Toxicity in Wheat by Influencing the Absorption and Translocation of Cadmium. *Bulletin of Environmental Contamination and Toxicology* 107 (2), 320-326.
- Li G, Meng X, Wang R, Mao G, Han L, Liu Y, Zhang S.** 2012. Dual-level regulation of ACC synthase activity by MPK3/MPK6 cascade and its downstream WRKY transcription factor during ethylene induction in *Arabidopsis*. *PLOS Genetics* 8 (6), e1002767.
- Li M, Hao PF, Cao FB.** 2017. Glutathione-induced alleviation of cadmium toxicity in *Zea mays*. *Plant Physiology and Biochemistry* 119, 240-249.
- Lin Y-F, Aarts MGM.** 2012. The molecular mechanism of zinc and cadmium stress response in plants. *Cellular and Molecular Life Sciences* 69 (19), 3187-3206.
- Liu H, Wang J, Liu J, Liu T, Xue S.** 2021. Hydrogen sulfide (H₂S) signaling in plant development and stress responses. *aBIOTECH* 2 (1), 32-63.
- Liu M, Huang Z, Xie K, Guo C, Wang Y, Wang X.** 2023. Mitostasis is the central biological hub underlying the response of plants to cadmium stress. *Journal of Hazardous Materials* 441, 129930, <https://doi.org/10.1016/j.jhazmat.2022.129930>.
- Liu X-M, Kim KE, Kim K-C, et al.** 2010. Cadmium activates *Arabidopsis* MPK3 and MPK6 via accumulation of reactive oxygen species. *Phytochemistry* 71 (5), 614-618.
- Liu Y, He C.** 2017. A review of redox signaling and the control of MAP kinase pathway in plants. *Redox Biology* 11, 192-204.
- Luo S, Tang Z, Yu J, Liao W, Xie J, Lv J, Liu Z, Calderón-Urrea A.** 2021. Hydrogen Sulfide Inhibits Cadmium-Induced Cell Death of Cucumber Seedling Root Tips by Protecting Mitochondrial Physiological Function. *Journal of Plant Growth Regulation*.
- Malerba M, Crosti P, Cerana R.** 2010. Ethylene is involved in stress responses induced by fusaric acid in sycamore cultured cells. *Journal of Plant Physiology* 167 (17), 1442-1447.
- Mendoza-Cózatl D, Loza-Tavera H, Hernández-Navarro A, Moreno-Sánchez R.** 2005. Sulfur assimilation and glutathione metabolism under cadmium stress in yeast, protists and plants. *Fems Microbiology Reviews* 29 (4), 653-671.
- Meng XX, Li L, De Clercq I, et al.** 2019. ANAC017 Coordinates Organellar Functions and Stress Responses by Reprogramming Retrograde Signaling. *Plant Physiology* 180 (1), 634-653.
- Meyer AJ, Dreyer A, Ugalde JM, Feitosa-Araujo E, Dietz K-J, Schwarzländer M.** 2021. Shifting paradigms and novel players in Cys-based redox regulation and ROS signaling in plants - and where to go next. *Biological Chemistry* 402 (3), 399-423.

Moseler A, Dhalleine T, Rouhier N, Couturier J. 2021. *Arabidopsis thaliana* 3-mercaptopyruvate sulfurtransferases interact with and are protected by reducing systems. *Journal of Biological Chemistry* 296, 100429.

Ng S, Ivanova A, Duncan O, et al. 2013. A Membrane-Bound NAC Transcription Factor, ANAC017, Mediates Mitochondrial Retrograde Signaling in *Arabidopsis*. *The Plant Cell* 25 (9), 3450-3471.

Ozgun R, Turkan I, Uzilday B, Sekmen AH. 2014. Endoplasmic reticulum stress triggers ROS signalling, changes the redox state, and regulates the antioxidant defence of *Arabidopsis thaliana*. *Journal of Experimental Botany* 65 (5), 1377-1390.

Ozgun R, Uzilday B, Sekmen AH, Turkan I. 2015. The effects of induced production of reactive oxygen species in organelles on endoplasmic reticulum stress and on the unfolded protein response in *Arabidopsis*. *Annals of Botany* 116 (4), 541-553.

Park J, Song WY, Ko D, Eom Y, Hansen TH, Schiller M, Lee TG, Martinoia E, Lee Y. 2012. The phytochelatin transporters AtABCC1 and AtABCC2 mediate tolerance to cadmium and mercury. *The Plant Journal* 69 (2), 278-288.

Parmar P, Kumari N, Sharma V. 2013. Structural and functional alterations in photosynthetic apparatus of plants under cadmium stress. *Botanical Studies* 54 (45), 6.

Pattyn J, Vaughan-Hirsch J, Van de Poel B. 2021. The regulation of ethylene biosynthesis: a complex multilevel control circuitry. *New Phytologist* 229 (2), 770-782.

Pérez-Martín M, Pérez-Pérez ME, Lemaire SD, Crespo JL. 2014. Oxidative stress contributes to autophagy induction in response to endoplasmic reticulum stress in *Chlamydomonas reinhardtii*. *Plant Physiology* 166 (2), 997-1008.

Qiao Z, Jing T, Liu Z, Zhang L, Jin Z, Liu D, Pei Y. 2015. H₂S acting as a downstream signaling molecule of SA regulates Cd tolerance in *Arabidopsis*. *Plant and Soil* 393 (1), 137-146.

Quadros IPS, Madeira NN, Loriato VAP, et al. 2022. Cadmium-mediated toxicity in plant cells is associated with the DCD/NRP-mediated cell death response. *Plant, Cell and Environment* 45 (2), 556-571.

Rentel MC, Lecourieux D, Ouaked F, et al. 2004. OXI1 kinase is necessary for oxidative burst-mediated signalling in *Arabidopsis*. *Nature* 427 (6977), 858-861.

Saini S, Kaur N, Pati PK. 2021. Phytohormones: Key players in the modulation of heavy metal stress tolerance in plants. *Ecotoxicology and Environmental Safety* 223, 14.

Schellingen K, Van Der Straeten D, Remans T, Loix C, Vangronsveld J, Cuypers A. 2015a. Ethylene biosynthesis is involved in the early oxidative challenge induced by moderate Cd exposure in *Arabidopsis thaliana*. *Environmental and Experimental Botany* 117, 1-11.

Schellingen K, Van Der Straeten D, Remans T, Vangronsveld J, Keunen E, Cuypers A. 2015b. Ethylene signalling is mediating the early cadmium-induced oxidative challenge in *Arabidopsis thaliana*. *Plant Science* 239, 137-146.

Schellingen K, Van Der Straeten D, Vandenbussche F, Prinsen E, Remans T, Vangronsveld J, Cuypers A. 2014. Cadmium-induced ethylene production and responses in *Arabidopsis thaliana* rely on *ACS2* and *ACS6* gene expression. *Bmc Plant Biology* 14, 14.

Schwarzländer M, Fricker MD, Sweetlove LJ. 2009. Monitoring the *in vivo* redox state of plant mitochondria: Effect of respiratory inhibitors, abiotic stress and assessment of recovery from oxidative challenge. *Biochimica Et Biophysica Acta-Bioenergetics* 1787 (5), 468-475.

Sehar Z, Gautam H, Iqbal N, Alvi AF, Jahan B, Fatma M, Albaqami M, Khan NA. 2022. The Functional Interplay between Ethylene, Hydrogen Sulfide, and Sulfur in Plant Heat Stress Tolerance. *Biomolecules* 12 (5).

Sharma R, Bhardwaj R, Handa N, et al. 2016. Chapter 10 - Responses of Phytochelatins and Metallothioneins in Alleviation of Heavy Metal Stress in Plants: An Overview. In: Ahmad P, ed. *Plant Metal Interaction: Emerging Remediation Techniques*: Elsevier, 263-283.

Shi H, Ye T, Chan Z. 2014. Nitric oxide-activated hydrogen sulfide is essential for cadmium stress response in bermudagrass (*Cynodon dactylon* (L). Pers.). *Plant Physiology and Biochemistry* 74, 99-107.

Signorelli S, Tarkowski LP, Van den Ende W, Bassham DC. 2019. Linking Autophagy to Abiotic and Biotic Stress Responses. *Trends in Plant Science* 24 (5), 413-430.

Simoni EB, Oliveira CC, Fraga OT, Reis PAB, Fontes EPB. 2022. Cell Death Signaling From Endoplasmic Reticulum Stress: Plant-Specific and Conserved Features. *Frontiers in Plant Science* 13, 15.

Song X, Yue X, Chen W, Jiang H, Han Y, Li X. 2019. Detection of Cadmium Risk to the Photosynthetic Performance of Hybrid *Pennisetum*. *Frontiers in Plant Science* 10.

Sterckeman T, Thomine S. 2020. Mechanisms of Cadmium Accumulation in Plants. *Critical Reviews in Plant Sciences* 39 (4), 322-359.

Su T, Li XZ, Yang MY, Shao Q, Zhao YX, Ma CL, Wang PP. 2020. Autophagy: An Intracellular Degradation Pathway Regulating Plant Survival and Stress Response. *Frontiers in Plant Science* 11, 16.

Sun J, Wang R, Zhang X, Yu Y, Zhao R, Li Z, Chen S. 2013. Hydrogen sulfide alleviates cadmium toxicity through regulations of cadmium transport across the plasma and vacuolar membranes in *Populus euphratica* cells. *Plant Physiology and Biochemistry* 65, 67-74.

Tamás L, Mistrík I, Zelinová V. 2016. Cadmium activates both diphenylethylideneiodonium- and rotenone-sensitive superoxide production in barley root tips. *Planta* 244 (6), 1277-1287.

Van Aken O, De Clercq I, Ivanova A, Law SR, Van Breusegem F, Millar AH, Whelan J. 2016. Mitochondrial and Chloroplast Stress Responses Are Modulated in Distinct Touch and Chemical Inhibition Phases. *Plant Physiology* 171 (3), 2150-2165.

Van Aken O, Pogson BJ. 2017. Convergence of mitochondrial and chloroplastic ANAC017/PAP-dependent retrograde signalling pathways and suppression of programmed cell death. *Cell Death and Differentiation* 24 (6), 955-960.

Vogel MO, Moore M, König K, Pecher P, Alsharafa K, Lee J, Dietz K-J. 2014. Fast retrograde signaling in response to high light involves metabolite export, MITOGEN-ACTIVATED PROTEIN KINASE6, and AP2/ERF transcription factors in *Arabidopsis*. *The Plant Cell* 26 (3), 1151-1165.

Vojtovič D, Luhová L, Petřivalský M. 2021. Something smells bad to plant pathogens: Production of hydrogen sulfide in plants and its role in plant defence responses. *Journal of Advanced Research* 27, 199-209.

Wang X, Auwerx J. 2017. Systems Phytohormone Responses to Mitochondrial Proteotoxic Stress. *Molecular Cell* 68 (3), 540-551.

Xi HM, Xu H, Xu WX, He ZY, Xu WZ, Ma M. 2016. A SAL1 Loss-of-Function *Arabidopsis* Mutant Exhibits Enhanced Cadmium Tolerance in Association with Alleviation of Endoplasmic Reticulum Stress. *Plant and Cell Physiology* 57 (6), 1210-1219.

Xiao M, Ma J, Li H, Jin H, Feng H. 2010. Effects of Hydrogen Sulfide on Alternative Pathway Respiration and Induction of Alternative Oxidase Gene Expression in Rice Suspension Cells. *Zeitschrift für Naturforschung C* 65 (7-8), 463-471.

Xu H, Xu WZ, Xi HM, Ma WW, He ZY, Ma M. 2013. The ER luminal binding protein (BiP) alleviates Cd²⁺-induced programmed cell death through endoplasmic reticulum stress-cell death signaling pathway in tobacco cells. *Journal of Plant Physiology* 170 (16), 1434-1441.

Yamaoka Y, Choi BY, Kim H, et al. 2018. Identification and functional study of the endoplasmic reticulum stress sensor IRE1 in *Chlamydomonas reinhardtii*. *The Plant Journal* 94 (1), 91-104.

Yang F, Zhang H, Wang Y, He G, Wang J, Guo D, Li T, Sun G, Zhang H. 2021. The role of antioxidant mechanism in photosynthesis under heavy metals Cd or Zn exposure in tobacco leaves. *Journal of Plant Interactions* 16 (1), 354-366.

Yang L, Zeng J, Wang P, Zhu J. 2018. Sodium hydrosulfide alleviates cadmium toxicity by changing cadmium chemical forms and increasing the activities of antioxidant enzymes in *salix*. *Environmental and Experimental Botany* 156, 161-169.

Yeh C-M, Chien P-S, Huang H-J. 2007. Distinct signalling pathways for induction of MAP kinase activities by cadmium and copper in rice roots. *Journal of Experimental Botany* 58 (3), 659-671.

Yu JP, Nickels R, McIntosh L. 2001. A genome approach to mitochondrial-nuclear communication in *Arabidopsis*. *Plant Physiology and Biochemistry* 39 (3-4), 345-353.

Yu Y, Zhou X, Zhu Z, Zhou K. 2019. Sodium Hydrosulfide Mitigates Cadmium Toxicity by Promoting Cadmium Retention and Inhibiting Its Translocation from Roots to Shoots in *Brassica napus*. *Journal of Agricultural and Food Chemistry* 67 (1), 433-440.

Yue JY, Wei XJ, Wang HZ. 2018. Cadmium tolerant and sensitive wheat lines: their differences in pollutant accumulation, cell damage, and autophagy. *Biologia Plantarum* 62 (2), 379-387.

Zechmann B. 2014. Compartment-specific importance of glutathione during abiotic and biotic stress. *Frontiers in Plant Science* 5, 11.

Zelinová V, Demecsová L, Tamás L. 2019. Impact of antimycin A and myxothiazol on cadmium-induced superoxide, hydrogen peroxide, and nitric oxide generation in barley root tip. *Protoplasma* 256 (5), 1375-1383.

Zhang C, Yin X, Gao K, Ge Y, Cheng W. 2013. Non-protein thiols and glutathione S-transferase alleviate Cd stress and reduce root-to-shoot translocation of Cd in rice. *Journal of Plant Nutrition and Soil Science* 176 (4), 626-633.

Zhang Q, Cai W, Ji T-T, Ye L, Lu Y-T, Yuan T-T. 2020. WRKY13 Enhances Cadmium Tolerance by Promoting *D-CYSTEINE DESULFHYDRASE* and Hydrogen Sulfide Production. *Plant Physiology* 183 (1), 345-357.

Zhu L, Du H, Wang W, Zhang W, Shen Y, Wan C, Chen J. 2019. Synergistic effect of nitric oxide with hydrogen sulfide on inhibition of ripening and softening of peach fruits during storage. *Scientia Horticulturae* 256, 108591.

Zhu T, Li L, Duan Q, Liu X, Chen M. 2021. Progress in our understanding of plant responses to the stress of heavy metal cadmium. *Plant Signaling & Behavior* 16 (1), 1836884.

Zhu T, Zou LJ, Li Y, Yao XH, Xu F, Deng XG, Zhang DW, Lin HH. 2018. Mitochondrial alternative oxidase-dependent autophagy involved in ethylene-mediated drought tolerance in *Solanum lycopersicum*. *Plant Biotechnology Journal* 16 (12), 2063-2076.

Zulfiqar U, Ayub A, Hussain S, Waraich EA, El-Esawi MA, Ishfaq M, Ahmad M, Ali N, Maqsood MF. 2022. Cadmium Toxicity in Plants: Recent Progress on Morpho-physiological Effects and Remediation Strategies. *Journal of Soil Science and Plant Nutrition* 22, 212-269.

Figure legends

Figure 1: Proposed sequence of events of the mild cadmium (Cd) stress response in plants, showing the glutathione (GSH)-dependent alarm phase, a subsequent signalling phase and an acclimation phase. The GSH-dependent alarm, characterised by a phytochelatin (PC)-induced GSH depletion, triggers ethylene (ET) production and signalling. The subsequent signalling phase is additionally associated with organellar stress responses, elevated reactive oxygen species (ROS) production and autophagy, which are intertwined. Glutathione production is gradually increased to control ROS levels and maintain PC production, while autophagy might be involved in resolving Cd-induced organellar stress. Following prolonged Cd exposure, signalling responses attenuate and both GSH and PC levels stabilise to reach an acclimation state. During this phase, hydrogen sulfide (H₂S) may be involved in maintaining GSH and PC production, as well as regulating signalling events. Full lines are based on established interactions, whereas dashed lines indicate interactions that are more speculative.

Figure 2: Hypothetical overview of responses upon cadmium (Cd) exposure in plants. symbols indicate stress responses elicited by Cd, depicting the altered redox environment as well as organellar stress. The (hypothetical) points at which hydrogen sulfide (H₂S) acts are indicated using blue plus (positive interaction) or minus (negative interaction) signs. Full lines are based on established interactions, whereas dashed lines are more speculative. ANAC017: no apical meristem/Arabidopsis transcription activation factor/cup-shaped cotyledon 17, ACC: 1-aminocyclopropane-1-carboxylic acid, ACO: ACC oxidase, ACS: ACC synthase, AOX: alternative oxidase, ET: ethylene, GSH: glutathione, MDS: mitochondrial dysfunction stimulon, MPK: mitogen-activated protein kinase, OXI: oxidative signal inducible, PC: phytochelatin, PCS: PC synthase, RBOH: respiratory burst oxidase homologue, ROS: reactive oxygen species, SAM: S-adenosylmethionine, UPR: unfolded protein response.



