Molecular and Cellular Aspects of Contaminant Toxicity in Plants: The Importance of Sulphur and Associated Signalling Pathways

Peer-reviewed author version

HENDRIX, Sophie; Schröder, Peter; KEUNEN, Els; Huber, Christian & CUYPERS, Ann (2017) Molecular and Cellular Aspects of Contaminant Toxicity in Plants: The Importance of Sulphur and Associated Signalling Pathways. In: Advances in Botanical Research, 83, p. 223-276.

DOI: 10.1016/bs.abr.2016.12.007
Handle: http://hdl.handle.net/1942/25667

Running title: Plant responses to contaminant exposure

Molecular and cellular aspects of contaminant toxicity in plants

Sophie Hendrix[a], Peter Schröder[b], Els Keunen[a], Christian Huber[b] and Ann Cuypers[a]

[a] Environmental Biology, Centre for Environmental Sciences, Hasselt University, Agoralaan Building D, 3590 Diepenbeek, Belgium

[b] Helmholtz Zentrum München, German Research Centre for Environmental Health, GmbH Research Unit Environmental Genomics, Ingolstaedter Landstrasse 1, 85764 Neuherberg, Germany

Abstract

Environmental contamination with metals and organic compounds poses a serious threat to human health. Investigating plant responses to these contaminants at the molecular and cellular level is crucial to optimise phytoremediation strategies to clean up contaminated soils. Two key players in plant stress responses are the sulphur-containing amino acids cysteine and methionine. Cysteine is an important constituent of the metal-chelating metallothioneins and is also the precursor for glutathione and subsequent phytochelatin synthesis. During stress conditions, glutathione is involved in (1) metal chelation, (2) xenobiotic detoxification and (3) antioxidative defence. The activated form of methionine, S-adenosylmethionine, is involved in the synthesis of ethylene and polyamines, both playing important roles in signal transduction. This review provides an overview

of sulphur assimilation and its conversion into basic metabolites essential for detoxification and signal transduction during metal- and organic contaminant-induced stress conditions in plants. Furthermore, the cross-talk between these pathways and their relation to the contaminant-induced oxidative challenge are discussed.

1 General introduction

Since the industrial revolution, but especially over the last decades, abiotic contaminants – mainly from agricultural and industrial origin – have emerged into the environment and are recognised to pose a serious threat to the environment and human health (Ibrahim, Hayyan, AlSaadi, Hayyan, & Ibrahim, 2016; Noguera-Oviedo & Aga, 2016). These contaminants can be categorised in different groups based on their physicochemical properties, but their persistence (e.g. metals) and degradability (e.g. organics) are determining factors for their residence in the environment. As a result, different strategies are needed to remediate the environment. Microbe-assisted phytoremediation is a promising technology to be implemented in the clean-up of soils polluted with metals and organics (Chirakkara, Cameselle, & Reddy, 2016; Gerhardt, Huang, Glick, & Greenberg, 2009; Ma, Oliveira, Freitas, & Zhang, 2016; Vangronsveld et al., 2009). However, to exploit this technology, it is important to unravel the underlying molecular and cellular responses of plants in order to minimise phytotoxic responses.

Besides growth reduction and retardation, morphological and physiological responses as a result of organics or metal stress have been extensively described in plants (Verkleij, Golan-Goldhirsh, Antosiewisz, Schwitzguébel, & Schröder, 2009). More recently, multiple studies have focussed on the underlying molecular mechanisms of these stress responses. To cope with toxic compounds, plants rely on different detoxification mechanisms such as (1) biotransformation, (2) conjugation and (3) sequestration, all of which should be highly coordinated to prevent cellular damage. In addition to the detoxification of excess contaminants, also other factors such as environmental conditions (e.g. temperature, humidity) determine the stress intensity perceived by the plant (Reichenauer & Germida, 2008). Pivotal modulators of plant metabolism upon multiple

environmental challenges are reactive oxygen species (ROS), involved in oxidative damage but also signalling. In response to the alteration of ROS levels, metabolic adjustments lead to a newly established cellular homeostasis essential for plant performance (Cuypers et al., 2016; Foyer & Noctor, 2005).

When considering cellular detoxification and regulation mechanisms, the sulphur-containing amino acids, cysteine and methionine, play an important role. Cysteine is a major constituent of the metal chelators metallothioneins (MTs), glutathione (GSH) and phytochelatins (PCs) (Anjum, Gill, & Gill, 2014). Additionally, GSH is often consumed in conjugation reactions of organic compounds prior to sequestration into the vacuole (Dixon, Skipsey, & Edwards, 2010). Besides its role in detoxification, GSH is a major cellular antioxidant in plants (Noctor et al., 2012). As such, it is clear that detoxification and regulation are closely interconnected in plant stress acclimation. Methionine, the other sulphur-containing amino acid, forms the basis of S-adenosylmethionine (SAM), a central component in plant metabolism. It is also the initial biosynthetic compound in the production of the stress hormone ethylene (Sauter, Moffatt, Saechao, Hell, & Wirtz, 2013). Besides ROS, ethylene is an important regulator of stress perception and is known to be involved in multiple responses induced by abiotic stress (Keunen, Schellingen, Vangronsveld, & Cuypers, 2016b). From this, it becomes clear that upon exposure to excess organics or metals, sulphur is an essential element in (1) detoxification pathways and (2) the regulation of plant stress responses. Therefore, the current review focusses on sulphur uptake and assimilation and its conversion into basic metabolites essential for detoxification and regulation mechanisms under organic and metal exposure in plants. Via these sulphur-containing metabolites, detoxification and regulation are strongly interconnected and are discussed in view of the oxidative challenge and signal transduction under stress conditions in plants.

2 Sulphur uptake and assimilation

Sulphur is an essential macronutrient for all organisms, serving different metabolic functions. It is incorporated into the amino acids cysteine and methionine, controlling the structure and biological activity of many proteins (Davidian & Kopriva, 2010; Gotor et al., 2015). Furthermore, it is an important component of several coenzymes and prosthetic groups and is involved in plant responses to both biotic and abiotic stress factors (Davidian & Kopriva, 2010; Gigolashvili & Kopriva, 2014; Romero et al., 2014). In soil, sulphur is mainly present as inorganic sulphate (SO_4^{2-}). In order to enter metabolic pathways, sulphate is assimilated in plants through a pathway consisting of (1) sulphate uptake, (2) sulphate activation, (3) sulphate reduction and (4) synthesis of cysteine (Fig. 1) (Droux, 2004; Ravilious & Jez, 2012).

[Insert Figure 1 here]

Sulphate is taken up by plants via the action of proton/sulphate cotransport systems (Gigolashvili & Kopriva, 2014; Takahashi, Kopriva, Giordano, Saito, & Hell, 2011). The plant family of sulphate transporters (SULTRs), comprised of 12 to 16 genes depending on the plant species, is subdivided into four functional groups based on sequence, biochemical characteristics and physiological function (Takahashi et al., 2011). The SULTR family is best characterised in the model organism *Arabidopsis thaliana* (Gigolashvili & Kopriva, 2014). The most important transporters involved in sulphate uptake in roots are the high-affinity SULTR1;1 and SULTR1;2 proteins (group 1). However, as the expression of the *SULTR1;1* gene in roots is much lower as compared to that of *SULTR1;2*, the latter is considered the most important transporter involved in plant sulphate uptake during normal sulphate supply (Gigolashvili & Kopriva, 2014; Rouached et al., 2008). During sulphate starvation however, *SULTR1;1* expression is strongly upregulated, a response that is likely

due to the presence of a sulphur-responsive (SURE) *cis* element in its promoter (Maruyama-Nakashita et al., 2005). Another known sulphate transporter is SULTR1;3, which is localised in phloem companion cells and involved in sulphate transport from source to sink (Yoshimoto, Inoue, Saito, Yamaya, & Takahashi, 2003). Furthermore, the low-affinity group 2 SULTRs facilitate long distance sulphate transport (Gigolashvili & Kopriva, 2014; Takahashi et al., 2000). The activity of SULTR2;1 is modulated by SULTR3;5, which does not transport sulphate itself (Kataoka, Hayashi, Yamaya, & Takahashi, 2004a). In contrast to SULTR3;5, other group 3 SULTRs function in sulphate import into plastids (Cao et al., 2013; Gigolashvili & Kopriva, 2014), as the enzymes necessary for sulphate reduction are exclusively present in these organelles (Davidian & Kopriva, 2010). In addition, transporters belonging to group 4 are localised in the tonoplast, releasing sulphate from vacuoles (Kataoka et al., 2004b; Takahashi et al., 2011).

Once taken up in plant cells, sulphate is activated in an adenylation reaction catalysed by ATP sulphurylase (ATPS). The resulting adenosine 5'-phosphosulphate (APS) is an important branching point in the sulphate assimilation pathway (Fig. 1). It can be phosphorylated by the action of APS kinase (APK), leading to the formation of 3'-phosphoadenosine 5'-phosphosulphate (PAPS), an important activated sulphate donor for many sulphation reactions in plant secondary metabolism. In the primary sulphate assimilation pathway, however, APS reductase (APR) reduces APS to sulphite (SO_3^{2-}), which is further reduced to sulphide (S^{2-}) by the ferredoxin-dependent sulphite reductase (SiR). Sulphide is subsequently used for the synthesis of cysteine (Droux, 2004; Takahashi et al., 2011). While ATPS is also present in the cytosol, the reductive steps of the sulphate assimilation pathway only take place in plastids (Davidian & Kopriva, 2010).

The final step in the assimilation pathway of reduced sulphate is the synthesis of cysteine (Fig. 1). This sulphur-containing amino acid is subsequently incorporated into different compounds

including MTs, GSH and PCs, important in metal chelation, detoxification of xenobiotics and antioxidative defence (Anjum et al., 2014). Furthermore, cysteine is an important building block for the synthesis of methionine, the second sulphur-containing amino acid (Wirtz & Droux, 2005). In the first and rate-limiting step of cysteine biosynthesis, O-acetylserine (OAS) is synthesised from L-serine and acetyl-coenzyme A by serine acetyl transferase (SAT) (Wirtz & Droux, 2005; Wirtz & Hell, 2006). Subsequently, cysteine is formed by substitution of the acetate of OAS with sulphide in a β-replacement reaction catalysed by OAS (thiol) lyase (OASTL). Both SAT and OASTL are present in the cytosol, plastids and mitochondria (Anjum et al., 2014). These enzymes were demonstrated to associate, forming the hetero-oligomeric cysteine synthase complex. This association with OASTL is necessary for the stability and activity of SAT, which is otherwise subjected to feedback inhibition by cysteine. In contrast, OASTL activity is silenced when bound to SAT, suggesting that this enzyme only functions as a chaperone-like subunit in the cysteine synthase complex (Wirtz et al., 2010; Wirtz & Hell, 2006). However, as cellular OASTL activity is 100 to 300 times higher as compared to SAT activity, free OASTL enzymes are present in cells and catalyse the formation of cysteine from sulphide and OAS released by the complex (Droux, 2004; Saito, 2000). It must be noted that several metabolites regulate the stability of the cysteine synthase complex. Under sulphur deprivation conditions, for example, increased OAS levels stimulate dissociation of the complex, thereby inhibiting SAT activity and further OAS accumulation. In contrast, the presence of sulphide counteracts the dissociation of SAT and OASTL, resulting in the maintenance of SAT activity as long as sulphide availability is not a limiting factor (Droux, Ruffet, Douce, & Job, 1998; Takahashi et al., 2011; Wirtz & Hell, 2006).

2.1 Response to metal stress

In addition to its regulation by internal stimuli, the sulphate assimilation pathway is affected by environmental factors including metal exposure (Ernst, Krauss, Verkleij, & Wesenberg, 2008; Na & Salt, 2011). Indeed, several metals were shown to influence the expression of genes encoding both high- and low-affinity sulphate transporters, thereby affecting sulphate uptake and assimilation. Nocito et al. (2006), for example, demonstrated that exposure to different concentrations of cadmium (Cd), copper (Cu) and zinc (Zn) increased the expression of *ST1;1*, a high-affinity sulphate transporter in *Zea mays*. As a result, sulphate uptake capacity in roots was significantly enhanced under all metal exposure conditions (Nocito et al., 2006). Furthermore, Zn was shown to enhance root *SULTR1;1*, *SULTR1;2*, *SULTR4;1* and *SULTR4;2* expression and sulphate uptake in *Brassica pekinensis* (Stuiver et al., 2014). Similarly, Dixit et al. (2016) demonstrated an upregulation of *SULTR1;1*, *SULTR1;2 SULTR2;2* and *SULTR4;1* expression in roots of *Oryza sativa* exposed to the metalloid arsenic (As), resulting in increased sulphur accumulation and translocation. As a consequence, cysteine, GSH and PC levels were higher in both roots and shoots (Dixit et al., 2016). However, exposure to chromium (Cr) clearly decreased the expression levels of *ST1;1* and significantly suppressed sulphate uptake in *Z. mays* (Schiavon et al., 2007). Furthermore, Cr was also shown to reduce sulphate uptake and negatively affect the transcription of several sulphate transporters including *SULTR1;3*, *SULTR2;1*, *SULTR3;2*, *SULTR3;5* and *SULTR4;1* in roots of *Brassica juncea* after 24 hours of exposure. In contrast, expression levels of *SULTR4;2* were significantly increased at this time point (Schiavon et al., 2012).

In addition to their effect on sulphate uptake, metals also influence transcript levels, protein abundance and activity of enzymes catalysing different reactions in the sulphate assimilation

pathway. For example, an increased activity of the sulphate-activating enzyme ATPS was reported in leaves of *A. thaliana* exposed to 50 µM Cd for 3 or 5 days (Bashir, Ahmad, Bagheri, Nauman, & Qureshi, 2013). Similar effects were demonstrated in *Sedum alfredii*, with Cd increasing root and shoot *ATPS* transcript levels in a concentration-dependent manner. Moreover, the expression of the *SAT* gene encoding the first enzyme in cysteine biosynthesis was also significantly upregulated by Cd exposure in the roots (Liang et al., 2014). In addition to Cd, also Cu was shown to affect sulphate assimilation, as demonstrated by a strongly increased *ATPS* expression in roots of both *A. thaliana* and *Arabidopsis halleri* (Weber, Trampczynska, & Clemens, 2006). Furthermore, Cu-induced increases in the protein abundance of APR, SAT and OASTL in *O. sativa* roots were reported by Song et al. (2013). By affecting enzymes involved in sulphate assimilation, several metal(loid)s including As (Dixit et al., 2016; Talukdar & Talukdar, 2014), Cd (Bashir et al., 2013; Liang et al., 2014; Nocito et al., 2006), Cr (Schiavon et al., 2012; Schiavon, Pilon-Smits, Wirtz, Hell, & Malagoli, 2008), Cu (Gajewska, Glowacki, Mazur, & Sklodowska, 2013), nickel (Ni) (Gajewska et al., 2013), lead (Pb) (Mandavian, Ghaderian, & Schat, 2016) and Zn (Stuiver et al., 2014) were shown to alter cysteine levels in a wide range of plant species.

However, it is important to note that plant responses to metal exposure depend on sulphate supply, as metal-induced effects observed during sulphate starvation conditions often differ from those observed during normal sulphate availability. Indeed, results of Bashir et al. (2015) indicate that under conditions of sulphate starvation, *B. juncea* displayed higher Cd-induced oxidative stress levels and an increased Cd sensitivity. Furthermore, sulphur addition was reported to increase Cd uptake in roots of *Brassica chinensis*, while inhibiting root-to-shoot translocation of the metal. Although the addition of sulphate to the growth medium increased plant Cd uptake, it also relieved the Cd-induced inhibition of root and shoot growth and significantly reduced malondialdehyde

(MDA) and superoxide ($O_2^{\bullet-}$) levels in both organs (Liang et al., 2016). Furthermore, decreases in *Corchorus olitorius* dry weight induced by aluminium (Al), Cd, Cu and Pb were alleviated by the addition of either K_2SO_4 or cysteine to the growth medium (Mazen, 2004). These results are of particular interest in the context of phytoremediation, as this soil remediation strategy strongly benefits from plants exhibiting a high metal uptake capacity and increased metal tolerance.

The involvement of sulphate uptake and assimilation in plant responses to metal stress is further supported by the observation that modification of genes involved in sulphate assimilation alters plant tolerance to a broad array of metals. Indeed, overexpression of *SAT* was shown to increase OAS, cysteine and GSH levels in *A. thaliana*, thereby increasing its resistance to Ni-induced growth inhibition (Freeman et al., 2004). Furthermore, overexpression of different *OASTL* isoforms increased Cd tolerance of both *A. thaliana* and *Nicotiana tabacum* (Dominguez-Solis, Gutierrez-Alcala, Romero, & Gotor, 2001; Dominguez-Solis et al., 2004; Harada, Choi, Tsuchisaka, Obata, & Sano, 2001; Kawashima et al., 2004; Ning, Zhang, Yao, & Yu, 2010). Interestingly, transgenic *Brassica napus* plants overexpressing *miR395* also showed diminished Cd-induced oxidative stress levels and were more tolerant to Cd. This miRNA is induced by sulphate starvation and controls the expression levels of *SULTR2;1* and three *ATPS* isoforms. However, expression of *miRNA395* is also increased under Cd exposure conditions, highlighting the similarity between plant responses to both stress factors (Gielen, Remans, Vangronsveld, & Cuypers, 2016; Zhang, Song, Shu, Zhang, & Yang, 2013).

Another finding indicating the importance of sulphate assimilation in plant responses to metal stress is the fact that transcript levels and activity of enzymes involved in sulphate uptake often differ between metal hyperaccumulators and their non-accumulator counterparts. Freeman et al. (2004), for example, reported strong positive correlations between OAS, cysteine and GSH levels

and shoot Ni concentrations in various *Thlaspi* hyperaccumulators and non-accumulators. Indeed, the metal-accumulating species *Thlaspi goesingense* displayed increased SAT activity and OAS, cysteine and GSH levels in comparison to the related non-accumulator *A. thaliana* under control conditions. When exposed to Ni, this resulted in an increased resistance of the former species against Ni-induced oxidative stress, as indicated by considerably lower concentrations of thiobarbituric acid-reactive substances (TBARS) (Freeman et al., 2004). Furthermore, metal-tolerant genotypes of the same species often exhibit increased sulphate assimilation capacities as compared to their metal-sensitive counterparts. For example, in *Lens culinaris*, an As-tolerant cultivar, showed strongly increased *SAT* and *OASTL* transcript levels and activities and significantly enhanced cysteine and GSH levels as compared to an As-sensitive cultivar exposed to As. Interestingly, As differently affected transcript levels of group 1 sulphate transporters between both cultivars, with *SULTR1;1* and *SULTR1;2* upregulation in the tolerant and downregulation in the sensitive cultivar after 24 hours of exposure (Talukdar & Talukdar, 2014). Similarly, Song et al. (2013) reported more pronounced Cu-induced increases in SAT and OASTL protein abundance in a Cu-tolerant as compared to a Cu-sensitive *O. sativa* variety. Furthermore, the induction of ATPS and OASTL activities by Cd exposure in a tolerant *B. chinensis* cultivar was less pronounced or even absent in its sensitive counterpart (Liang et al., 2016). In conclusion, it is obvious that sulphate uptake and assimilation play a prominent role in plant responses to metal stress. This can be explained by the crucial role of cysteine-containing compounds in metal chelation and detoxification, as discussed in section 3.

2.2 Response to organic contaminants

While our knowledge on the effects of organic environmental contaminants on sulphur assimilation is scarce, the effects of herbicides and safeners – used to selectively protect crops from herbicide damage – have been frequently described (Abu-Qare & Duncan, 2002; Hirase & Molin, 2003). In 1985, the lab of Lamoureux described that pretreatment of *Z. mays* with low levels of the chloroacetamide herbicide 2-chloro-N,N-di-2-propenylacetamide protected them from the herbicide when it was applied later at higher concentrations by increasing GSH synthesis (Ezra, Rusness, Lamoureux, & Stephenson, 1985). Similarly, dichloroacetamide herbicide antidotes were shown to enhance sulphate metabolism in *Z. mays* roots by Adams et al. (1983). Diclormid and benoxacor both induced ATPS, whereas flurazole acted on OASTL (Hirase & Molin, 2003). Such an effect was also observed a few years later for *Sorghum bicolor* by Gronwald and co-workers (1987). In 1990, evidence for a direct interaction of N,N-diallyl-2,2-dichloroacetamide and 4-dichloroacetyl-3,4-dihydro-3-methyl-2H-1,4-benzo-oxazin (CGA 154 281) with sulphate assimilation and GSH contents was presented in *Z. mays* (Farago & Brunold, 1990). Similar to the safener CGA 154 281, cysteine formation and GSH synthesis were also induced by the safener 1-dichloroacetyl-hexahydro-3,3,8-trimethyl-pyrrolo-[1,2-]-pyrimidine-6-(2H)-one-(dicyclonone) (BAS 145 138) in *Z. mays*. (Kocsy et al., 2001). These specific effects of safeners on sulphate assimilation have been attributed to their influence on oxidative stress levels, but are not completely unravelled yet. Just recently, the effect of three herbicide safeners (mefenpyr-diethyl, fenchlorazole-ethyl and dichlormid) on the content of sulphur-containing metabolites in Fe-deficient barley was investigated (Bartucca et al., 2016). All three safeners were effectively inducing ATPS activity, but the effect on OASTL activity was dependent on concentration as well as exposure time. An initial reduction of OASTL activity was followed by a strong induction. The

authors speculate that safeners initially induce some membrane damage and the generation of hydrogen peroxide (H_2O_2) in an oxidative burst, which leads to the activation of defence genes. Different from herbicidal metabolic action injuring plants, the inertness of safeners to plants does not cause toxicity. Hence, the initial decrease in OASTL activity after treatment would be regarded as an unspecific response of the plant to stress. Thereafter, the safening action prevails and the chemicals activate defence responses inducing OASTL activity, leading to enhanced GSH availability (Bartucca et al., 2016).

3 Thiols play an important role in detoxification

3.1 Metallothioneins

Metallothioneins are cysteine-rich low-molecular-weight (< 10 kDa) metal-chelating proteins in many organisms (Fig. 1) (Anjum et al., 2015; Freisinger, 2008). Plant MTs are divided into four groups based on the number and arrangement of their cysteine residues (Cobbett & Goldsbrough, 2002). Type 1-3 MTs contain two cysteine-rich domains, connected by a cysteine-poor linker region, the length of which depends on the specific MT type and the plant species (Leszczyszyn, Imam, & Blindauer, 2013). The cysteine-rich α- and β-domains are involved in metal binding. The α-domain is located at the C-terminus of the protein and contains six cysteine residues arranged according to the consensus sequence CxCxxxCxCxxCxC, where x represents any other amino acid than cysteine. The β-domain is present at the N-terminus and has a more variable amino acid sequence. It generally contains six cysteine residues in MT1 proteins, eight in MT2 proteins and four in MT3 proteins. Type 4 MTs – also referred to as E_c proteins – can be distinguished from

other MT classes based on the presence of three cysteine-rich domains separated by two linker regions. Furthermore, they are characterised by two highly conserved histidine residues in the central cysteine-rich domain (Freisinger, 2008, 2011). In contrast to PCs, which are also important metal chelators in plant cells (see section 3.2.1), MTs are encoded by genes and thus are products of mRNA translation (Anjum et al., 2015). While almost all MT genes contain an intron near the N-terminal cysteine-rich domain, it is interesting to note that the exact position of this intron varies according to the specific type of MT encoded (Cobbett & Goldsbrough, 2002).

Different types of MTs display distinct spatiotemporal expression patterns. In general, type 1 MTs are more strongly expressed in roots as compared to shoots, whereas the opposite holds true for type 2 MTs (Guo, Bundithya, & Goldsbrough, 2003). Although type 3 MTs are also present in leaves, they are mainly expressed in ripening, fleshy fruits (Clendennen & May, 1997; Moyle, Fairbairn, Ripi, Crowe, & Botella, 2005). In contrast, MTs of type 4 are exclusively localised in developing seeds, implying a role in metal storage and accumulation there (Guo et al., 2003). As proposed by Ren et al. (2012), these MTs could provide a means to store Zn required for seed germination. Furthermore, different MT genes are strongly upregulated in ageing leaves, suggesting their involvement in leaf senescence. During this process, MTs possibly serve a dual function. On one hand, they could protect cells against metal toxicity arising from the breakdown of different metal-containing cellular components. On the other hand, they could also be involved in translocating the released metals to non-senescing plant tissues (Guo et al., 2003; Leszczyszyn et al., 2013).

Although many questions remain with regard to the different functions of plant MTs, their role in metal chelation is well established. Metallothioneins have the ability to bind both mono- and divalent metal ions in typical metal-thiolate clusters, characterised by high thermodynamic and low

kinetic stability. As a consequence, metals are tightly bound by MTs, but part of the metal ions can be readily relocated to other proteins (Hassinen, Tervahauta, Schat, & Karenlampi, 2011). Both essential and non-essential metals are chelated by MTs, indicating their involvement in nutrient homeostasis as well as metal detoxification. The metals Zn, Cu and Cd are bound to MTs with the highest affinity (Blindauer & Leszczyszyn, 2010). The importance of MTs in detoxifying excess metals is highlighted by their induction under metal exposure conditions, as discussed in the following section (3.1.1). However, many other biotic and abiotic stress factors including pathogen attack (Dauch & Jabaji-Hare, 2006), wounding (Razem & Bernards, 2002), light (Chen et al., 2003), drought (Li et al., 2016) and low temperature (Zhu et al., 2009) also induce the expression of *MT* genes, suggesting additional roles besides metal chelation. Transcriptional responses of MTs to different stress factors are possibly mediated by the presence of upstream regulatory elements in the promoter regions of genes encoding these proteins. Indeed, metal-, antioxidant-, wounding-and stress-responsive elements were identified in the promoters of type 1, 2 and 3 MTs. Furthermore, elements responsive to different phytohormones including ethylene, methyl jasmonate, gibberellic acid and salicylic acid were also found in MT promoters, suggesting hormonal regulation of MT expression (Leszczyszyn et al., 2013).

Interestingly, MTs also exhibit antioxidant properties. This is illustrated in *N. tabacum*, where ectopic expression of a type 1 MT from *O. sativa* clearly decreased H_2O_2 accumulation during salinity stress (Kumar et al., 2012). Similar results were obtained by Xue et al. (2009), reporting increased stress tolerance and decreased H_2O_2 levels in transgenic *N. tabacum* plants overexpressing the *Gossypium hirsutum MT3a* gene as compared to the wildtype (WT) exposed to cold, salt and drought stress. Moreover, H_2O_2 levels were significantly higher in an *A. thaliana* T-DNA insertion line lacking functional MT2a as compared to the WT subjected to drought and cold

stress (Zhu et al., 2009). Additional evidence supporting an antioxidative function for MTs is provided by the observation that exposure to ROS or ROS-inducing stress factors (e.g. paraquat) increased the expression of several *MT* genes in many plant species including *Quercus suber* (Mir et al., 2004), *G. hirsutum* (Xue et al., 2009), *Ipomoea batatas* (Kim, Jeong, Ahn, Lee, & Kwak, 2014) and *O. sativa* (Liu et al., 2015a). The antioxidative function of MTs can be explained by the fact that the cysteine residues responsible for metal scavenging also have the capability to reduce ROS. During metal exposure conditions, MTs could also exert an indirect antioxidative function by chelating redox-active metals such as Cu, thereby preventing ROS formation as a consequence of Fenton and Haber-Weiss reactions (Leszczyszyn et al., 2013).

3.1.1 Response to metal stress

As mentioned before, metal exposure induces MT expression in numerous plant species. Exposure to Cu, for example, induced the expression of *MT1a*, *MT2a*, *MT2b* and *MT3* in different organs of *A. thaliana*. This increased transcription was especially pronounced in leaf trichomes, which are often reported to accumulate large amounts of metal ions (Guo et al., 2003). Similarly, Cd exposure significantly induced the expression of *MT2a* in *A. thaliana* roots and leaves (Jozefczak et al., 2014). In another study, 24 hours exposure to Cd, Cu or Zn was shown to induce the expression of *MT1* in *Cajanus cajan* (Sekhar, Priyanka, Reddy, & Rao, 2011). Furthermore, these metals also increased *MT3* transcription levels in leaves of *Porteresia coarctata* (Usha, Keeran, Harikrishnan, Kavitha, & Parida, 2011). In addition to Cd, Cu and Zn, which are most often associated with MT binding, other metals were shown to induce MT expression as well. Exposure to Pb, for example, strongly induced *MT2a* and *MT2b* expression in shoots of *Hirschfeldia incana* (Auguy et al., 2016). Similarly, *MT1* and *MT2* expression levels were elevated in As-exposed *O. sativa* (Nath et al.,

2014). Furthermore, also Fe (Ahn et al., 2012), Mn (Ahn et al., 2012; Zhao et al., 2012), mercury (Hg) (Venkatachalam, Srivastava, Raghothama, & Sahi, 2009) and Cr (Gautam et al., 2012) were shown to induce the expression of different *MT* genes in a broad range of plant species. The induction of *MT* expression in metal-exposed plants suggests a role for these proteins in metal stress defence responses.

A positive role for MTs in metal tolerance is also suggested by several studies reporting differences in *MT* expression between metal-sensitive plants and their tolerant counterparts. Indeed, *MT3* transcript levels were approximately 2.5-fold higher in the metal hyperaccumulator *Thlaspi caerulescens* as compared to the non-accumulator *A. thaliana* grown under control conditions. Furthermore, the cysteine positions in the MT amino acid sequence of both species were different, predicting a smaller metal binding cavity in *A. thaliana* as compared to *T. caerulescens* (Roosens, Bernard, Leplae, & Verbruggen, 2004). Interestingly, *MT2a*, *MT2b* and *MT3* expression levels were also 1.5- to 4-fold higher in the metal hyperaccumulator *A. halleri* as compared to those in *A. thaliana* (Chiang, Lo, & Yeh, 2006). In addition, *MT2b* expression was considerably higher in two independently evolved Cu-tolerant *Silene paradoxa* populations as compared to a Cu-sensitive population, both under control conditions and after Cu exposure. This effect is probably related to the presence of multiple *MT2b* gene copies in both Cu-tolerant populations (Mengoni et al., 2003). Furthermore, Hassinen et al. (2009) reported that *MT2* and *MT3* were more highly expressed in metallicolous *T. caerulescens* accessions as compared to a non-metallicolous accession. However, Zn accumulation and *MT* transcript levels did not co-segregate, implying that MTs are not the major determinants of Zn accumulation in these plants.

A role for MTs in plant defence against metal stress is also supported by the fact that plants overexpressing different *MT* genes often display enhanced metal tolerance. Overexpression of a

putative MT from *Colocasia esculenta* (*CeMT*), for example, reduced the negative effects of Cd, Cu and Zn exposure on root growth of *N. tabacum* seedlings. Furthermore, it significantly increased metal accumulation in these plants. The positive function of this MT during metal stress could be related to its metal-chelating function, but could also be associated with its antioxidant properties. This is confirmed by significantly reduced H_2O_2 and lipid peroxidation levels in the *CeMT*-overexpressing plants as compared to their WT counterparts after 24 hours of metal exposure (Kim, Jung, Kim, & Bae, 2013). Similarly, overexpression of *MT2* from *S. alfredii* resulted in increased Cd and Zn tolerance and accumulation in *N. tabacum*. Again, this response was accompanied by significantly decreased H_2O_2 levels in metal-exposed transgenic tobacco plants as compared to the WT. Furthermore, when exposed to Cd and Zn, the *MT2* overexpressing plants also displayed increased SOD, CAT and POD activities in comparison to WT plants (Zhang et al., 2014a). In addition, overexpression of MTs from yeast and mammals can also increase metal tolerance and/or accumulation in plants (Daghan, Arslan, Uygur, & Koleli, 2013; Ruiz, Alvarez, Torres, Roman, & Daniell, 2011; Vrbova et al., 2013) and is proposed to be an interesting phytoremediation strategy.

Although MTs are important players in plant metal stress responses, it should be noted that their responses depend on many factors, including the plant species, tissue and specific metal considered. Schiller et al. (2014), for example, demonstrated that leaf expression levels of *MT1a* were upregulated by Cu and Zn exposure and downregulated by Cd exposure in *Hordeum vulgare*. Similarly, transcript levels of *MT2c* and *MT3* increased in response to Cu, while they decreased in leaves of Cd-exposed plants. In contrast, expression levels of these *MTs* remained unchanged in response to Zn. Furthermore – as mentioned above – expression levels of different MTs were strongly tissue-dependent, with *MT1b* and *MT4* transcripts exclusively detected in roots and grains respectively (Schiller et al., 2014). Taken together, these data point towards distinct physiological

functions for different MT isoforms. A first step towards unravelling these functions involves the analysis of MTs at the protein level. This remains a challenge, however, as plant MTs are very prone to proteolysis, hampering the application of proteomics approaches (Blindauer & Leszczyszyn, 2010).

3.2 Glutathione

Glutathione is the most abundant non-protein thiol in almost all aerobic species, occurring at intracellular concentrations of 0.5 to 10 mM. It is a tripeptide consisting of γ-glutamate, cysteine and glycine and is synthesised in two ATP-dependent steps (Fig. 1). First, a peptide bond is formed between γ-glutamate and cysteine, producing γ-glutamylcysteine (γ-EC). This reaction is catalysed by γ-EC synthetase (γ-ECS; GSH1), also known as glutamate–cysteine ligase (GCL), and constitutes the rate-limiting step in GSH biosynthesis. Subsequently, GSH is formed by the addition of glycine to γ-EC in a reaction catalysed by glutathione synthetase (GSH-S; GSH2) (Noctor et al., 2012). While GSH1 is exclusively localised in chloroplasts, GSH2 is also present in the cytosol, with cytosolic GSH2 activity strongly exceeding that in chloroplasts (Wachter, Wolf, Steininger, Bogs, & Rausch, 2005). Transport of γ-EC into the cytosol, necessary for cytosolic GSH production, is mediated by chloroquine-resistance transporter-like transporters (CLTs) in the plastid envelope (Maughan et al., 2010). Once synthesised, GSH is distributed to different organelles by the action of plastidal CLTs and transporters in mitochondrial and nuclear membranes (Zechmann, 2014). Mitochondria, chloroplasts and the cytosol have the highest GSH abundance in plant cells (Noctor et al., 2012). In addition to its intracellular transport, GSH is also transported between cells and is one of the major reduced sulphur forms translocated in the phloem (Noctor et al., 2012).

An important determinant of GSH biosynthesis is GSH1 activity, which is regulated at three levels. First, GSH1 activity is subject to feedback inhibition by GSH itself. Under stress conditions, GSH is consumed and the feedback inhibition is alleviated, thereby increasing GSH synthesis. In addition, GSH1 is transcriptionally regulated via the interaction of a redox-sensitive repressor-binding protein with the 5'-untranslated region of its encoding gene (Noctor, Gomez, Vanacker, & Foyer, 2002). Furthermore, GSH1 is post-transcriptionally modulated by the cellular redox state. Under oxidising conditions, it functions as a homodimeric enzyme with two intermolecular disulphide bonds. Reducing conditions however, disrupt one of these disulphide bonds, thereby altering the dimer interface and shifting the enzyme to its less active monomeric form (Hothorn et al., 2006). Whether this mechanism is responsible for GSH1 feedback inhibition by GSH is currently unknown (Jozefczak, Remans, Vangronsveld, & Cuypers, 2012). It is interesting to note that GSH biosynthesis is tightly coupled to sulphate assimilation, as stress-induced GSH accumulation is often accompanied by increased sulphate uptake and transcriptional upregulation of sulphate assimilation genes including *APR* and *SAT* (Noctor et al., 2012; Queval et al., 2009; Smith, Kendall, Keys, Turner, & Lea, 1985).

In plants, GSH is involved in many processes including cell cycle regulation and defence against biotic and abiotic stress factors (Noctor et al., 2012). The essential role of GSH in plant physiology is underlined by the observation that plants lacking GSH due to a knockout mutation in either *GSH1* or *GSH2* display a lethal phenotype (Cairns, Pasternak, Wachter, Cobbett, & Meyer, 2006; Lim, Meyer, & Cobbett, 2011). In contrast, plants with lowered GSH levels are viable but show an altered phenotype as compared to WT plants. For example, the *root meristemless 1-1* (*rml1-1*) mutant contains less than 5% of WT GSH levels and thereby fails to develop a root apical meristem (Cheng, Seeley, & Sung, 1995; Vernoux et al., 2000). Other GSH-deficient mutants such as

cadmium-sensitive 2-1 (*cad2-1*), *regulator of ascorbate peroxidase 1-1* (*rax1-1*) and *phytoalexin-deficient 2-1* (*pad2-1*) display GSH levels between 25 and 50% of WT levels. Even though these plants develop normally, they are more sensitive to environmental stress factors, emphasising the crucial role of GSH during stress conditions (Ball et al., 2004; Howden, Andersen, Goldsbrough, & Cobbett, 1995; Parisy et al., 2007).

In this review, we will focus on the most important functions of GSH in stress responses induced by metals and organic contaminants. These include (1) metal chelation by PCs, (2) detoxification of xenobiotics by glutathione transferases (GSTs) and (3) antioxidative defence (Fig. 1).

3.2.1 Glutathione in chelation: Phytochelatins

An important role of GSH during stress relies on its function as a precursor of PCs. These molecules are important metal scavengers in plant cells and are characterised by a typical $(\gamma\text{-Glu-Cys})_n\text{-Gly}$ structure, with n ranging from 2 to 11 (Zagorchev, Seal, Kranner, & Odjakova, 2013). Phytochelatins are synthesised by PC synthase (PCS) in a two-step reaction. In the first step, the cysteine-glycine peptide bond of a donor GSH molecule is cleaved, yielding γ-EC and glycine. Subsequently, the γ-EC unit is transferred to an acceptor molecule – either another GSH molecule or an oligomeric PC peptide – in a transpeptidation reaction. Even though its encoding gene is constitutively expressed, PCS activity is dependent on the presence of metals. While Cd is the most important inducer of PCS activity, other metal(loid)s including As, Cu, Hg, Ni, Pb and Zn were also reported to increase PC synthesis (Anjum et al., 2014). The metal-induced activation of PCS is related to the presence of four highly conserved cysteine residues in its N-terminal domain, which also contains the active site. The C-terminal domain, in contrast, is rich

in cysteine residues that possibly bind metal ions in order to transfer them to the N-terminal active site (Vestergaard et al., 2008). Interestingly, Cd-PC complexes were demonstrated to be translocated into the vacuole, possibly reducing Cd-induced damage to other cellular compartments (Cobbett & Goldsbrough, 2002). In *A. thaliana*, this vacuolar translocation is likely mediated by the ATP binding cassette (ABC) C3 (ABCC3) transporter, which is transcriptionally induced upon Cd exposure (Brunetti et al., 2015).

As mentioned above, PC synthesis is induced by several metals. The importance of PCs in metal-induced plant responses is further supported by the fact that the PC-deficient *A. thaliana* mutants *cad1-3* and *cad1-6* – carrying a mutation in the *PCS1* gene – display an enhanced sensitivity towards several metals such as Cd, Pb and Zn (Fischer, Kuhnlenz, Thieme, Schmidt, & Clemens, 2014; Tennstedt, Peisker, Bottcher, Trampczynska, & Clemens, 2009). Furthermore, differences in the extent of PC synthesis between metal-sensitive and -tolerant varieties are often observed. Indeed, a Cd-tolerant *Triticum aestivum* cultivar displayed stronger Cd-induced increases in GSH levels and *PCS* expression in comparison to a Cd-sensitive cultivar (Kumari, Parmar, & Sharma, 2015). Similarly, *PCS1* expression and total PC levels were significantly increased by As exposure in an As-tolerant *O. sativa* cultivar, while they remained unaffected in its As-sensitive counterpart. In addition, As-induced increases in GSH, cysteine and methionine levels were also more pronounced in the tolerant as compared to the sensitive cultivar (Begum et al., 2016).

As PCs are efficient metal chelators, transgenic plants with increased PC levels are often able to accumulate high metal concentrations. For example, *N. tabacum* plants overexpressing *PCS1* displayed an enhanced accumulation of Cd, Cu, Ni and Zn as compared to WT plants. These plants even accumulated higher metal concentrations as compared to the metal hyperaccumulator *T.*

478 *caerulescens* (Martínez et al., 2006). The use of transgenic plants with increased PC production
479 could therefore offer a promising strategy for the phytoremediation of metal-polluted soils.
480 However, it should be noted that overproduction of PCs is not always beneficial for plants, as it
481 can cause a strong decrease of cellular GSH levels. Since GSH also serves important antioxidative
482 functions (see section 3.2.3), GSH depletion possibly results in oxidative stress and cellular
483 damage. This was shown by Jozefczak and co-workers (2014), who reported a rapid increase in PC
484 levels in roots and leaves of *A. thaliana* plants exposed to 5 and 10 µM Cd. In roots, this response
485 coincided with a fast (2 hours) reduction of GSH levels. Despite the activation of alternative
486 antioxidative defence systems after 24 hours, decreased biomass and increased lipid peroxidation
487 were observed in Cd-exposed plants (Jozefczak et al., 2014). These results indicate that plant metal
488 tolerance is not only related to the production of PCs, but also to the ability to prevent the resulting
489 GSH depletion. Therefore, the use of transgenic plants overexpressing both *PCS* and GSH
490 biosynthesis genes could be more efficient to remediate metal-contaminated areas (Seth et al.,
491 2012).

492

493 *3.2.2* *Glutathione in detoxification: glutathione transferases*

494 Another important function of GSH is related to its involvement in detoxification reactions
495 mediated by GSTs (Fig. 1). These enzymes catalyse the conjugation of xenobiotics to GSH by
496 addition or substitution reactions in order to reduce their toxicity and increase their water solubility
497 (Coleman, Blake-Kalff, & Davies, 1997; Dixon et al., 2010). As GSTs possess broad and
498 overlapping substrate specificities, they are able to detoxify a chemically diverse array of
499 compounds. Xenobiotics susceptible to GSH conjugation include electrophilic herbicides, several
500 drugs and organic contaminants (Cole, Cummins, Hatton, Dixon, & Edwards, 1997; Frova, 2003).

Generally, these compounds are characterised by the presence of two carbon atoms coupled by a double bond, adjacent to an electron withdrawing group (Frova, 2003). Given the reactivity of these molecules, conjugation to GSH occurs spontaneously but is speeded up considerably by GST activity (Coleman et al., 1997). In addition to their role in GSH conjugation reactions, GSTs also serve a broad range of other catalytic as well as non-catalytic functions (Frova, 2003). While the molecular structure and catalytic mechanisms of GSTs have been studied by several research groups, their natural functions remains largely obscure. An overview of natural GST functions elucidated so far is provided in Table 1. It is interesting to note that the first functions ascribed to GSTs were all related to conjugation (e.g. of xenobiotics), whereas more sophisticated functions were only discovered more recently.

[Insert Table 1 here]

Even though most GSTs are soluble proteins present in the cytosol, they have also been detected in several organelles including chloroplasts, mitochondria, peroxisomes, the vacuole and the nucleus. Furthermore, microsomal GSTs with herbicide detoxifying activities have been characterized in *Pachyrhizus erosus* (Belford, Dorfler, Stampfl, & Schroder, 2004) and in lower plants (Pflugmacher, Schroder, & Sandermann, 2000). Microsomal GSTs belonging to the 'Membrane Associated Proteins involved in Eicosanoid and Glutathione metabolism' (MAPEG) family have been characterised as well (Oztetik, 2008). It is important to note that cytosolic GSTs are very abundant, constituting up to 2% of all soluble proteins in plants (Scalla & Roulet, 2002).

To date, plant GSTs are subdivided into several classes according to a classification system proposed by Droog (1997) and subsequently refined by Edwards and co-workers (2000). Using this system, GSTs are classified based on gene organisation (number and position of introns), amino acid similarity and conservation of specific residues in the protein. The phi (GSTF) and tau

(GSTU) classes constitute the largest classes of plant GSTs and are plant-specific. Interestingly, they are also predominantly active in xenobiotic detoxification (Frova 2006, Edwards et al. 2011). Indeed, phi class GSTs have been co-crystallised with a range of herbicide and other xenobiotic conjugates (Prade, Huber, & Bieseler, 1998). In contrast, the zeta (GSTZ) and theta (GSTT) families are much smaller and are related to mammalian GSTs. While GSTs belonging to these classes display GSH-conjugating activities, other classes including the lambda GSTs (GSTL) and dehydroascorbate reductases (DHARs) have no known ability to conjugate or detoxify synthetic compounds. Instead, they seem to have a redox-related role (Dixon, Davis, & Edwards, 2002).

Structurally, most cytosolic GSTs occur as dimers consisting of two subunits (approximately 25 kDa each), each encoded by one gene (Oztetik, 2008). Although homodimers and heterodimers can be formed, dimerisation has only been observed between GSTs from the same class (Armstrong, 1997). An exception are the DHARs and lambda GSTs, which function as monomers (Dixon et al., 2010). Each GST subunit is characterised by a common overall structure with a well-defined N-terminal GSH binding domain (G-site) and a less specific C-terminal domain binding the hydrophobic co-substrate (H-site). Most GST classes including the phi, tau, zeta and theta class contain a serine in the active site, responsible for the formation and stabilisation of the reactive thiolate anion from GSH, which is the target for the nucleophilic attack of an electrophilic substrate (Dixon et al., 2010). In DHARs and lambda GSTs however, this serine is replaced by a cysteine that forms a mixed disulphide with GSH. The catalytic cysteine performs a nucleophilic attack on GSH-conjugated substrates. Under these conditions, the catalytic cysteine of the GST becomes glutathionylated, thereby deconjugating the substrate that is subsequently released. Regeneration of the resulting glutathionylated GST forms requires a GSH molecule, forming GSSG as another end product. While the reduced GSTs can be used in another catalytic cycle, GSSG is reduced back

to GSH by glutathione reductase (GR) at the expense of NADPH (Lallement, Brouwer, Keech, Hecker, & Rouhier, 2014).

In general, phi and tau GST-catalysed substitution reactions change the parent compound to an extent that the toxicity of the xenobiotic is significantly lowered (Schroder & Collins, 2002). Along with the resulting increase in polarity, GSH addition is a very effective means of detoxification, especially when the electrophilic centre of the target molecule is a leaving group. In some situations, however, the formation of GS-conjugates could be disadvantageous to plants when they accumulate in high amounts. In *Picea abies* for example, it is clearly proven that GS-conjugates accumulating in the cytosol inhibit the stress-reducing activity of GR (Schroder & Pflugmacher, 1996). Furthermore, when detoxification reactions withdraw GSH from the cellular pool, GSH depletion due to exaggerated utilisation in conjugation reactions or inhibition of GSH supply could become a problem, particularly when the xenobiotic conjugates still remain reactive and/or are not efficiently stabilised (Brazier-Hicks et al., 2008). However, GS-conjugate formation has generally been accepted to be beneficial to plants (Edwards et al., 2000; Schröder, 2006).

Furthermore, conjugation of substrates with GSH allows them to be transported into the vacuole by ATP-binding cassette transporters (Mohanasundaram et al., 2015; Theodoulou, 2000). This removal of GS-conjugates from the cytosol is of ample importance, as they can inhibit the activities of GSTs as well as other GSH-dependent enzymes (cfr. *supra*) (Coleman et al., 1997). Once present in the vacuole, GS-conjugates can undergo further metabolism, as discussed by Coleman et al. (1997).

Interestingly, GSTs are induced by a broad range of biotic and abiotic stress conditions, including exposure to organic contaminants. The relevance of this induction is mostly related to direct detoxification of xenobiotics, but can also be explained by the fact that certain GSTs detoxify

products of lipid peroxidation and oxidative DNA damage. This characteristic of GSTs also possibly underlies their involvement in plant responses to metal stress (Frova, 2003).

3.2.2.1 Response to stress conditions

As mentioned above, GSTs are important players in plant responses to organic compounds. In this context, especially herbicides are known to affect plant GST levels and activities. For example, glyphosate significantly increased GST activities in *Pisum sativum* roots and leaves (Miteva, Ivanov, & Alexieva, 2010). Similarly, exposure to the chloroacetamide herbicide metazachlor induced GST activity in *B. napus* leaves (Vercampt, Koleva, Vassilev, Vangronsveld, & Cuypers, 2016). A role for GSTs in herbicide detoxification is further supported by the enhanced tolerance of *N. tabacum* plants overexpressing a *Citrus cinensis* tau class GST to the diphenyl ether herbicide fluorodifen. Interestingly, salt and drought tolerance were also increased in the overexpressor plants. However, this effect was not due to scavenging of oxidative stress by-products, suggesting additional GST-mediated mechanisms provoking tolerance to these stress conditions (Lo Cicero, Madesis, Tsaftaris, & Lo Piero, 2015). Even though GSTs play an important role in detoxifying herbicides, their activities are not always induced in herbicide-exposed plants. Indeed, the photosynthetic herbicide isoproturon reduced GST activity in *Z. mays* leaves, indicating that certain herbicides function as GST inhibitors (Alla, Hassan, & El-Bastawisy, 2008).

Herbicide safeners often exert their function by increasing the rate of GSH conjugation in several crop species (Abu-Qare & Duncan, 2002; Davies & Caseley, 1999). For example, the safener benoxacor induced the activity of a tau class GST in *Festuca arundinacea* (Del Buono, Scarponi, & Espen, 2007). Furthermore, the safener cloquintocet-mexyl was shown to increase the protein

abundance of several GSTs belonging to the phi, tau and lambda classes in the roots and coleoptile of *Triticum tauschii* seedlings (Zhang, Xu, Lambert, & Riechers, 2007). However, it should be noted that safeners could also increase the GSH conjugation rate in weeds competing with the target crop. Mefenpyr diethyl and fenchlorazole ethyl safeners, for example, were shown to increase the protein abundance of certain phi and lambda GSTs in the weed *Alopecurus myosuroides* treated with the graminicidal herbicide fenoxaprop ethyl (Cummins, Bryant, & Edwards, 2009). In addition, the gene encoding GSTU26 was transcriptionally induced upon exposure to the chloroacetanilide herbicides alachlor and metolachlor and the safener benoxacor in *A. thaliana* seedlings. In contrast, expression levels of *GSTF9* were not affected under these conditions, indicating that specific GST isoforms are responsive to specific safeners and/or herbicides (Nutricati, Miceli, Blando, & De Bellis, 2006). Similarly, different safeners were shown to transcriptionally induce several phi and theta class GSTs in *A. thaliana*. Nonetheless, this response did not protect the plants from herbicide-induced damage, suggesting that other players besides GSTs are responsible for the differences in safener-induced herbicide tolerance between plant species (DeRidder, Dixon, Beussman, Edwards, & Goldsbrough, 2002).

Furthermore, it should be noted that GSTs can bioactivate instead of detoxify certain organic compounds. Indeed, two examples of toxic GS-conjugates in plants refer to GST-mediated isomerisation involved in herbicide bioactivation of isourazoles and thiadiazolidines to toxic urazoles and triazolidines respectively (Edwards et al., 2000).

In addition to herbicides and safeners, metal exposure is also known to affect GSTs in plants. As mentioned, this observation is likely related to the involvement of GSTs in detoxifying products resulting from oxidative damage to cellular macromolecules. Indeed, exposure to Cd, Cu, cobalt (Co), Hg, Ni and Zn significantly increased GST activity in root tips of *H. vulgare*. Interestingly,

615 this response was also induced by salt, cold, drought and H_2O_2 exposure (Halušková,
616 Valentovičová, Huttová, Mistrík, & Tamás, 2009). Furthermore, Cd was shown to induce GST
617 activity in roots and leaves of *Ricinus communis*. This Cd-induced increase in GST activity was
618 also observed in *B. napus* roots. As Cd exposure significantly increased MDA concentrations in
619 both plant species, the induction of GST activity could be targeted towards detoxifying lipid
620 peroxidation products (Bauddh, Kumar, Srivastava, Singh, & Tripathi, 2016). In addition, Kumar
621 et al. (2013) reported that gene expression levels of *GSTL1* and *GSTL2* were increased by As, Cd
622 and Pb exposure in *O. sativa* seedlings, while they were not affected by Cr. In contrast, *GSTL3*
623 expression was induced by As and Pb, but not by Cd. Taken together, these data indicate that metal-
624 induced transcriptional responses of GSTs depend on the metal and GST isoform under study
625 (Kumar et al., 2013; Lyubenova & Schroder, 2011).

626

627 *3.2.3* *Glutathione in antioxidative defence*

628 Glutathione, as the most abundant essential non-enzymatic antioxidant in plant cells, contributes
629 to antioxidative defence in several ways. First of all, it can directly react with singlet oxygen ($^1O_2$),
630 H_2O_2 and hydroxyl radicals ($^{\bullet}OH$) (Gill & Tuteja, 2010b). In addition, GSH plays an important role
631 in the antioxidative ascorbate (AsA)-GSH cycle, also referred to as the Foyer-Halliwell-Asada
632 pathway. In this cycle, H_2O_2 is reduced to H_2O by the action of ascorbate peroxidase (APX),
633 simultaneously oxidising AsA to dehydroascorbate (DHA). The latter molecule is again reduced
634 to AsA by the action of DHAR, using GSH as an electron donor (Fig. 1) (Foyer & Noctor, 2011).
635 Furthermore, GSH also contributes to antioxidative defence as an electron donor for glutathione
636 peroxidase (GPX), catalysing the reduction of H_2O_2, organic peroxides and lipid peroxides (Anjum

et al., 2012; Gill & Tuteja, 2010b). In addition, it reduces glutaredoxins (GRXs), which are involved in several processes including the regeneration of antioxidative enzymes (Rouhier, 2010).

In all reactions described above, GSH donates an electron – derived from the cysteine residue of its thiol group – to another molecule and thereby becomes reactive. It then readily reacts with another reactive GSH molecule, forming glutathione disulphide (GSSG). Subsequently, GSSG can be reduced to GSH by the action of the NADPH-dependent GR enzyme (Fig. 1). Plants possess two GR-encoding genes: (1) *GR1*, present in the cytosol and (2) *GR2*, dually targeted to plastids and mitochondria (Noctor et al., 2012). As GR is constitutively active, more than 90 percent of the cellular GSH pool is in its reduced form under control conditions. Under stress conditions however, GSH demand can exceed GR activity, thereby decreasing the GSH/GSSG ratio (Jozefczak et al., 2012). As the most abundant redox couple in plant cells, GSH/GSSG play a crucial role in redox regulation (Noctor et al., 2012). However, the relevance of the GSH/GSSG redox potential as driving force of biological processes has to be critically discussed (Flohe, 2013).

Plant GSH levels and redox state are often affected by metal(loid) exposure (Jozefczak et al., 2012). For example, Dixit et al. (2016) demonstrated that exposure to 25 and 50 µM As significantly increased GSH and GSSG levels in both roots and shoots of *O. sativa* plants. The observed oxidation of GSH was related to the activation of the AsA-GSH cycle, as indicated by significantly increased APX activities in both organs. However, the GSH/GSSG ratio remained unaffected after As exposure. This is probably due to the observed increases in the activities of GR and enzymes involved in sulphate assimilation and GSH biosynthesis such as SAT and GSH1. It is interesting to note that under all conditions, GSH levels were positively correlated with sulphur concentrations in the growth medium, while GSSG levels decreased with increasing sulphur levels. These data indicate that antioxidative defence in As-exposed plants is more efficient under high sulphur

conditions (Dixit et al., 2016). Furthermore, the GSH redox state was significantly shifted towards a more reduced state in leaves of *A. thaliana* exposed to Zn concentrations ranging from 100 to 500 µM, despite a significant decrease of GR activity. However, the increased GSH/GSSG ratio could be explained by an increased GSH synthesis, as Zn significantly increased total GSH equivalents and free GSH levels. In contrast, Zn slightly decreased the GSH/GSSG ratio in roots, possibly as a result of the observed decrease in root GR activity. Furthermore, PC synthesis was strongly induced, thereby decreasing free GSH levels available for antioxidative defence reactions in roots of Zn-exposed plants (Remans et al., 2012). In addition, Cuypers et al. (2011) reported that both Cd and Cu exposure shifted the GSH redox state in *A. thaliana* roots towards a more oxidised state, which was much more pronounced in Cu-exposed plants. In contrast, the GSH/GSSG ratio was not significantly affected by either metal in the leaves (Cuypers et al., 2011). Furthermore, Nocito et al. (2006) reported that Cd exposure caused significantly decreased total GSH levels in roots of *Z. mays*. While reduced GSH levels strongly diminished, GSSG concentrations significantly increased in roots of Cd-exposed plants, thereby shifting the cellular redox balance towards a more oxidised state. Zinc exposure, in contrast, did not alter the GSH/GSSG ratio, as it increased both GSH and GSSG levels to the same extent. Again, exposure to Cu caused the most dramatic effects, as it decreased the GSH/GSSG ratio by more than 20-fold (Nocito et al., 2006). Taken together, these data indicate that metal-induced effects related to the GSH/GSSG ratio depend on many factors including the chemical properties of the metal, its concentration, the exposure duration and the plant species and organ considered.

As mentioned above, the role of GSH in plants exposed to organic contaminants is mainly related to its involvement in GST-mediated xenobiotic detoxification. In addition, GSH possibly functions in antioxidative defence reactions triggered by organic compounds such as herbicides. Indeed,

683 exposure to metazachlor for 14 days increased the activities of APX and GR in leaves of *B. napus*,
684 suggesting activation of the AsA-GSH cycle. This assumption was further supported by the
685 observation that the GSH redox state was significantly shifted towards its oxidised form. In
686 addition, activities of other antioxidative enzymes including SOD and CAT were also induced by
687 exposure to metazachlor (Vercampt et al., 2016). Similarly, exposure to the recommended field
688 dose of metribuzin increased GSSG levels and slightly decreased the GSH/GSSG ratio in leaves of
689 *Z. mays*, possibly as a result of the observed decrease in GR activity. In contrast, treatment with
690 the recommended field dose of pretilachlor increased GR activity and the GSH/GSSG ratio. These
691 results possibly explain the fact that the induction of H_2O_2 levels and lipid peroxidation was more
692 pronounced in plants exposed to metribuzin as compared to pretilachlor. Taken together, these data
693 indicate that herbicide-induced antioxidative plant responses depend on the specific herbicide
694 applied (Alla, Badawi, Hassan, El-Bastawisy, & Badran, 2008).

695

696 **4 Thiols play an important role in cellular regulation**

697 ***4.1 Methionine and S-adenosylmethionine synthesis***

698 In addition to its function as a building block of proteins and in particular MTs, GSH and PCs,
699 cysteine also plays an important role in the biosynthesis of the second sulphur-containing amino
700 acid methionine. Like lysine and threonine, methionine belongs to the family of aspartate-derived
701 amino acids (Hesse et al., 2001). It is composed of (1) a sulphur atom derived from cysteine, (2)
702 the nitrogen/carbon skeleton from a phosphorylated serine and (3) a folate-derived methyl group
703 (Wirtz & Droux, 2005). The synthesis of methionine comprises three steps (Fig. 1). In the first step,

cystathionine γ-synthase (CGS) catalyses the formation of the thioether cystathionine from its substrates cysteine and O-homophosphoserine (OPH). Subsequently, cystathionine β-lyase (CBL) cleaves cystathionine to homocysteine in a β-cleavage reaction. These two steps exclusively take place in plastids and are together referred to as the transsulphuration pathway (Droux, 2004; Wirtz & Droux, 2005). In the third and final step of methionine synthesis, methionine is formed through the methylation of homocysteine, which is catalysed by a vitamin B12-independent methionine synthase (MS), using methyltetrahydrofolate as the methyl donor (Fig. 1) (Takahashi et al., 2011). This enzyme is present in both plastids and the cytosol. Whereas the plastidal MS isoform is only involved in *de novo* methionine synthesis, the cytosolic isoform is suggested to also mediate the recycling of homocysteine resulting from the hydrolysis of S-adenosylhomocysteine (Gigolashvili & Kopriva, 2014; Wirtz & Droux, 2005).

More than 80% of the synthesised methionine is used for the production of SAM (Ravanel, Gakiere, Job, & Douce, 1998). This metabolite is produced from methionine and ATP in a cytosolic reaction catalysed by SAM synthetase (SAMS) (Fig. 1). Subsequently, SAM serves as methyl donor in a broad array of methylation reactions catalysed by methyltransferases. Furthermore, SAM is also the precursor for the synthesis of ethylene, polyamines, nicotianamine, phytosiderophores and biotin (Roje, 2006).

The regulation of methionine and SAM synthesis mainly takes place at the level of the CGS enzyme. Although CGS is not subject to feedback inhibition by methionine or its metabolites at the activity level, it is post-transcriptionally regulated by SAM (Amir, 2010). When SAM is present, it induces a temporary arrest of the translation elongation process of the Methionine Overaccumulation 1 (MTO1) domain in the CGS mRNA, causing degradation of the mRNA upstream of the stalled ribosome. As a result, 5'-truncated RNA species are produced, causing the

decay of the transcript (Amir, 2010; Onouchi et al., 2005). The importance of the tight regulation of CGS is highlighted by the observation that plants with increased or decreased CGS levels display severe morphological phenotypes (Amir, 2010).

Furthermore, control of methionine production also occurs at the level of OPH, which is the last common intermediate for methionine and threonine biosynthesis. Although CGS and threonine synthase (TS) both use OPH as a substrate, the affinity of TS for OPH strongly exceeds that of CGS. In addition, SAM enhances the activity and substrate affinity of TS. Therefore, OPH mainly flows to the threonine synthesis pathway when methionine and SAM levels are sufficiently high. When SAM levels decline, however, TS activity decreases and OPH supply to the methionine synthesis pathway increases (Takahashi et al., 2011).

In addition to cysteine, also methionine levels can be affected in metal-exposed plants. Indeed, exposure to 120 µM Ni was demonstrated to increase methionine concentrations in roots and shoots of *Matricaria chamomilla* (Kovacik, Klejdus, Hedbavny, & Backor, 2009). Furthermore, exposure to different sublethal Cd concentrations for 24 and 72 hours significantly increased methionine concentrations in both roots and leaves of *A. thaliana* (Keunen et al., 2016a). In addition, MS protein abundance was significantly increased by Cd exposure in *T. aestivum* leaves. This response was accompanied by an increased level of 1-aminocyclopropane-1-carboxylate (ACC) synthase (ACS), an intermediate in the ethylene biosynthesis pathway (Fig 1). Interestingly, similar effects were induced by exposure to trichlorobenzene, suggesting that both metals and organic contaminants can affect methionine and ethylene biosynthesis pathways in plants (Ge et al., 2009). In contrast, Barkla et al. (2014) reported that Zn decreased MS abundance in leaves of *A. thaliana*. In addition, serine hydroxymethyltransferase levels were also reduced in Zn-exposed plants. This enzyme catalyses the conversion of glycine to serine, thereby yielding methyl units that are

channelled into the methionine biosynthesis pathway. The Zn-induced decrease of this enzyme possibly results in a decreased methionine concentration. The authors hypothesise that this mechanism protects Zn-stressed cells by attenuating the initiation of protein translation, thereby preventing the synthesis of misfolded proteins (Barkla et al., 2014). Furthermore, a Zn-induced decrease of methionine levels was also observed in shoots of *N. tabacum* (Pavlíková et al., 2014). Exposure to As, however, increased methionine concentrations in *O. sativa*. This response was more pronounced in a genotype accumulating higher As levels. A similar response was observed for cysteine and other stress-responsive amino acids such as proline, glycine and glutamate. Furthermore, clear correlations were reported between the levels of these amino acids, the extent of lipid peroxidation and the activities of antioxidative enzymes, suggesting that their levels are increased as a defence mechanism against As-induced oxidative stress (Dave et al., 2013).

Further evidence supporting a role for methionine in plant responses to metal stress is again derived from comparing metal-tolerant and sensitive species. Begum et al. (2016) reported that As exposure significantly increased methionine levels in a tolerant *O. sativa* genotype, whereas they were not affected in an As-sensitive genotype. Similar results were demonstrated for cysteine and GSH, underlining the importance of sulphur metabolism in plant metal tolerance (Begum et al., 2016). Similarly, Liang et al. (2014) demonstrated that Cd increased root methionine levels in a hyperaccumulating *S. alfredii* ecotype, while this response was absent in its non-hyperaccumulating counterpart. Furthermore, *SAMS* expression levels were only significantly enhanced by Cd exposure in roots and shoots of the hyperaccumulator plants. Taken together, these data point towards a role for SAM in mediating Cd tolerance in *S. alfredii* (Liang et al., 2014). Furthermore, MS and SAMS protein abundance were significantly increased by Cu exposure in roots of a metallicolous population of *Agrostis capillaris*, while they were not affected in a non-

773 metallicolous population, further supporting a role for methionine and SAM in plant metal
774 tolerance (Hego et al., 2014).

775 Whereas the effects of metals on methionine and SAM biosynthesis are relatively well studied,
776 information regarding the influence of organic contaminants on these processes is scarce and
777 therefore constitutes an interesting topic for future research. In this regard, evidence has been
778 presented that SAM-methyltransferase is involved in the detoxification of the herbicide fluorodifen
779 in *Picea*. The fluorodifen metabolite 2-nitro-4-trifluoromethyl-thiophenol was rapidly converted
780 into the corresponding 2-nitro-4-trifluoromethyl-thioanisole, a volatile end product of the
781 degradation pathway in needles of *P. abies*, *Picea glauca* and *Picea pungens* (Lamoureux, Rusness,
782 & Schroder, 1993). A similar pathway was demonstrated for the metabolism of
783 pentachloronitrobenzene in *Allium cepa* (Lamoureux & Rusness, 1980).

784

785 ***4.2 Ethylene: hormonal regulation***

786 As mentioned above, thiols represent important precursors in a large number of cellular processes
787 and regulatory networks. As an activated form of methionine, SAM is a key player in the synthesis
788 of ethylene and polyamines (PAs). As a precursor of ethylene, SAM is directly linked to hormonal
789 signalling.

790 In the ethylene biosynthesis pathway, SAM is converted to ACC by the ACS enzyme belonging to
791 the class of pyridoxal phosphate (PLP)-dependent enzymes utilising vitamin B6 as a cofactor (Fig.
792 1). This reaction involves the release of 5'-methylthioadenosine (MTA), which is recycled back to
793 methionine in the so-called Yang Cycle (Murr & Yang, 1975). The ACS enzyme is located in the
794 cytosol and encoded by a multigene family of 12 members in *Arabidopsis*, from which 8 encode

functional proteins (Van de Poel & Van Der Straeten, 2014). Expression of different members of this large gene family is tissue-dependent and single isoforms are specifically involved in distinct physiological or developmental tasks (Tsuchisaka & Theologis, 2004). Furthermore, complex interactions are known to occur between different ACS members (Tsuchisaka et al., 2009).

The second step in ethylene synthesis consists of the oxidation of ACC to ethylene via ACC oxidase (ACO) in the presence of oxygen (Fig. 1) (Kende, 1993). As a member of the superfamily of dioxygenases, ACO requires Fe^{2+} as cofactor and bicarbonate as activator (Zhang, Ren, Clifton, & Schofield, 2004). Information on its cellular localisation remains unclear: it could be localised either in the cytosol (Chung, Chou, Kuang, Charng, & Yang, 2002; Hudgins, Ralph, Franceschi, & Bohlmann, 2006) or the plasma membrane (Ramassamy, Olmos, Bouzayen, Pech, & Latche, 1998; Rombaldi et al., 1994). Even though ACS is considered the rate-limiting step of ethylene biosynthesis in plants, there are reports that point into the direction of ACO limiting ethylene synthesis under certain conditions, e.g. post-climacteric fruit ripening in tomato (Van de Poel & Van Der Straeten, 2014). In plants, ethylene synthesis is regulated by a variety of internal and external signals, often with ACS as main target (for a review, see Van de Poel and Van Der Straeten (2014) and Keunen et al. (2016b)).

Many reports have shown enhanced ethylene production upon metal exposure in plants, with the extent depending on the metal and its applied concentration. From all inorganic ions, Cd probably causes the strongest induction of ethylene production in plants (Keunen et al., 2016b). While this was shown for several species under short time exposure conditions, longer Cd exposure decreased ethylene concentrations in *A. thaliana* (Carrio-Segui, Garcia-Molina, Sanz, & Penarrubia, 2015). Summing up literature data, an intimate relationship exists between metal stress and ethylene production in plants. In addition, more and more reports suggest an implication of ethylene

818 signalling in plant adaptation and tolerance to metal stress (Keunen et al., 2016b; Thao et al., 2015).
819 As discussed in section 5, extensive cross-talk is observed between ethylene and other key players
820 in the plant metal stress response such as ROS and GSH.

821 In contrast with the vast amount of data available on the involvement of ethylene in plant metal
822 stress responses, knowledge on its role in plants exposed to organic contaminants is limited.
823 Nonetheless, Kumerova et al. (2012) have demonstrated a significant stimulation of ethylene
824 release in germinating *Z. mays* and *P. sativum* seeds during exposure to the polycyclic aromatic
825 hydrocarbon fluoranthene. In addition, seed germination was significantly inhibited by this
826 compound in both species (Kummerova et al., 2012).

827

828 ### *4.3 Polyamines: hormone-like signalling compounds*

829 Next to ethylene synthesis, SAM is also a precursor for the synthesis of polyamines, representing
830 a group of low-molecular-weight polycationic amines ubiquitous in all living organisms (Liu,
831 Wang, Wu, Gong, & Moriguchi, 2015b). In plants, the positively charged polyamines are either
832 bound to negatively charged molecules or conjugated to small molecules and proteins but also
833 occur as free forms (Walters, 2003). Polyamines are involved in a large number of cellular
834 functions and regulatory processes, including the stabilisation of proteins and other biomolecules,
835 the regulation of cell division, growth and differentiation or senescence as well as general adaptive
836 stress responses (Bouchereau, Aziz, Larher, & Martin-Tanguy, 1999; Groppa & Benavides, 2008;
837 Walters, 2003).

838 Putrescine (Put), spermidine (Spd) and spermine (Spm) are the three major polyamines and
839 therefore most studied in plants. They can be present in free, soluble conjugated and insoluble

bound forms. In plants, polyamine synthesis is well studied and based on the two precursors L-ornithine and L-arginine from which Put is generated by the catalytic actions of ornithine decarboxylase (ODC) and arginine decarboxylase (ADC). From Put, both Spd and Spm are formed, with a strong connection to SAM (Fig. 1) (Sauter et al., 2013). In a first step, SAM is decarboxylated to decarboxylated SAM (dcSAM) by SAM decarboxylase (SAMDC). This metabolite then provides the aminopropyl group for the conversion of Put into Spd by Spd synthase. Spermidine is then further converted into Spm by Spm synthase, again using dcSAM as an aminopropyl donor (Fig. 1) (Liu et al. 2015). From this reaction, MTA is released and feeded back into the SAM cycle where SAM is synthesised from methionine by SAM synthase (SAMS) (Sauter et al., 2013).

Apart from *de novo* synthesis of polyamines, it is worth mentioning that there is catabolism of these molecules, involving both Cu-containing diamine oxidases (CuAOs) as well as FAD-dependent polyamine oxidases (PAOs) (Cona, Rea, Angelini, Federico, & Tavladoraki, 2006; Liu et al., 2015b).

The relationship between abiotic stress and altered (mostly elevated) polyamine levels is well established and known for a long time. Nevertheless, their actual function still remains unclear. Although polyamines could protect cells against abiotic stress, for example due to their antioxidative properties, they could also cause cell damage due to the production of H_2O_2 by their catabolism (Minocha, Majumdar, & Minocha, 2014).

As indicated in Fig. 1, SAM represents a direct link between ethylene and polyamine synthesis, allowing plants to directly switch between both pathways. Interestingly, polyamines and ethylene display counteracting functions in plants. Whereas ethylene is known to induce senescence, polyamines play the opposite role by decelerating chlorophyll loss and cell membrane degradation

as well as inducing protease and RNase activity (Kusano, Berberich, Tateda, & Takahashi, 2008). This is due to their ability to neutralise acids, their antioxidative properties as well as their potential to stabilise cell walls and membranes (Gill & Tuteja, 2010a).

Different reports indicate a role for polyamines in plant metal stress responses. For example, exposure to Cu and Cd led to an increase in ADC and ODC activity in *T. aestivum* leaves. This effect was stronger under Cd exposure, where Put levels were elevated up to 3-fold as compared to control levels. Spermidine concentration was not affected by any of the metals, whereas Spm levels were significantly reduced. Interestingly, externally applied Spm reduced the formation of H_2O_2 and led to a reduction of TBARS levels as well. A potential antioxidative function of Spm could be concluded from these results, although the actual mechanism of protection is still unclear (Groppa, Tomaro, & Benavides, 2007).

Although it is well established that in plants many organic pollutants cause the formation of ROS, there is only little information available on how the polyamine metabolism is affected by these xenobiotic compounds. Nevertheless, Burritt (2008) could link phenanthrene exposure to the synthesis of polyamines. Phenanthrene is a polycyclic and highly toxic aromatic hydrocarbon that is often found in aquatic environments. Whereas data on its effect on aquatic macrophytes are scarce, polycyclic aromatic hydrocarbons often induce oxidative stress in plants. The involvement of polyamines in the protection of plants against oxidative stress could be shown for *Riccia fluitans* exposed to phenanthrene. Concentrations up to 500 µM led to an induction of oxidative stress to which the plants responded with an increase in polyamine synthesis linked to elevated ADC and SAMDC activity. Chemical inhibition of these enzymes caused an inhibition of plant recovery, while externally applied polyamines could reduce the negative effects caused by phenanthrene exposure (Burritt, 2008). More recent studies suggest the application of polyamines as priming

886 agents prior to all sorts of abiotic stress, also including metal exposure (Savvides, Ali, Tester, &
887 Fotopoulos, 2016). This opens the window to more extensive research on the involvement of
888 polyamines in plant responses to metals as well as organic pollutants.

889

890 **5 Interaction between detoxification and signal transduction pathways**

891 As discussed in the previous sections of this review, sulphur-containing metabolites play an
892 important role in plant responses to metal stress. While compounds directly derived from cysteine
893 (e.g. GSH, PCs and MTs) are involved in metal chelation and ROS detoxification, molecules
894 derived from methionine and its primary metabolite SAM (e.g. ethylene and polyamines) mainly
895 function in cellular signal transduction. However, it should be noted that different components of
896 the detoxification and signal transduction pathways interact with each other under both
897 physiological and stress conditions, including metal exposure (Fig. 2).

898 [Insert Figure 2 here]

899 For example, cross-talk exists between ethylene and other players involved in plant responses to
900 metal stress, as recently reviewed by Keunen et al. (2016b). Indeed, several studies demonstrate a
901 role for ethylene in the oxidative burst induced by a broad range of stress conditions. Cao et al.
902 (2006) demonstrated that paraquat-induced increases in $O_2^{\bullet-}$ and H_2O_2 levels were less pronounced
903 in an *ethylene insensitive 2-1* (*ein2-1*) mutant as compared to those in WT *A. thaliana* plants. As a
904 consequence, the extent of lipid peroxidation was significantly lower in the mutant (Cao et al.,
905 2006). Similar responses were described in *ein2-1* mutants exposed to Al (Zhang, He, Zhao, Huang,
906 & Hao, 2014b). In addition, blocking of ethylene biosynthesis and/or signalling resulted in a

907 reduced extent of H_2O_2 production in camptothecin-exposed *Lycopersicon esculentum* suspension
908 cells (de Jong, Yakimova, Kapchina, & Woltering, 2002) and Cd-exposed *Phaseolus coccineus*
909 roots (Maksymiec, 2011). Furthermore, inhibition of ethylene biosynthesis limited Cd-induced $O_2^{\bullet-}$
910 accumulation in root hair tips of *B. napus*, suggesting that ethylene is an upstream regulator of $O_2^{\bullet-}$
911 generation (Sun & Guo, 2013). The effect of ethylene on $O_2^{\bullet-}$ production is possibly mediated by
912 its interaction with ROS-producing NADPH oxidases, also referred to as respiratory burst oxidase
913 homologues (RBOHs). This is confirmed by the observation that H_2O_2 production induced by the
914 ethylene releasing compound ethephon in *I. batatas* was limited by treatment with the NADPH
915 inhibitor diphenyleneiodonium (Chen, Huang, Huang, Chow, & Lin, 2013). This relationship
916 between ethylene and NADPH oxidases is also supported under metal exposure conditions, as Hg-
917 induced increases in apoplastic H_2O_2 accumulation and NADPH oxidase activity in apical root
918 segments of *Medicago sativa* were reduced by treatment with an ethylene signalling inhibitor
919 (Montero-Palmero, Martin-Barranco, Escobar, & Hernandez, 2014). Furthermore, Keunen et al.
920 (2015) reported that the Cd-induced *RBOHC* upregulation in several *A. thaliana* ethylene
921 biosynthesis and signal transduction mutants was less pronounced as compared to that in WT
922 plants.

923 In addition to its effects on ROS production, ethylene is also linked to several players of the
924 antioxidative defence system. It interacts with both enzymatic antioxidants such as SOD and CAT
925 and non-enzymatic antioxidants such as AsA and α-tocopherol (Keunen et al., 2016b). In the
926 context of this review, the interaction between ethylene and GSH is of particular interest. The cross-
927 talk between these two compounds is probably related to the link of ethylene biosynthesis and
928 signalling with sulphate assimilation (Fig. 2), as was recently reviewed by Iqbal et al. (2013) and
929 Wawrzynska et al. (2015). Indeed, sulphur nutrition has been shown to modulate plant responses

to a diverse array of stress factors by increasing ethylene production (Wawrzynska et al., 2015). Nazar et al. (2014), for example, demonstrated that excess sulphur alleviated the effects of salt stress in *B. juncea*. However, this response was absent when ethylene synthesis was inhibited, suggesting a role for ethylene in the sulphur-induced salt stress alleviation (Nazar et al., 2014). Furthermore, several genes involved in ethylene signalling were strongly upregulated in sulphate-treated *Vitis vinifera* (Giraud, Ivanova, Gordon, Whelan, & Considine, 2012). In addition to sulphur excess, sulphur limitation was also shown to increase ethylene concentrations and the expression of ethylene-related genes in *N. tabacum* plants (Lewandowska et al., 2010; Moniuszko et al., 2013).

Interestingly, the interplay between ethylene and sulphate assimilation also functions in the other direction, with ethylene levels affecting several proteins of the sulphate assimilation pathway (Fig. 2). Indeed, it was shown that ethephon significantly induced ATPS activity and sulphur content in *B. juncea* (Iqbal, Khan, Nazar, & da Silva, 2012). Furthermore, treatment of *A. thaliana* with 200 µM ACC was shown to induce APR at the transcriptional and activity level (Koprivova, North, & Kopriva, 2008). As GSH synthesis depends on sulphur availability for the production of its precursor cysteine, ethylene possibly modulates the sulphate assimilation pathway in order to meet the increasing demand for GSH during stress conditions (Keunen et al., 2016b).

The cross-talk between ethylene and GSH is further supported by the observation that application of exogenous GSH in *A. thaliana* increased both gene expression levels and protein abundance of ACS2 and ERF2, involved in ethylene biosynthesis and signal transduction respectively (Sinha et al., 2015). Furthermore, the ethephon-induced increased ROS production in sweet potato was attenuated when plants were treated with exogenous GSH (Chen et al., 2013). In addition, expression levels of ethylene-related genes are often affected in transgenic plants with altered GSH levels (Keunen et al., 2016b). Schnaubelt et al. (2015), for example, reported several *ERF* genes to

be significantly upregulated in the severely GSH-deficient *rml1-1 A. thaliana* mutant as compared to WT plants. In contrast, *ERF2* expression was significantly downregulated in the *A. thaliana cad2-1* mutant, which is characterised by a milder GSH deficiency (Han, Mhamdi, Chaouch, & Noctor, 2013). Furthermore, transgenic tobacco plants with an enhanced GSH content displayed a significant upregulation of genes involved in ethylene biosynthesis and signal transduction (Ghanta et al., 2014). Similarly, Datta et al. (2015) reported that transgenic *A. thaliana* plants with elevated GSH levels displayed increased ACS2, ACS6 and ACO1 transcription levels and protein abundance as compared to WT plants, while the opposite response was observed in the GSH-deficient *pad2-1* mutant. The authors demonstrated that the GSH-induced upregulation of *ACS2* and *ACS6* was mediated by a WRKY33-related mechanism, whereas the increased *ACO1* expression was due to an increased stability of its encoding mRNA. Interestingly, application of exogenous GSH also increased the tolerance of WT *A. thaliana* plants to necrotrophic infection and salt stress, while it did not affect the stress tolerance of *ein2-1* mutants deficient in ethylene signalling. These data suggest that GSH induces plant tolerance to different stress factors by an ethylene-dependent mechanism (Datta et al., 2015).

Interestingly, the relationship between ethylene and GSH is also supported under metal exposure conditions (Keunen et al., 2016b). Indeed, ethephon application was shown to increase GSH concentrations in *B. juncea* exposed to Cd (Masood, Iqbal, & Khan, 2012), Ni and Zn (Khan & Khan, 2014). Furthermore, Schellingen et al. (2015) reported that the Cd-induced upregulation of genes involved in GSH biosynthesis was significantly weaker in an *acs2-1/6-1* knockout mutant as compared to WT *A. thaliana* plants, suggesting that ethylene biosynthesis is essential for the induction of efficient GSH-dependent defence responses upon Cd exposure (Schellingen et al., 2015). Similarly, the crucial role of EIN2 in Pb tolerance in *A. thaliana* was also demonstrated to

be partially related to its stimulating effect on GSH levels (Cao et al., 2009). It should be noted that the interaction between ethylene and GSH under metal exposure conditions also functions in the other direction, with GSH levels affecting ethylene signalling. Indeed, Hasan et al. (2016) reported that the Cd-induced upregulation of *ERF1* and *ERF2* was more pronounced in plants supplied with 5 mM GSH, while it was weaker in plants treated with the GSH biosynthesis inhibitor buthionine sulphoximine.

In addition to ethylene, polyamines are also subject to cross-talk with the antioxidative defence system during stress conditions. Addition of Spd to *Solanum lycopersicum* exposed to chilling stress, for example, strongly enhanced the chilling-induced increase in GSH and AsA levels. Furthermore, it significantly increased the GSH/GSSG and AsA/DHA ratios, possibly as a result of the observed induction of enzymes involved in the AsA-GSH cycle at the transcriptional and/or activity level. While the enzymatic activities of SOD, POD and CAT were decreased by chilling stress, this effect was reversed by adding Spd, with activities even increasing those measured in control plants. These data possibly explain the observed reduction of chilling-induced $O_2^{\bullet-}$ and H_2O_2 production by addition of exogenous Spd (Diao, Song, & Qi, 2015). Similarly, positive effects of polyamine treatment on the antioxidative defence system were also reported in salt-exposed *O. sativa* (Jain, Vart, Verma, & Malhotra, 2015) and *Cucumis sativus* (Shu, Yuan, Guo, Sun, & Yuan, 2013) and *Glycine max.* subjected to osmotic stress (Radhakrishnan & Lee, 2013).

Polyamines were also shown to positively affect several components of the antioxidative defence system in metal-exposed plants. Indeed, Spd treatment was shown to further enhance the Cr-induced increase in GSH levels in *Rhapus sativus*. Furthermore, Spd significantly alleviated Cr-induced lipid peroxidation and H_2O_2 concentrations. In contrast, it counteracted the increase in CAT activity caused by Cr exposure. Overall, exogenous Spd increased plant tolerance to Cr, as

indicated by its positive effects on root and shoot length and fresh weight of Cr-exposed plants (Choudhary, Kanwar, Bhardwaj, Yu, & Tran, 2012). Similarly, Rady and Hemida (2015) reported that addition of either Spd or Spm counteracted the negative effects of Cd exposure on several growth parameters of *T. aestivum* plants. This effect was likely related to the fact that polyamine application attenuated Cd-induced effects on leaf GSH levels and SOD, CAT and APX activity. As a result, Cd-induced lipid peroxidation and H_2O_2 production were reduced in leaves of polyamine-treated plants. However, these data should be interpreted with caution, as the application of Spd and Spm also significantly reduced shoot Cd concentrations potentially explaining the observed attenuation of metal stress (Rady & Hemida, 2015).

Taken together, available data indicate that significant cross-talk exists between cysteine-related detoxification and SAM-related signal transduction pathways under different stress conditions including exposure to metals and possibly also organic contaminants. This interaction should not be surprising as the sulphur atom of cysteine is used to synthesise methionine and its primary metabolite SAM, thereby linking both pathways. However, further research is needed to fully elucidate the mechanisms connecting both pathways (Fig. 2).

6 Concluding remarks and future perspectives

The sulphur-containing amino acids cysteine and methionine play crucial roles in plant responses to metals and organic contaminants through their incorporation into the primary metabolites GSH and SAM. Whereas GSH is primarily involved in chelation and detoxification mechanisms, SAM mainly contributes to signal transduction reactions mediated by ethylene and polyamines.

Significant cross-talk exists between these detoxification and signal transduction pathways, which is explained by the fact that cysteine is a precursor for both GSH and methionine synthesis. Furthermore, ROS appear to play an important role in mediating the interaction between both pathways. Whereas the effects of metal exposure on plant detoxification and signal transduction pathways are relatively well described, information regarding the influence of organic contaminants is rather scarce and mostly limited to herbicides and safeners, indicating a need for further research. Elucidating plant responses to metals and organic compounds can significantly contribute to optimising phytoremediation strategies for the clean-up of contaminated soils. In this strategy, special attention should be given to sulphur availability in the environment as it affects sulphate assimilation and downstream detoxification and regulation pathways that are important factors controlling metal uptake and translocation as well as phytotoxic responses. With view to the importance of sulphur nutrition and the internal regulation of sulphur-dependent metabolic pathways for plant health and performance, future research on breeding and practical application of plants should focus on ways to establish stable and adaptive sulphur metabolism in crops. This could be achieved by molecular approaches, fostering plant-microbe interactions or the use of sustainable agrochemical amendments.

7 References

Abu-Qare, A. W., & Duncan, H. J. (2002). Herbicide safeners: Uses, limitations, metabolism, and mechanisms of action. *Chemosphere, 48*, 965-974.

Adams, C. A., Blee, E., & Casida, J. E. (1983). Dichloroacetamide herbicide antidotes enhance sulfate metabolism in corn roots. *Pesticide Biochemistry and Physiology, 19*, 350-360.

Ahn, Y. O., Kim, S. H., Lee, J., Kim, H., Lee, H.-S., & Kwak, S.-S. (2012). Three *Brassica rapa* metallothionein genes are differentially regulated under various stress conditions. *Molecular Biology Reports, 39*, 2059-2067.

Alla, M. M. N., Badawi, A. H. M., Hassan, N. M., El-Bastawisy, Z. M., & Badran, E. G. (2008). Herbicide tolerance in maize is related to increased levels of glutathione and glutathione-associated enzymes. *Acta Physiologiae Plantarum, 30*, 371-379.

Alla, M. M. N., Hassan, N. M., & El-Bastawisy, Z. M. (2008). Changes in antioxidants and kinetics of glutathione-S-transferase of maize in response to isoproturon treatment. *Plant Biosystems, 142*, 5-16.

Amir, R. (2010). Current understanding of the factors regulating methionine content in vegetative tissues of higher plants. *Amino Acids, 39*, 917-931.

Anjum, N. A., Ahmad, I., Mohmood, I., Pacheco, M., Duarte, A. C., Pereira, E., et al. (2012). Modulation of glutathione and its related enzymes in plants' responses to toxic metals and metalloids - A review. *Environmental and Experimental Botany, 75*, 307-324.

Anjum, N. A., Gill, S. S., & Gill, R. (2014). Cysteine - Jack of all glutathione-based plant stress defence trades. In N. A. Anjum, S. S. Gill, & R. Gill (Eds.), *Plant adaptation to environmental change - Significance of amino acids and their derivatives* (pp. 35-52). London: CAB International.

Anjum, N. A., Hasanuzzaman, M., Hossein, M. A., Thangavel, P., Roychoudhury, A., Gill, S. S., et al. (2015). Jacks of metal/metalloid chelation trade in plants - an overview. *Frontiers in Plant Science, 6*, 1-17.

Armstrong, R. N. (1997). Structure, catalytic mechanism, and evolution of the glutathione transferases. *Chemical Research in Toxicology, 10*, 2-18.

Auguy, F., Mouna, F., Moulin, P., El Mzibri, M., Abdelaziz, S., Filali-Maltouf, A., et al. (2016). Transcriptome changes in *Hirschfeldia incana* in response to lead exposure. *Frontiers in Plant Science, 6*, 1-13.

Ball, L., Accotto, G. P., Bechtold, U., Creissen, G., Funck, D., Jimenez, A., et al. (2004). Evidence for a direct link between glutathione biosynthesis and stress defense gene expression in *Arabidopsis*. *Plant Cell, 16*, 2448-2462.

Barkla, B. J., Vera-Estrella, R., Miranda-Vergara, M. C., & Pantoja, O. (2014). Quantitative proteomics of heavy metal exposure in *Arabidopsis thaliana* reveals alterations in one-carbon metabolism enzymes upon exposure to zinc. *Journal of Proteomics, 111*, 128-138.

Bartucca, M. L., Celletti, S., Astolfi, S., Mimmo, T., Cesco, S., Panfili, I., et al. (2016). Effect of three safeners on sulfur assimilation and iron deficiency response in barley (*Hordeum vulgare*) plants. *Pest Management Science*.

Bashir, H., Ahmad, J., Bagheri, R., Nauman, M., & Qureshi, M. I. (2013). Limited sulfur resource forces *Arabidopsis thaliana* to shift towards non-sulfur tolerance under cadmium stress. *Environmental and Experimental Botany, 94*, 19-32.

Bashir, H., Ibrahim, M. M., Bagheri, R., Ahmad, J., Arif, I. A., Baig, M. A., et al. (2015). Influence of sulfur and cadmium on antioxidants, phytochelatins and growth in Indian mustard. *Aob Plants, 7*, 1-13.

Bauddh, K., Kumar, A., Srivastava, S., Singh, R. P., & Tripathi, R. D. (2016). A study on the effect of cadmium on the antioxidative defense system and alteration in different functional groups in castor bean and Indian mustard. *Archives of Agronomy and Soil Science, 62*, 877-891.

Begum, M. C., Islam, M. S., Islam, M., Amin, R., Parvez, M. S., & Kabir, A. H. (2016). Biochemical and molecular responses underlying differential arsenic tolerance in rice (*Oryza sativa* L.). *Plant Physiology and Biochemistry, 104*, 266-277.

Belford, E. J., Dorfler, U., Stampfl, A., & Schroder, P. (2004). Microsomal detoxification enzymes in yam bean [*Pachyrhizus erosus* (L.) urban]. *Z Naturforsch C, 59*, 693-700.

Blindauer, C. A., & Leszczyszyn, O. I. (2010). Metallothioneins: unparalleled diversity in structures and functions for metal ion homeostasis and more. *Natural Product Reports, 27*, 720-741.

Bouchereau, A., Aziz, A., Larher, F., & Martin-Tanguy, J. (1999). Polyamines and environmental challenges: recent development. *Plant Science, 140*, 103-125.

Brazier-Hicks, M., Evans, K. M., Cunningham, O. D., Hodgson, D. R. W., Steel, P. G., & Edwards, R. (2008). Catabolism of glutathione conjugates in *Arabidopsis thaliana* - Role in metabolic reactivation of the herbicide safener fenclorim. *Journal of Biological Chemistry, 283*, 21102-21112.

Brunetti, P., Zanella, L., De Paolis, A., Di Litta, D., Cecchetti, V., Falasca, G., et al. (2015). Cadmium-inducible expression of the ABC-type transporter AtABCC3 increases phytochelatin-mediated cadmium tolerance in *Arabidopsis*. *Journal of Experimental Botany, 66*, 3815-3829.

1106 Burritt, D. J. (2008). The polycyclic aromatic hydrocarbon phenanthrene causes oxidative stress
1107 and alters polyamine metabolism in the aquatic liverwort *Riccia fluitans* L. *Plant Cell and*
1108 *Environment, 31*, 1416-1431.
1109 Cairns, N. G., Pasternak, M., Wachter, A., Cobbett, C. S., & Meyer, A. J. (2006). Maturation of
1110 *Arabidopsis* seeds is dependent on glutathione biosynthesis within the embryo. *Plant*
1111 *Physiology, 141*, 446-455.
1112 Cao, M. J., Wang, Z., Wirtz, M., Hell, R., Oliver, D. J., & Xiang, C. B. (2013). SULTR3;1 is a
1113 chloroplast-localized sulfate transporter in *Arabidopsis thaliana*. *Plant Journal, 73*, 607-
1114 616.
1115 Cao, S. Q., Chen, Z. Y., Liu, G. Q., Jiang, L., Yuan, H. B., Ren, G., et al. (2009). The *Arabidopsis*
1116 Ethylene-Insensitive 2 gene is required for lead resistance. *Plant Physiology and*
1117 *Biochemistry, 47*, 308-312.
1118 Cao, S. Q., Jiang, S. T., & Zhang, R. X. (2006). Evidence for a role of Ethylene-Insensitive 2 gene
1119 in the regulation of the oxidative stress response in *Arabidopsis*. *Acta Physiologiae*
1120 *Plantarum, 28*, 417-425.
1121 Carrio-Segui, A., Garcia-Molina, A., Sanz, A., & Penarrubia, L. (2015). Defective copper transport
1122 in the *copt5* mutant affects cadmium tolerance. *Plant and Cell Physiology, 56*, 442-454.
1123 Chen, H. J., Hou, W. C., Yang, C. Y., Huang, D. J., Liu, J. S., & Lin, Y. H. (2003). Molecular
1124 cloning of two metallothionein-like protein genes with differential expression patterns from
1125 sweet potato (*Ipomoea batatas*) leaves. *Journal of Plant Physiology, 160*, 547-555.
1126 Chen, H. J., Huang, C. S., Huang, G. J., Chow, T. J., & Lin, Y. H. (2013). NADPH oxidase inhibitor
1127 diphenyleneiodonium and reduced glutathione mitigate ethephon-mediated leaf
1128 senescence, H_2O_2 elevation and senescence-associated gene expression in sweet potato
1129 *(Ipomoea batatas*). *Journal of Plant Physiology, 170*, 1471-1483.

1130 Cheng, J. C., Seeley, K. A., & Sung, Z. R. (1995). *RML1* and *RML2, Arabidopsis* genes required
1131 for cell proliferation at the root tip. *Plant Physiology, 107*, 365-376.
1132 Chiang, H. C., Lo, J. C., & Yeh, K. C. (2006). Genes associated with heavy metal tolerance and
1133 accumulation in Zn/Cd hyperaccumulator *Arabidopsis halleri*: A genomic survey with
1134 cDNA microarray. *Environmental Science & Technology, 40*, 6792-6798.
1135 Chirakkara, R. A., Cameselle, C., & Reddy, K. R. (2016). Assessing the applicability of
1136 phytoremediation of soils with mixed organic and heavy metal contaminants. *Reviews in*
1137 *Environmental Science and Bio/Technology, 15*, 299-326.
1138 Choudhary, S. P., Kanwar, M., Bhardwaj, R., Yu, J. Q., & Tran, L. S. P. (2012). Chromium stress
1139 mitigation by polyamine-brassinosteroid application involves phytohormonal and
1140 physiological strategies in *Raphanus sativus* L. *Plos One, 7*, 11.
1141 Chung, M. C., Chou, S. J., Kuang, L. Y., Charng, Y. Y., & Yang, S. F. (2002). Subcellular
1142 localization of 1-aminocyclopropane-1-carboxylic acid oxidase in apple fruit. *Plant and*
1143 *Cell Physiology, 43*, 549-554.
1144 Clendennen, S. K., & May, G. D. (1997). Differential gene expression in ripening banana fruit.
1145 *Plant Physiology, 115*, 463-469.
1146 Cobbett, C., & Goldsbrough, P. (2002). Phytochelatins and metallothioneins: Roles in heavy metal
1147 detoxification and homeostasis. *Annual Review of Plant Biology, 53*, 159-182.
1148 Cole, D. J., Cummins, I., Hatton, P. J., Dixon, D. P., & Edwards, R. (1997). Glutathione
1149 transferases in crops and major weeds. In K. K. Hatzios (Ed.), *Regulation of enzymatic*
1150 *systems detoxifying xenobiotics in plants* (Vol. 37, pp. 139-154). Dordrecht: Kluwer
1151 Academic Publishers.

1152 Coleman, J., Blake-Kalff, M., & Davies, E. (1997). Detoxification of xenobiotics by plants:
1153 chemical modification and vacuolar compartmentation. *Trends in Plant Science, 2*, 144-
1154 151.
1155 Cona, A., Rea, G., Angelini, R., Federico, R., & Tavladoraki, P. (2006). Functions of amine
1156 oxidases in plant development and defence. *Trends in Plant Science, 11*, 80-88.
1157 Cummins, I., Bryant, D. N., & Edwards, R. (2009). Safener responsiveness and multiple herbicide
1158 resistance in the weed black-grass (*Alopecurus myosuroides*). *Plant Biotechnology Journal,*
1159 *7*, 807-820.
1160 Cummins, I., Dixon, D. P., Freitag-Pohl, S., Skipsey, M., & Edwards, R. (2011). Multiple roles for
1161 plant glutathione transferases in xenobiotic detoxification. *Drug Metabolism Reviews, 43*,
1162 266-280.
1163 Cuypers, A., Hendrix, S., dos Reis, R. A., De Smet, S., Deckers, J., Gielen, H., et al. (2016).
1164 Hydrogen peroxide, signaling in disguise during metal phytotoxicity. *Frontiers in Plant*
1165 *Science, 7*, 1-25.
1166 Cuypers, A., Smeets, K., Ruytinx, J., Opdenakker, K., Keunen, E., Remans, T., et al. (2011). The
1167 cellular redox state as a modulator in cadmium and copper responses in *Arabidopsis*
1168 *thaliana* seedlings. *J Plant Physiol, 168*, 309-316.
1169 Daghan, H., Arslan, M., Uygur, V., & Koleli, N. (2013). Transformation of tobacco with *ScMTII*
1170 gene-enhanced cadmium and zinc accumulation. *Clean-Soil Air Water, 41*, 503-509.
1171 Datta, R., Kumar, D., Sultana, A., Hazra, S., Bhattacharyya, D., & Chattopadhyay, S. (2015).
1172 Glutathione regulates 1-aminocyclopropane-1-carboxylate synthase transcription via
1173 WRKY33 and 1-aminocyclopropane-1-carboxylate oxidase by modulating messenger
1174 RNA stability to induce ethylene synthesis during stress. *Plant Physiology, 169*, 2963-2981.

1175 Dauch, A. L., & Jabaji-Hare, S. H. (2006). Metallothionein and bZIP transcription factor genes
1176 from velvetleaf and their differential expression following *Colletotrichum coccodes*
1177 infection. *Phytopathology, 96*, 1116-1123.
1178 Dave, R., Tripathi, R. D., Dwivedi, S., Tripathi, P., Dixit, G., Sharma, Y. K., et al. (2013). Arsenate
1179 and arsenite exposure modulate antioxidants and amino acids in contrasting arsenic
1180 accumulating rice (*Oryza sativa* L.) genotypes. *Journal of Hazardous Materials, 262*, 1123-
1181 1131.
1182 Davidian, J. C., & Kopriva, S. (2010). Regulation of sulfate uptake and assimilation - the same or
1183 not the same? *Molecular Plant, 3*, 314-325.
1184 Davies, J., & Caseley, J. C. (1999). Herbicide safeners: a review. *Pesticide Science, 55*, 1043-1058.
1185 de Jong, A. J., Yakimova, E. T., Kapchina, V. M., & Woltering, E. J. (2002). A critical role for
1186 ethylene in hydrogen peroxide release during programmed cell death in tomato suspension
1187 cells. *Planta, 214*, 537-545.
1188 Dean, J. V., Devarenne, T. P., Lee, I. S., & Orlofsky, L. E. (1995). Properties of a maize glutathione
1189 S-transferase that conjugates coumaric acid and other phenylpropanoids. *Plant Physiology,*
1190 *108*, 985-994.
1191 Del Buono, D., Scarponi, L., & Espen, L. (2007). Glutathione S-transferases in *Festuca*
1192 *arundinacea*: Identification, characterization and inducibility by safener benoxacor.
1193 *Phytochemistry, 68*, 2614-2624.
1194 DeRidder, B. P., Dixon, D. P., Beussman, D. J., Edwards, R., & Goldsbrough, P. B. (2002).
1195 Induction of glutathione S-transferases in *Arabidopsis* by herbicide safeners. *Plant*
1196 *Physiology, 130*, 1497-1505.
1197 Dhindsa, R. S. (1991). Drought stress, enzymes of glutathione metabolism, oxidation injury, and
1198 protein synthesis in *Tortula ruralis*. *Plant Physiology, 95*, 648-651.

1199 Diao, Q. N., Song, Y. J., & Qi, H. Y. (2015). Exogenous spermidine enhances chilling tolerance of
1200 tomato (*Solanum lycopersicum* L.) seedlings via involvement in polyamines metabolism
1201 and physiological parameter levels. *Acta Physiologiae Plantarum, 37*, 15.
1202 Diesperger, H., & Sandermann, H. (1979). Soluble and microsomal glutathione S-transferase
1203 activities in pea seedlings (*Pisum sativum* L.). *Planta, 146*, 643-648.
1204 Dixit, G., Singh, A. P., Kumar, A., Mishra, S., Dwivedi, S., Kumar, S., et al. (2016). Reduced
1205 arsenic accumulation in rice (*Oryza sativa* L.) shoot involves sulfur mediated improved
1206 thiol metabolism, antioxidant system and altered arsenic transporters. *Plant Physiology and*
1207 *Biochemistry, 99*, 86-96.
1208 Dixon, D. P., Davis, B. G., & Edwards, R. (2002). Functional divergence in the glutathione
1209 transferase superfamily in plants - Identification of two classes with putative functions in
1210 redox homeostasis in *Arabidopsis thaliana*. *Journal of Biological Chemistry, 277*, 30859-
1211 30869.
1212 Dixon, D. P., & Edwards, R. (2010). Glutathione transferases. *The Arabidopsis Book* (Vol. 8, pp.
1213 e0131).
1214 Dixon, D. P., Skipsey, M., & Edwards, R. (2010). Roles for glutathione transferases in plant
1215 secondary metabolism. *Phytochemistry, 71*, 338-350.
1216 Dominguez-Solis, J. R., Gutierrez-Alcala, G., Romero, L. C., & Gotor, C. (2001). The cytosolic
1217 O-acetylserine(thiol)lyase gene is regulated by heavy metals and can function in cadmium
1218 tolerance. *Journal of Biological Chemistry, 276*, 9297-9302.
1219 Dominguez-Solis, J. R., Lopez-Martin, M. C., Ager, F. J., Ynsa, M. D., Romero, L. C., & Gotor,
1220 C. (2004). Increased cysteine availability is essential for cadmium tolerance and
1221 accumulation in *Arabidopsis thaliana*. *Plant Biotechnology Journal, 2*, 469-476.

1222 Droog, F. (1997). Plant glutathione S-transferases, a tale of theta and tau. *Journal of Plant Growth*
1223 *Regulation, 16*, 95-107.
1224 Droux, M. (2004). Sulfur assimilation and the role of sulfur in plant metabolism: a survey.
1225 *Photosynthesis Research, 79*, 331-348.
1226 Droux, M., Ruffet, M. L., Douce, R., & Job, D. (1998). Interactions between serine
1227 acetyltransferase and O-acetylserine (thiol) lyase in higher plants - Structural and kinetic
1228 properties of the free and bound enzymes. *European Journal of Biochemistry, 255*, 235-
1229 245.
1230 Dudler, R., Hertig, C., Rebmann, G., Bull, J., & Mauch, F. (1991). A pathogen-induced wheat gene
1231 encodes a protein homologous to glutathione-S-transferases. *Molecular Plant-Microbe*
1232 *Interactions, 4*, 14-18.
1233 Edwards, R., Dixon, D. P., & Walbot, V. (2000). Plant glutathione S-transferases: enzymes with
1234 multiple functions in sickness and in health. *Trends in Plant Science, 5*, 193-198.
1235 Edwards, R., & Dixon, R. A. (1991). Glutathione S-cinnamoyl transferases in plants.
1236 *Phytochemistry, 30*, 79-84.
1237 Ernst, W. H. O., Krauss, G. J., Verkleij, J. A. C., & Wesenberg, D. (2008). Interaction of heavy
1238 metals with the sulphur metabolism in angiosperms from an ecological point of view. *Plant*
1239 *Cell and Environment, 31*, 123-143.
1240 Ezra, G., Rusness, D. G., Lamoureux, G. L., & Stephenson, G. R. (1985). The effect of CDAA
1241 (N,N,-diallyl-2-chloroacetamide) pretreatments on subsequent CDAA injury to corn (*Zea*
1242 *mays* L.). *Pesticide Biochemistry and Physiology, 23*, 108-115.
1243 Farago, S., & Brunold, C. (1990). Regulation of assimilatory sulfate reduction by herbicide
1244 safeners in *Zea mays* L. *Plant Physiology, 94*, 1808-1812.

1245 Fischer, S., Kuhnlenz, T., Thieme, M., Schmidt, H., & Clemens, S. (2014). Analysis of plant Pb
1246 tolerance at realistic submicromolar concentrations demonstrates the role of phytochelatin
1247 synthesis for Pb detoxification. *Environmental Science & Technology, 48*, 7552-7559.
1248 Flohe, L. (2013). The fairytale of the GSSG/GSH redox potential. *Biochimica Et Biophysica Acta-*
1249 *General Subjects, 1830*, 3139-3142.
1250 Foyer, C. H., & Noctor, G. (2005). Oxidant and antioxidant signalling in plants: a re-evaluation of
1251 the concept of oxidative stress in a physiological context. *Plant Cell and Environment, 28*,
1252 1056-1071.
1253 Foyer, C. H., & Noctor, G. (2011). Ascorbate and glutathione: The heart of the redox hub. *Plant*
1254 *Physiology, 155*, 2-18.
1255 Freeman, J. L., Persans, M. W., Nieman, K., Albrecht, C., Peer, W., Pickering, I. J., et al. (2004).
1256 Increased glutathione biosynthesis plays a role in nickel tolerance in *Thlaspi* nickel
1257 hyperaccumulators. *Plant Cell, 16*, 2176-2191.
1258 Freisinger, E. (2008). Plant MTs - long neglected members of the metallothionein superfamily.
1259 *Dalton Transactions*, 6663-6675.
1260 Freisinger, E. (2011). Structural features specific to plant metallothioneins. *Journal of Biological*
1261 *Inorganic Chemistry, 16*, 1035-1045.
1262 Frova, C. (2003). The plant glutathione transferase gene family: genomic structure, functions,
1263 expression and evolution. *Physiologia Plantarum, 119*, 469-479.
1264 Gajewska, E., Glowacki, R., Mazur, J., & Sklodowska, M. (2013). Differential response of wheat
1265 roots to Cu, Ni and Cd treatment: oxidative stress and defense reactions. *Plant Growth*
1266 *Regulation, 71*, 13-20.
1267 Gautam, N., Verma, P. K., Verma, S., Tripathi, R. D., Trivedi, P. K., Adhikari, B., et al. (2012).
1268 Genome-wide identification of rice class I metallothionein gene: tissue expression patterns

and induction in response to heavy metal stress. *Functional & Integrative Genomics, 12*, 635-647.

Ge, C., Ding, Y., Wang, Z., Wan, D., Wang, Y., Shang, Q., et al. (2009). Responses of wheat seedlings to cadmium, mercury and trichlorobenzene stresses. *Journal of Environmental Sciences, 21*, 806-813.

Gerhardt, K. E., Huang, X.-D., Glick, B. R., & Greenberg, B. M. (2009). Phytoremediation and rhizoremediation of organic soil contaminants: Potential and challenges. *Plant Science, 176*, 20-30.

Ghanta, S., Datta, R., Bhattacharyya, D., Sinha, R., Kumar, D., Hazra, S., et al. (2014). Multistep involvement of glutathione with salicylic acid and ethylene to combat environmental stress. *Journal of Plant Physiology, 171*, 940-950.

Gielen, H., Remans, T., Vangronsveld, J., & Cuypers, A. (2016). Toxicity responses of Cu and Cd: the involvement of miRNAs and the transcription factor SPL7. *BMC Plant Biology, 16*, 16.

Gigolashvili, T., & Kopriva, S. (2014). Transporters in plant sulfur metabolism. *Frontiers in Plant Science, 5*, 1-16.

Gill, S. S., & Tuteja, N. (2010a). Polyamines and abiotic stress tolerance in plants. *Plant Signaling & Behavior, 5*, 26-33.

Gill, S. S., & Tuteja, N. (2010b). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry, 48*, 909-930.

Giraud, E., Ivanova, A., Gordon, C. S., Whelan, J., & Considine, M. J. (2012). Sulphur dioxide evokes a large scale reprogramming of the grape berry transcriptome associated with oxidative signalling and biotic defence responses. *Plant Cell and Environment, 35*, 405-417.

1292 Gotor, C., Laureano-Marin, A. M., Moreno, I., Aroca, A., Garcia, I., & Romero, L. C. (2015).
1293 Signaling in the plant cytosol: cysteine or sulfide? *Amino Acids, 47*, 2155-2164.
1294 Gronwald, J. W., Fuerst, E. P., Eberlein, C. V., & Egli, M. A. (1987). Effect of herbicide antidotes
1295 on glutathione content and glutathione S-transferase activity of *Sorghum* shoots. *Pesticide*
1296 *Biochemistry and Physiology, 29*, 66-76.
1297 Groppa, M. D., & Benavides, M. P. (2008). Polyamines and abiotic stress: recent advances. *Amino*
1298 *Acids, 34*, 35-45.
1299 Groppa, M. D., Tomaro, M. L., & Benavides, M. P. (2007). Polyamines and heavy metal stress:
1300 the antioxidant behavior of spermine in cadmium- and copper-treated wheat leaves.
1301 *Biometals, 20*, 185-195.
1302 Guo, W. J., Bundithya, W., & Goldsbrough, P. B. (2003). Characterization of the *Arabidopsis*
1303 metallothionein gene family: tissue-specific expression and induction during senescence
1304 and in response to copper. *New Phytologist, 159*, 369-381.
1305 Halušková, L. u., Valentovičová, K., Huttová, J., Mistrík, I., & Tamás, L. (2009). Effect of abiotic
1306 stresses on glutathione peroxidase and glutathione S-transferase activity in barley root tips.
1307 *Plant Physiology and Biochemistry, 47*, 1069-1074.
1308 Han, Y., Mhamdi, A., Chaouch, S., & Noctor, G. (2013). Regulation of basal and oxidative stress-
1309 triggered jasmonic acid-related gene expression by glutathione. *Plant Cell and*
1310 *Environment, 36*, 1135-1146.
1311 Harada, E., Choi, Y. E., Tsuchisaka, A., Obata, H., & Sano, H. (2001). Transgenic tobacco plants
1312 expressing a rice cysteine synthase gene are tolerant to toxic levels of cadmium. *Journal of*
1313 *Plant Physiology, 158*, 655-661.
1314 Hasan, M. K., Liu, C., Wang, F., Ahammed, G. J., Zhou, J., Xu, M.-X., et al. (2016). Glutathione-
1315 mediated regulation of nitric oxide, S-nitrosothiol and redox homeostasis confers cadmium

tolerance by inducing transcription factors and stress response genes in tomato. *Chemosphere, 161*, 536-545.

Hassinen, V. H., Tervahauta, A. I., Schat, H., & Karenlampi, S. O. (2011). Plant metallothioneins - metal chelators with ROS scavenging activity? *Plant Biology, 13*, 225-232.

Hassinen, V. H., Tuomainen, M., Peraniemi, S., Schat, H., Karenlampi, S. O., & Tervahauta, A. I. (2009). Metallothioneins 2 and 3 contribute to the metal-adapted phenotype but are not directly linked to Zn accumulation in the metal hyperaccumulator, *Thlaspi caerulescens*. *Journal of Experimental Botany, 60*, 187-196.

Hego, E., Bes, C. M., Bedon, F., Palagi, P. M., Chaumeil, P., Barre, A., et al. (2014). Differential accumulation of soluble proteins in roots of metallicolous and nonmetallicolous populations of *Agrostis capillaris* L. exposed to Cu. *Proteomics, 14*, 1746-1758.

Hesse, H., Kreft, O., Maimann, S., Zeh, M., Willmitzer, L., & Höfgen, R. (2001). Approaches towards understanding methionine biosynthesis in higher plants. *Amino Acids, 20*, 281-289.

Hirase, K., & Molin, W. T. (2003). Sulfur assimilation in plants and weed control: Potential targets for novel herbicides and action sites of certain safeners. *Weed Biology and Management, 3*, 147-157.

Hothorn, M., Wachter, A., Gromes, R., Stuwe, T., Rausch, T., & Scheffzek, K. (2006). Structural basis for the redox control of plant glutamate cysteine ligase. *Journal of Biological Chemistry, 281*, 27557-27565.

Howden, R., Andersen, C. R., Goldsbrough, P. B., & Cobbett, C. S. (1995). A cadmium-sensitive, glutathione-deficient mutant of *Arabidopsis thaliana*. *Plant Physiology, 107*, 1067-1073.

Hudgins, J. W., Ralph, S. G., Franceschi, V. R., & Bohlmann, J. (2006). Ethylene in induced conifer defense: cDNA cloning, protein expression, and cellular and subcellular localization of 1-

aminocyclopropane-1-carboxylate oxidase in resin duct and phenolic parenchyma cells. *Planta, 224*, 865-877.

Ibrahim, R. K., Hayyan, M., AlSaadi, M. A., Hayyan, A., & Ibrahim, S. (2016). Environmental application of nanotechnology: air, soil, and water. *Environmental Science and Pollution Research, 23*, 13754-13788.

Iqbal, N., Khan, N. A., Nazar, R., & da Silva, J. A. T. (2012). Ethylene-stimulated photosynthesis results from increased nitrogen and sulfur assimilation in mustard types that differ in photosynthetic capacity. *Environmental and Experimental Botany, 78*, 84-90.

Iqbal, N., Masood, A., Khan, M. I., Asgher, M., Fatma, M., & Khan, N. A. (2013). Cross-talk between sulfur assimilation and ethylene signaling in plants. *Plant Signal Behav, 8*, e22478.

Jain, V., Vart, S., Verma, E., & Malhotra, S. P. (2015). Spermine reduces salinity-induced oxidative damage by enhancing antioxidative system and decreasing lipid peroxidation in rice seedlings. *Journal of Plant Biochemistry and Biotechnology, 24*, 316-323.

Jozefczak, M., Keunen, E., Schat, H., Bliek, M., Hernandez, L. E., Carleer, R., et al. (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*, 3145-3175.

Kataoka, T., Hayashi, N., Yamaya, T., & Takahashi, H. (2004a). Root-to-shoot transport of sulfate in *Arabidopsis*. Evidence for the role of SULTR3;5 as a component of low-affinity sulfate transport system in the root vasculature. *Plant Physiology, 136*, 4198-4204.

1361 Kataoka, T., Watanabe-Takahashi, A., Hayashi, N., Ohnishi, M., Mimura, T., Buchner, P., et al.
1362 (2004b). Vacuolar sulfate transporters are essential determinants controlling internal
1363 distribution of sulfate in *Arabidopsis*. *Plant Cell, 16*, 2693-2704.
1364 Kawashima, C. G., Noji, M., Nakamura, M., Ogra, Y., Suzuki, K. T., & Saito, K. (2004). Heavy
1365 metal tolerance of transgenic tobacco plants over-expressing cysteine synthase.
1366 *Biotechnology Letters, 26*, 153-157.
1367 Kende, H. (1993). Ethylene biosynthesis. *Annual Review of Plant Physiology and Plant Molecular*
1368 *Biology, 44*, 283-307.
1369 Keunen, E., Florez-Sarasa, I., Obata, T., Jozefczak, M., Remans, T., Vangronsveld, J., et al.
1370 (2016a). Metabolic responses of *Arabidopsis thaliana* roots and leaves to sublethal
1371 cadmium exposure are differentially influenced by ALTERNATIVE OXIDASE1a.
1372 *Environmental and Experimental Botany, 124*, 64-78.
1373 Keunen, E., Schellingen, K., Van Der Straeten, D., Remans, T., Colpaert, J., Vangronsveld, J., et
1374 al. (2015). ALTERNATIVE OXIDASE1a modulates the oxidative challenge during
1375 moderate Cd exposure in *Arabidopsis thaliana* leaves. *Journal of Experimental Botany, 66*,
1376 2967-2977.
1377 Keunen, E., Schellingen, K., Vangronsveld, J., & Cuypers, A. (2016b). Ethylene and metal stress:
1378 Small molecule, big impact. *Frontiers in Plant Science, 7*, 1-18.
1379 Khan, M. I. R., & Khan, N. A. (2014). Ethylene reverses photosynthetic inhibition by nickel and
1380 zinc in mustard through changes in PS II activity, photosynthetic nitrogen use efficiency,
1381 and antioxidant metabolism. *Protoplasma, 251*, 1007-1019.
1382 Kim, S. H., Jeong, J. C., Ahn, Y. O., Lee, H. S., & Kwak, S. S. (2014). Differential responses of
1383 three sweetpotato metallothionein genes to abiotic stress and heavy metals. *Molecular*
1384 *Biology Reports, 41*, 6957-6966.

1385 Kim, Y. O., Jung, S., Kim, K., & Bae, H. J. (2013). Role of pCeMT, a putative metallothionein
1386 from *Colocasia esculenta*, in response to metal stress. *Plant Physiology and Biochemistry,*
1387 *64*, 25-32.
1388 Kocsy, G., von Ballmoos, P., Ruegsegger, A., Szalai, G., Galiba, G., & Brunold, C. (2001).
1389 Increasing the glutathione content in a chilling-sensitive maize genotype using safeners
1390 increased protection against chilling-induced injury. *Plant Physiology, 127*, 1147-1156.
1391 Koprivova, A., North, K. A., & Kopriva, S. (2008). Complex signaling network in regulation of
1392 adenosine 5'-phosphosulfate reductase by salt stress in *Arabidopsis* roots. *Plant Physiology,*
1393 *146*, 1408-1420.
1394 Kovacik, J., Klejdus, B., Hedbavny, J., & Backor, M. (2009). Nickel uptake and its effect on some
1395 nutrient levels, amino acid contents and oxidative status in *Matricaria chamomilla* plants.
1396 *Water, Air, & Soil Pollution, 202*, 199-209.
1397 Kumar, G., Kushwaha, H. R., Panjabi-Sabharwal, V., Kumari, S., Joshi, R., Karan, R., et al. (2012).
1398 Clustered metallothionein genes are co-regulated in rice and ectopic expression of *OsMT1e-*
1399 *P* confers multiple abiotic stress tolerance in tobacco via ROS scavenging. *BMC Plant*
1400 *Biology, 12*, 1-15.
1401 Kumar, S., Asif, M. H., Chakrabarty, D., Tripathi, R. D., Dubey, R. S., & Trivedi, P. K. (2013).
1402 Differential expression of rice lambda class GST gene family members during plant growth,
1403 development, and in response to stress conditions. *Plant Molecular Biology Reporter, 31*,
1404 569-580.
1405 Kumari, N., Parmar, P., & Sharma, V. (2015). Differential gene expression in two contrasting
1406 wheat cultivars under cadmium stress. *Biologia Plantarum, 59*, 701-707.

Kummerova, M., Zezulka, S., Vanova, L., & Fiserova, H. (2012). Effect of organic pollutant treatment on the growth of pea and maize seedlings. *Central European Journal of Biology, 7*, 159-166.

Kusano, T., Berberich, T., Tateda, C., & Takahashi, Y. (2008). Polyamines: essential factors for growth and survival. *Planta, 228*, 367-381.

Lallement, P. A., Brouwer, B., Keech, O., Hecker, A., & Rouhier, N. (2014). The still mysterious roles of cysteine-containing glutathione transferases in plants. *Frontiers in Pharmacology, 5*, 1-22.

Lamoureux, G. L., & Frear, D. S. (1987). Current problems, trends and developments in pesticide metabolism in plants. In R. Greenhalgh & T. R. Roberts (Eds.), *Pesticide science and biotechnology* (pp. 455-463). Oxford: Blackwell Scientific.

Lamoureux, G. L., & Rusness, D. G. (1980). In vitro metabolism of pentachloronitrobenzene to pentachloromethylthiobenzene by onion: Characterization of glutathione S-transferase, cysteine C-S lyase, and S-adenosylmethionine methyl transferase activities. *Pesticide Biochemistry and Physiology, 14*, 50-61.

Lamoureux, G. L., & Rusness, D. G. (1989). The role of glutathione and glutathione S-transferases in pesticide metabolism, selectivity and mode of action in plants and insects. In D. Dolphin, R. Poulson, & O. Avramovic (Eds.), *Glutathione: Chemical, biochemical, and medical aspects* (Vol. IIIB, pp. 153-196). New York: Wiley & Sons.

Lamoureux, G. L., Rusness, D. G., & Schroder, P. (1993). Metabolism of a diphenylether herbicide to a volatile thioanisole and a polar sulfonic-acid metabolite in spruce (*Picea*). *Pesticide Biochemistry and Physiology, 47*, 8-20.

1429 Leszczyszyn, O. I., Imam, H. T., & Blindauer, C. A. (2013). Diversity and distribution of plant
1430 metallothioneins: a review of structure, properties and functions. *Metallomics, 5*, 1146-
1431 1169.
1432 Levine, A., Tenhaken, R., Dixon, R., & Lamb, C. (1994). H_2O_2 from the oxidative burst
1433 orchestrates the plant hypersensitive disease resistance response. *Cell, 79*, 583-593.
1434 Lewandowska, M., Wawrzynska, A., Moniuszko, G., Lukomska, J., Zientara, K., Piecho, M., et al.
1435 (2010). A contribution to identification of novel regulators of plant response to sulfur
1436 deficiency: characteristics of a tobacco gene *UP9C*, its protein product and the effects of
1437 *UP9C* silencing. *Molecular Plant, 3*, 347-360.
1438 Li, Z., Zhang, Y., Zhang, X. Q., Peng, Y., Merewitz, E., Ma, X., et al. (2016). The alterations of
1439 endogenous polyamines and phytohormones induced by exogenous application of
1440 spermidine regulate antioxidant metabolism, metallothionein and relevant genes conferring
1441 drought tolerance in white clover. *Environmental and Experimental Botany, 124*, 22-38.
1442 Li, Z. S., Alfenito, M., Rea, P. A., Walbot, V., & Dixon, R. A. (1997). Vacuolar uptake of the
1443 phytoalexin medicarpin by the glutathione conjugate pump. *Phytochemistry, 45*, 689-693.
1444 Liang, J., Shohag, M. J. I., Yang, X. E., Tian, S. K., Zhang, Y. B., Feng, Y., et al. (2014). Role of
1445 sulfur assimilation pathway in cadmium hyperaccumulation by *Sedum alfredii* Hance.
1446 *Ecotoxicology and Environmental Safety, 100*, 159-165.
1447 Liang, T. S., Ding, H., Wang, G. D., Kang, J. Q., Pang, H. X., & Lv, J. Y. (2016). Sulfur decreases
1448 cadmium translocation and enhances cadmium tolerance by promoting sulfur assimilation
1449 and glutathione metabolism in *Brassica chinensis* L. *Ecotoxicology and Environmental*
1450 *Safety, 124*, 129-137.
1451 Lim, B., Meyer, A. J., & Cobbett, C. S. (2011). Development of glutathione-deficient embryos in
1452 *Arabidopsis* is influenced by the maternal level of glutathione. *Plant Biology, 13*, 693-697.

Liu, J., Shi, X. T., Qian, M., Zheng, L. Q., Lian, C. L., Xia, Y., et al. (2015a). Copper-induced hydrogen peroxide upregulation of a metallothionein gene, *OsMT2c*, from *Oryza sativa* L. confers copper tolerance in *Arabidopsis thaliana*. *Journal of Hazardous Materials, 294*, 99-108.

Liu, J. H., Wang, W., Wu, H., Gong, X. Q., & Moriguchi, T. (2015b). Polyamines function in stress tolerance: from synthesis to regulation. *Frontiers in Plant Science, 6*, 1-10.

Lo Cicero, L., Madesis, P., Tsaftaris, A., & Lo Piero, A. R. (2015). Tobacco plants over-expressing the sweet orange tau glutathione transferases (CsGSTUs) acquire tolerance to the diphenyl ether herbicide fluorodifen and to salt and drought stresses. *Phytochemistry, 116*, 69-77.

Loyall, L., Uchida, K., Braun, S., Furuya, M., & Frohnmeyer, H. (2000). Glutathione and a UV light-induced glutathione S-transferase are involved in signaling to chalcone synthase in cell cultures. *Plant Cell, 12*, 1939-1950.

Lyubenova, L., & Schroder, P. (2011). Plants for waste water treatment - Effects of heavy metals on the detoxification system of *Typha latifolia*. *Bioresource Technology, 102*, 996-1004.

Ma, Y., Oliveira, R. S., Freitas, H., & Zhang, C. (2016). Biochemical and molecular mechanisms of plant-microbe-metal interactions: Relevance for phytoremediation. *Frontiers in Plant Science, 7*, 1-19.

Maksymiec, W. (2011). Effects of jasmonate and some other signalling factors on bean and onion growth during the initial phase of cadmium action. *Biologia Plantarum, 55*, 112-118.

Mandavian, K., Ghaderian, S. M., & Schat, H. (2016). Pb accumulation, Pb tolerance, antioxidants, thiols, and organic acids in metallicolous and non-metallicolous *Peganum harmala* L. under Pb exposure. *Environmental and Experimental Botany, 126*, 21-31.

Mannervik, B., & Danielson, U. H. (1988). Glutathione transferases - Structure and catalytic activity. *Crc Critical Reviews in Biochemistry, 23*, 283-337.

Marrs, K. A. (1996). The functions and regulation of glutathione S-transferases in plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, *47*, 127-158.

Marrs, K. A., Alfenito, M. R., Lloyd, A. M., & Walbot, V. (1995). A glutathione S-transferase involved in vacuolar transfer encoded by the maize gene *Bronze-2*. *Nature, 375*, 397-400.

Martínez, M., Bernal, P., Almela, C., Vélez, D., García-Agustín, P., Serrano, R., et al. (2006). An engineered plant that accumulates higher levels of heavy metals than *Thlaspi caerulescens*, with yields of 100 times more biomass in mine soils. *Chemosphere, 64*, 478-485.

Martinoia, E., Grill, E., Tommasini, R., Kreuz, K., & Amrhein, N. (1993). ATP-dependent glutathione S-conjugate export pump in the vacuolar membrane of plants. *Nature, 364*, 247-249.

Maruyama-Nakashita, A., Nakamura, Y., Watanabe-Takahashi, A., Inoue, E., Yamaya, T., & Takahashi, H. (2005). Identification of a novel cis-acting element conferring sulfur deficiency response in *Arabidopsis* roots. *Plant Journal, 42*, 305-314.

Masood, A., Iqbal, N., & Khan, N. A. (2012). Role of ethylene in alleviation of cadmium-induced photosynthetic capacity inhibition by sulphur in mustard. *Plant Cell and Environment, 35*, 524-533.

Mauch, F., & Dudler, R. (1993). Differential induction of distinct glutathione-S-transferases of wheat by xenobiotics and by pathogen attack. *Plant Physiology, 102*, 1193-1201.

Maughan, S. C., Pasternak, M., Cairns, N., Kiddle, G., Brach, T., Jarvis, R., et al. (2010). Plant homologs of the *Plasmodium falciparum* chloroquine-resistance transporter, PfCRT, are required for glutathione homeostasis and stress responses. *Proceedings of the National Academy of Sciences of the United States of America, 107*, 2331-2336.

Mazen, A. M. A. (2004). Accumulation of four metals in tissues of *Corchorus olitorius* and possible mechanisms of their tolerance. *Biologia Plantarum, 48*, 267-272.

Mengoni, A., Gonnelli, C., Hakvoort, H. W. J., Galardi, F., Bazzicalupo, M., Gabbrielli, R., et al. (2003). Evolution of copper-tolerance and increased expression of a 2b-type metallothionein gene in *Silene paradoxa* L. populations. *Plant and Soil, 257*, 451-457.

Meyer, R. C., Goldsbrough, P. B., & Woodson, W. R. (1991). An ethylene-responsive flower senescence-related gene from carnation encodes a protein homologous to glutathione S-transferases. *Plant Molecular Biology, 17*, 277-281.

Minocha, R., Majumdar, R., & Minocha, S. C. (2014). Polyamines and abiotic stress in plants: a complex relationship. *Frontiers in Plant Science, 5*, 1-17.

Mir, G., Domenech, J., Huguet, G., Guo, W. J., Goldsbrough, P., Atrian, S., et al. (2004). A plant type 2 metallothionein (MT) from cork tissue responds to oxidative stress. *Journal of Experimental Botany, 55*, 2483-2493.

Miteva, L. P. E., Ivanov, S. V., & Alexieva, V. S. (2010). Alterations in glutathione pool and some related enzymes in leaves and roots of pea plants treated with the herbicide glyphosate. *Russian Journal of Plant Physiology, 57*, 131-136.

Mohanasundaram, K. A., Haworth, N. L., Grover, M. P., Crowley, T. M., Goscinski, A., & Wouters, M. A. (2015). Potential role of glutathione in evolution of thiol-based redox signaling sites in proteins. *Frontiers in Pharmacology, 6*, 1-15.

Moniuszko, G., Skoneczny, M., Zientara-Rytter, K., Wawrzynska, A., Glow, D., Cristescu, S. M., et al. (2013). Tobacco LSU-like protein couples sulphur-deficiency response with ethylene signalling pathway. *Journal of Experimental Botany, 64*, 5173-5182.

Montero-Palmero, M. B., Martin-Barranco, A., Escobar, C., & Hernandez, L. E. (2014). Early transcriptional responses to mercury: a role for ethylene in mercury-induced stress. *New Phytologist, 201*, 116-130.

Morgenstern, R., Depierre, J. W., & Jornvall, H. (1985). Microsomal glutathione transferase - Primary structure. *Journal of Biological Chemistry, 260*, 3976-3983.

Moyle, R., Fairbairn, D. J., Ripi, J., Crowe, M., & Botella, J. R. (2005). Developing pineapple fruit has a small transcriptome dominated by metallothionein. *Journal of Experimental Botany, 56*, 101-112.

Murr, D. P., & Yang, S. F. (1975). Conversion of 5'-methylthioadenosine to methionine by apple tissue. *Phytochemistry, 14*, 1291-1292.

Na, G., & Salt, D. E. (2011). The role of sulfur assimilation and sulfur-containing compounds in trace element homeostasis in plants. *Environmental and Experimental Botany, 72*, 18-25.

Nath, S., Panda, P., Mishra, S., Dey, M., Choudhury, S., Sahoo, L., et al. (2014). Arsenic stress in rice: Redox consequences and regulation by iron. *Plant Physiology and Biochemistry, 80*, 203-210.

Nazar, R., Khan, M. I. R., Iqbal, N., Masood, A., & Khan, N. A. (2014). Involvement of ethylene in reversal of salt-inhibited photosynthesis by sulfur in mustard. *Physiologia Plantarum, 152*, 331-344.

Ning, H. X., Zhang, C. H., Yao, Y., & Yu, D. Y. (2010). Overexpression of a soybean O-acetylserine (thiol) lyase-encoding gene *GmOASTL4* in tobacco increases cysteine levels and enhances tolerance to cadmium stress. *Biotechnology Letters, 32*, 557-564.

Nocito, F. F., Lancilli, C., Crema, B., Fourcroy, P., Davidian, J. C., & Sacchi, G. A. (2006). Heavy metal stress and sulfate uptake in maize roots. *Plant Physiology, 141*, 1138-1148.

Noctor, G., Gomez, L., Vanacker, H., & Foyer, C. H. (2002). Interactions between biosynthesis, compartmentation and transport in the control of glutathione homeostasis and signalling. *Journal of Experimental Botany, 53*, 1283-1304.

Noctor, G., Mhamdi, A., Chaouch, S., Han, Y., Neukermans, J., Marquez-Garcia, B., et al. (2012). Glutathione in plants: an integrated overview. *Plant Cell Environ, 35*, 454-484.

Noguera-Oviedo, K., & Aga, D. S. (2016). Lessons learned from more than two decades of research on emerging contaminants in the environment. *Journal of Hazardous Materials, 316*, 242-251.

Nutricati, E., Miceli, A., Blando, F., & De Bellis, L. (2006). Characterization of two *Arabidopsis thaliana* glutathione S-transferases. *Plant Cell Reports, 25*, 997-1005.

Onouchi, H., Nagami, Y., Haraguchi, Y., Nakamoto, M., Nishimura, Y., Sakurai, R., et al. (2005). Nascent peptide-mediated translation elongation arrest coupled with mRNA degradation in the *CGS1* gene of *Arabidopsis*. *Genes & Development, 19*, 1799-1810.

Oztetik, E. (2008). A tale of plant Glutathione S-transferases: Since 1970. *Botanical Review, 74*, 419-437.

Parisy, V., Poinssot, B., Owsianowski, L., Buchala, A., Glazebrook, J., & Mauch, F. (2007). Identification of PAD2 as a gamma-glutamylcysteine synthetase highlights the importance of glutathione in disease resistance of *Arabidopsis*. *Plant Journal, 49*, 159-172.

Pavlíková, D., Zemanová, V., Procházková, D., Pavlík, M., Száková, J., & Wilhelmová, N. (2014). The long-term effect of zinc soil contamination on selected free amino acids playing an important role in plant adaptation to stress and senescence. *Ecotoxicology and Environmental Safety, 100*, 166-170.

Pflugmacher, S., Schroder, P., & Sandermann, H. (2000). Taxonomic distribution of plant glutathione S-transferases acting on xenobiotics. *Phytochemistry, 54*, 267-273.

Prade, L., Huber, R., & Bieseler, B. (1998). Structures of herbicides in complex with their detoxifying enzyme glutathione S-transferase - explanations for the selectivity of the enzyme in plants. *Structure, 6*, 1445-1452.

Queval, G., Thominet, D., Vanacker, H., Miginiac-Maslow, M., Gakiere, B., & Noctor, G. (2009). H_2O_2-activated up-regulation of glutathione in *Arabidopsis* involves induction of genes encoding enzymes involved in cysteine synthesis in the chloroplast. *Molecular Plant, 2*, 344-356.

Radhakrishnan, R., & Lee, I. J. (2013). Spermine promotes acclimation to osmotic stress by modifying antioxidant, abscisic acid, and jasmonic acid signals in soybean. *Journal of Plant Growth Regulation, 32*, 22-30.

Rady, M. M., & Hemida, K. A. (2015). Modulation of cadmium toxicity and enhancing cadmium-tolerance in wheat seedlings by exogenous application of polyamines. *Ecotoxicology and Environmental Safety, 119*, 178-185.

Ramassamy, S., Olmos, E., Bouzayen, M., Pech, J. C., & Latche, A. (1998). 1-aminocyclopropane-1-carboxylate oxidase of apple fruit is periplasmic. *Journal of Experimental Botany, 49*, 1909-1915.

Ravanel, S., Gakiere, B., Job, D., & Douce, R. (1998). The specific features of methionine biosynthesis and metabolism in plants. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 7805-7812.

Ravilious, G. E., & Jez, J. M. (2012). Structural biology of plant sulfur metabolism: From assimilation to biosynthesis. *Natural Product Reports, 29*, 1138-1152.

Razem, F. A., & Bernards, M. A. (2002). Hydrogen peroxide is required for poly(phenolic) domain formation during wound-induced suberization. *Journal of Agricultural and Food Chemistry, 50*, 1009-1015.

Reichenauer, T. G., & Germida, J. J. (2008). Phytoremediation of organic contaminants in soil and groundwater. *ChemSusChem, 1*, 708-717.

1594 Remans, T., Opdenakker, K., Guisez, Y., Carleer, R., Schat, H., Vangronsveld, J., et al. (2012).
1595 Exposure of *Arabidopsis thaliana* to excess Zn reveals a Zn-specific oxidative stress
1596 signature. *Environmental and Experimental Botany, 84*, 61-71.
1597 Ren, Y. J., Liu, Y., Chen, H. Y., Li, G., Zhang, X. L., & Zhao, J. (2012). Type 4 metallothionein
1598 genes are involved in regulating Zn ion accumulation in late embryo and in controlling
1599 early seedling growth in *Arabidopsis*. *Plant Cell and Environment, 35*, 770-789.
1600 Roje, S. (2006). S-Adenosyl-L-methionine: Beyond the universal methyl group donor.
1601 *Phytochemistry, 67*, 1686-1698.
1602 Rombaldi, C., Lelievre, J. M., Latche, A., Petitprez, M., Bouzayen, M., & Pech, J. C. (1994).
1603 Immunocytolocalization of 1-aminocyclopropane-1-carboxylic acid oxidase in tomato and
1604 apple fruit. *Planta, 192*, 453-460.
1605 Romero, L. C., Aroca, M. A., Laureano-Marin, A. M., Moreno, I., Garcia, I., & Gotor, C. (2014).
1606 Cysteine and cysteine-related signaling pathways in *Arabidopsis thaliana*. *Molecular Plant,*
1607 *7*, 264-276.
1608 Roosens, N. H., Bernard, C., Leplae, R., & Verbruggen, N. (2004). Evidence for copper
1609 homeostasis function of metallothionein (MT3) in the hyperaccumulator *Thlaspi*
1610 *caerulescens*. *FEBS Letters, 577*, 9-16.
1611 Rouached, H., Wirtz, M., Alary, R., Hell, R., Arpat, A. B., Davidian, J. C., et al. (2008). Differential
1612 regulation of the expression of two high-affinity sulfate transporters, SULTR1.1 and
1613 SULTR1.2, in *Arabidopsis*. *Plant Physiology*, *147*, 897-911.
1614 Rouhier, N. (2010). Plant glutaredoxins: pivotal players in redox biology and iron-sulphur centre
1615 assembly. *New Phytologist, 186*, 365-372.

Ruiz, O. N., Alvarez, D., Torres, C., Roman, L., & Daniell, H. (2011). Metallothionein expression in chloroplasts enhances mercury accumulation and phytoremediation capability. *Plant Biotechnology Journal, 9*, 609-617.

Saito, K. (2000). Regulation of sulfate transport and synthesis of sulfur-containing amino acids. *Current Opinion in Plant Biology, 3*, 188-195.

Sauter, M., Moffatt, B., Saechao, M. C., Hell, R., & Wirtz, M. (2013). Methionine salvage and S-adenosylmethionine: Essential links between sulfur, ethylene and polyamine biosynthesis. *Biochemical Journal, 451*, 145-154.

Savvides, A., Ali, S., Tester, M., & Fotopoulos, V. (2016). Chemical priming of plants against multiple abiotic stresses: Mission possible? *Trends in Plant Science, 21*, 329-340.

Scalla, R., & Roulet, A. (2002). Cloning and characterization of a glutathione S-transferase induced by a herbicide safener in barley (*Hordeum vulgare*). *Physiologia Plantarum, 116*, 336-344.

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

Schiavon, M., Galla, G., Wirtz, M., Pilon-Smits, E. A. H., Telatin, V., Quaggiotti, S., et al. (2012). Transcriptome profiling of genes differentially modulated by sulfur and chromium identifies potential targets for phytoremediation and reveals a complex S-Cr interplay on sulfate transport regulation in *B. juncea*. *Journal of Hazardous Materials, 239*, 192-205.

Schiavon, M., Pilon-Smits, E. A. H., Wirtz, M., Hell, R., & Malagoli, M. (2008). Interactions between chromium and sulfur metabolism in *Brassica juncea*. *Journal of Environmental Quality, 37*, 1536-1545.

Schiavon, M., Wirtz, M., Borsa, P., Quaggicitti, S., Hell, R., & Malagoli, M. (2007). Chromate differentially affects the expression of a high-affinity sulfate transporter and isoforms of components of the sulfate assimilatory pathway in *Zea mays* (L.). *Plant Biology, 9*, 662-671.

Schiller, M., Hegelund, J. N., Pedas, P., Kichey, T., Laursen, K. H., Husted, S., et al. (2014). Barley metallothioneins differ in ontogenetic pattern and response to metals. *Plant Cell and Environment, 37*, 353-367.

Schnaubelt, D., Queval, G., Dong, Y. P., Diaz-Vivancos, P., Makgopa, M. E., Howell, G., et al. (2015). Low glutathione regulates gene expression and the redox potentials of the nucleus and cytosol in *Arabidopsis thaliana*. *Plant Cell and Environment, 38*, 266-279.

Schröder, P. (2006). Enzymes transferring biomolecules to organic foreign compounds: A role for glucosyltransferase and glutathione S-transferase in phytoremediation. In M. Mackova, D. Dowling, & T. Macek (Eds.), *Phytoremediation and rhizoremediation* (pp. 133-142). Dordrecht: Springer Netherlands.

Schroder, P., & Collins, C. (2002). Conjugating enzymes involved in xenobiotic metabolism of organic xenobiotics in plants. *International Journal of Phytoremediation, 4*, 247-265.

Schroder, P., & Pflugmacher, S. (1996). Induction of glutathione S-transferase activity in Norway spruce by xenobiotics and herbicide safeners. *Journal of Applied Botany-Angewandte Botanik, 70*, 97-100.

Sekhar, K., Priyanka, B., Reddy, V. D., & Rao, K. V. (2011). Metallothionein 1 (CcMT1) of pigeonpea (*Cajanus cajan*, L.) confers enhanced tolerance to copper and cadmium in *Escherichia coli* and *Arabidopsis thaliana*. *Environmental and Experimental Botany, 72*, 131-139.

Seth, C. S., Remans, T., Keunen, E., Jozefczak, M., Gielen, H., Opdenakker, K., et al. (2012). Phytoextraction of toxic metals: a central role for glutathione. *Plant Cell Environ, 35*, 334-346.

Shu, S., Yuan, L. Y., Guo, S. R., Sun, J., & Yuan, Y. H. (2013). Effects of exogenous spermine on chlorophyll fluorescence, antioxidant system and ultrastructure of chloroplasts in *Cucumis sativus* L. under salt stress. *Plant Physiology and Biochemistry, 63*, 209-216.

Singh, B. R., & Shaw, R. W. (1988). Selective-inhibition of oat glutathione-S-transferase activity by tetrapyrroles. *FEBS Letters, 234*, 379-382.

Sinha, R., Kumar, D., Datta, R., Hazra, S., Bhattacharyya, D., Mazumdar, A. B., et al. (2015). Integrated transcriptomic and proteomic analysis of *Arabidopsis thaliana* exposed to glutathione unravels its role in plant defense. *Plant Cell Tissue and Organ Culture, 120*, 975-988.

Smith, I. K., Kendall, A. C., Keys, A. J., Turner, J. C., & Lea, P. J. (1985). The regulation of the biosynthesis of glutathione in leaves of barley (*Hordeum vulgare* L.). *Plant Science, 41*, 11-17.

Sommer, A., & Boger, P. (1999). Characterization of recombinant corn glutathione S-transferase isoforms I, II, III, and IV. *Pesticide Biochemistry and Physiology, 63*, 127-138.

Song, Y. F., Cui, J., Zhang, H. X., Wang, G. P., Zhao, F. J., & Shen, Z. G. (2013). Proteomic analysis of copper stress responses in the roots of two rice (*Oryza sativa* L.) varieties differing in Cu tolerance. *Plant and Soil, 366*, 647-658.

Stuiver, C. E. E., Posthumus, F. S., Parmar, S., Shahbaz, M., Hawkesford, M. J., & De Kok, L. J. (2014). Zinc exposure has differential effects on uptake and metabolism of sulfur and nitrogen in Chinese cabbage. *Journal of Plant Nutrition and Soil Science, 177*, 748-757.

Sun, X., & Guo, L. Z. (2013). Relationship between cadmium-induced root subapical hair development and ethylene biosynthesis in oilseed rape seedlings. *Acta Biologica Cracoviensia Series Botanica, 55*, 68-75.

Takahashi, H., Kopriva, S., Giordano, M., Saito, K., & Hell, R. (2011). Sulfur assimilation in photosynthetic organisms: Molecular functions and regulations of transporters and assimilatory enzymes. *Annual Review of Plant Biology, 62*, 157-184.

Takahashi, H., Watanabe-Takahashi, A., Smith, F. W., Blake-Kalff, M., Hawkesford, M. J., & Saito, K. (2000). The roles of three functional sulphate transporters involved in uptake and translocation of sulphate in *Arabidopsis thaliana*. *Plant Journal, 23*, 171-182.

Takahashi, Y., & Nagata, T. (1992). parB: An auxin-regulated gene encoding glutathione S-transferase. *Proceedings of the National Academy of Sciences of the United States of America, 89*, 56-59.

Talukdar, D., & Talukdar, T. (2014). Coordinated response of sulfate transport, cysteine biosynthesis, and glutathione-mediated antioxidant defense in lentil (*Lens culinaris* Medik.) genotypes exposed to arsenic. *Protoplasma, 251*, 839-855.

Tennstedt, P., Peisker, D., Bottcher, C., Trampczynska, A., & Clemens, S. (2009). Phytochelatin synthesis is essential for the detoxification of excess zinc and contributes significantly to the accumulation of zinc. *Plant Physiology, 149*, 938-948.

Thao, N. P., Khan, M. I. R., Thu, N. B. A., Hoang, X. L. T., Asgher, M., Khan, N. A., et al. (2015). Role of ethylene and its cross talk with other signaling molecules in plant responses to heavy metal stress. *Plant Physiology, 169*, 73-84.

Theodoulou, F. L. (2000). Plant ABC transporters. *Biochimica Et Biophysica Acta-Biomembranes, 1465*, 79-103.

Tsuchisaka, A., & Theologis, A. (2004). Unique and overlapping expression patterns among the *Arabidopsis* 1-amino-cyclopropane-1-carboxylate synthase gene family members. *Plant Physiology, 136*, 2982-3000.

Tsuchisaka, A., Yu, G. X., Jin, H. L., Alonso, J. M., Ecker, J. R., Zhang, X. M., et al. (2009). A combinatorial interplay among the 1-aminocyclopropane-1-carboxylate isoforms regulates ethylene biosynthesis in *Arabidopsis thaliana*. *Genetics, 183*, 979-1003.

Usha, B., Keeran, N. S., Harikrishnan, M., Kavitha, K., & Parida, A. (2011). Characterization of a type 3 metallothionein isolated from *Porteresia coarctata*. *Biologia Plantarum, 55*, 119-124.

Van de Poel, B., & Van Der Straeten, D. (2014). 1-aminocyclopropane-1-carboxylic acid (ACC) in plants: more than just the precursor of ethylene! *Frontiers in Plant Science, 5*, 11.

Vangronsveld, J., Herzig, R., Weyens, N., Boulet, J., Adriaensen, K., Ruttens, A., et al. (2009). Phytoremediation of contaminated soils and groundwater: lessons from the field. *Environmental Science and Pollution Research, 16*, 765-794.

Venkatachalam, P., Srivastava, A. K., Raghothama, K. G., & Sahi, S. V. (2009). Genes induced in response to mercury-ion-exposure in heavy metal hyperaccumulator *Sesbania drummondii*. *Environmental Science & Technology, 43*, 843-850.

Vercampt, H., Koleva, L., Vassilev, A., Vangronsveld, J., & Cuypers, A. (2016). Short-term phytotoxicity in *Brassica napus* (L.) in response to pre-emergently applied metazachlor: A microcosm study. *Environ Toxicol Chem*.

Verkleij, J. A. C., Golan-Goldhirsh, A., Antosiewisz, D. M., Schwitzguébel, J.-P., & Schröder, P. (2009). Dualities in plant tolerance to pollutants and their uptake and translocation to the upper plant parts. *Environmental and Experimental Botany, 67*, 10-22.

Vernoux, T., Wilson, R. C., Seeley, K. A., Reichheld, J. P., Muroy, S., Brown, S., et al. (2000). The *ROOT MERISTEMLESS1/CADMIUM SENSITIVE2* gene defines a glutathione-dependent pathway involved in initiation and maintenance of cell division during postembryonic root development. *Plant Cell, 12*, 97-110.

Vestergaard, M., Matsumoto, S., Nishikori, S., Shiraki, K., Hirata, K., & Takagi, M. (2008). Chelation of cadmium ions by phytochelatin synthase: Role of the cystein-rich C-terminal. *Analytical Sciences, 24*, 277-281.

Vrbova, M., Kotrba, P., Horacek, J., Smykal, P., Svabova, L., Vetrovcova, M., et al. (2013). Enhanced accumulation of cadmium in *Linum usitatissimum* L. plants due to overproduction of metallothionein alpha-domain as a fusion to beta-glucuronidase protein. *Plant Cell Tissue and Organ Culture, 112*, 321-330.

Wachter, A., Wolf, S., Steininger, H., Bogs, J., & Rausch, T. (2005). Differential targeting of GSH1 and GSH2 is achieved by multiple transcription initiation: implications for the compartmentation of glutathione biosynthesis in the Brassicaceae. *Plant Journal, 41*, 15-30.

Walters, D. (2003). Resistance to plant pathogens: possible roles for free polyamines and polyamine catabolism. *New Phytologist, 159*, 109-115.

Wawrzynska, A., Moniuszko, G., & Sirko, A. (2015). Links between ethylene and sulfur nutrition - A regulatory interplay or just metabolite association? *Frontiers in Plant Science, 6*, 1-7.

Weber, M., Trampczynska, A., & Clemens, S. (2006). Comparative transcriptome analysis of toxic metal responses in *Arabidopsis thaliana* and the Cd^{2+}-hypertolerant facultative metallophyte *Arabidopsis halleri*. *Plant Cell and Environment, 29*, 950-963.

Wirtz, M., Birke, H., Heeg, C., Muller, C., Hosp, F., Throm, C., et al. (2010). Structure and function of the hetero-oligomeric cysteine synthase complex in plants. *Journal of Biological Chemistry, 285*, 32810-32817.

Wirtz, M., & Droux, M. (2005). Synthesis of the sulfur amino acids: cysteine and methionine. *Photosynthesis Research, 86*, 345-362.

Wirtz, M., & Hell, R. (2006). Functional analysis of the cysteine synthase protein complex from plants: structural, biochemical and regulatory properties. *Journal of Plant Physiology, 163*, 273-286.

Xue, T. T., Li, X. Z., Zhu, W., Wu, C. G., Yang, G. G., & Zheng, C. C. (2009). Cotton metallothionein GhMT3a, a reactive oxygen species scavenger, increased tolerance against abiotic stress in transgenic tobacco and yeast. *Journal of Experimental Botany, 60*, 339-349.

Yoshimoto, N., Inoue, E., Saito, K., Yamaya, T., & Takahashi, H. (2003). Phloem-localizing sulfate transporter, Sultr1;3, mediates re-distribution of sulfur from source to sink organs in *Arabidopsis*. *Plant Physiology, 131*, 1511-1517.

Zagorchev, L., Seal, C. E., Kranner, I., & Odjakova, M. (2013). A central role for thiols in plant tolerance to abiotic stress. *International Journal of Molecular Sciences, 14*, 7405-7432.

Zechmann, B. (2014). Compartment specific importance of glutathione during abiotic and biotic stress. *Frontiers in Plant Science, 5*, 1-11.

Zettl, R., Schell, J., & Palme, K. (1994). Photoaffinity-labeling of *Arabidopsis thaliana* plasma membrane vesicles by 5-azido-[7-^{3}H]indole-3-acetic acid: Identification of a glutathione S-transferase. *Proceedings of the National Academy of Sciences of the United States of America, 91*, 689-693.

Zhang, J., Zhang, M., Tian, S. K., Lu, L. L., Shohag, M. J. I., & Yang, X. E. (2014a). Metallothionein 2 (SaMT2) from *Sedum alfredii* Hance confers increased Cd tolerance and accumulation in yeast and tobacco. *Plos One, 9*, 1-10.

Zhang, L. W., Song, J. B., Shu, X. X., Zhang, Y., & Yang, Z. M. (2013). miR395 is involved in detoxification of cadmium in *Brassica napus*. *Journal of Hazardous Materials, 250*, 204-211.

Zhang, Q., Xu, F., Lambert, K. N., & Riechers, D. E. (2007). Safeners coordinately induce the expression of multiple proteins and MRP transcripts involved in herbicide metabolism and detoxification in *Triticum tauschii* seedling tissues. *Proteomics, 7*, 1261-1278.

Zhang, Y. Y., He, Q. Q., Zhao, S. Y., Huang, L. L., & Hao, L. (2014b). *Arabidopsis ein2-1* and *npr1-1* response to Al stress. *Bulletin of Environmental Contamination and Toxicology, 93*, 78-83.

Zhang, Z. H., Ren, J. S., Clifton, I. J., & Schofield, C. J. (2004). Crystal structure and mechanistic implications of 1-aminocyclopropane-1-carboxylic acid oxidase - The ethylene-forming enzyme. *Chemistry & Biology, 11*, 1383-1394.

Zhao, H. J., Wu, L. Q., Chai, T. Y., Zhang, Y. X., Tan, J. J., & Ma, S. W. (2012). The effects of copper, manganese and zinc on plant growth and elemental accumulation in the manganese-hyperaccumulator *Phytolacca americana*. *Journal of Plant Physiology, 169*, 1243-1252.

Zhu, W., Zhao, D. X., Miao, Q., Xue, T. T., Li, X. Z., & Zheng, C. C. (2009). *Arabidopsis thaliana* metallothionein, AtMT2a, mediates ROS balance during oxidative stress. *Journal of Plant Biology, 52*, 585-592.

8 Tables

Table 1: Natural functions of GSTs in plants.

Postulated natural role for GSTs	Reference
Conjugation of endogenous metabolites	Diesperger & Sandermann, 1979 Edwards & Dixon, 1991 Dean et al., 1995
Conjugation of DNA degradation products	Morgenstern et al., 1985
Conjugation of phytohormones (auxin, ethylene, gibberellic acid)	Lamoureux & Frear, 1987 Meyer et al., 1991 Takahashi & Nagata, 1992 Zettl et al., 1994
Detoxification of lipid hydroperoxides	Mannervik & Danielson, 1988 Marrs, 1996 Sommer & Böger, 1999
Transport of (thio-)phenols, chlorophyllin and anthocyans	Singh & Shaw, 1988 Martinoia et al., 1993
Regulation of GSH pool	Lamoureux & Rusness, 1989
Detoxification of fungal toxins and pathogen defence	Dudler et al., 1991 Mauch & Dudler, 1993
Increase of drought tolerance	Dhindsa, 1991

Antioxidative protective protein	Levine et al., 1994
Conjugation and transport of phytoalexins and similar compounds	Li et al., 1997 Marrs et al., 1995
Regulation of UV-induced genes	Loyall et al., 2000
Transporter proteins for secondary metabolites and their unstable intermediates	Dixon et al., 2010
Synthesis of glucosinolates; conjugation, transport and storage of reactive oxylipins, phenolics and flavonoids	Dixon et al., 2010 Dixon & Edwards, 2010
Isomerisation and peroxidation	Cummins et al., 2011
Deglutathionation of GS-conjugates	Lallement et al., 2014

1801

1802

1803

9 Figure legends

Fig. 1: Biosynthesis pathway of sulphur-derived cellular compounds and their involvement in metal scavenging, xenobiotic detoxification, antioxidative defence and signal transduction. Abbreviations: acetyl-CoA, acetyl-coenzyme A; ACC, 1-aminocyclopropane-1-carboxylic acid; ACO, ACC oxidase; ACS, ACC synthase; APK, APS kinase; APR, APS reductase; APS, 5'-phosphosulphate; APX, ascorbate peroxidase; AsA, ascorbate; ATPS, ATP sulphurylase; CBL, cystathionine β-lyase; CGS, cystathionine γ-synthase; Cys, cysteine; dcSAM, decarboxylated SAM; DHA, dehydroascorbate; DHAR, DHA reductase; γ-EC, γ-glutamylcysteine; γ-ECS, γ-EC synthetase; γ-Glu, γ-glutamate; Gly, glycine; GR, glutathione reductase; GS-conjugate, glutathione S-conjugate; GSH, glutathione; GSH-S, glutathione synthetase; GSSG, glutathione disulphide; GST, glutathione transferase; H_2O, water; H_2O_2, hydrogen peroxide; HCys, homocysteine; L-Ser, L-serine; Met, methionine; MS, methionine synthase; MT, metallothionein; OAS, O-acetylserine; OASTL, OAS (thiol) lyase; OPH, O-homophosphoserine; PAPS, 3'-phosphoadenosine 5'-phosphosulphate; PC, phytochelatin; PCS, phytochelatin synthase; Put, putrescine; S^{2-}, sulphide; SAM, S-adenosylmethionine; SAMDC, SAM decarboxylase; SAMS, SAM synthetase; SAT, serine acetyl transferase; SiR, sulphite reductase; SO_3^{2-}, sulphite; SO_4^{2-}, sulphate; Spd, spermidine; Spd synthase, spermidine synthase; Spm, spermine; Spm synthase, spermine synthase; SULTR, sulphate transporter.

Fig. 2: Cross-talk between detoxification and signal transduction pathways in plants. Significant cross-talk exists between glutathione-mediated detoxification mechanisms and ethylene- and polyamine-related signal transduction pathways in plants, both under physiological

and metal exposure conditions. Reactive oxygen species are put forward as central players in this interaction. Furthermore, several components involved in detoxification and signal transduction pathways are known to affect sulphur uptake and assimilation. Abbreviations: Cys, cysteine; GSH, glutathione; GSSG, glutathione disulphide; GST, glutathione transferase; Met, methionine; PC, phytochelatin; ROS, reactive oxygen species; SAM, S-adenosylmethionine.