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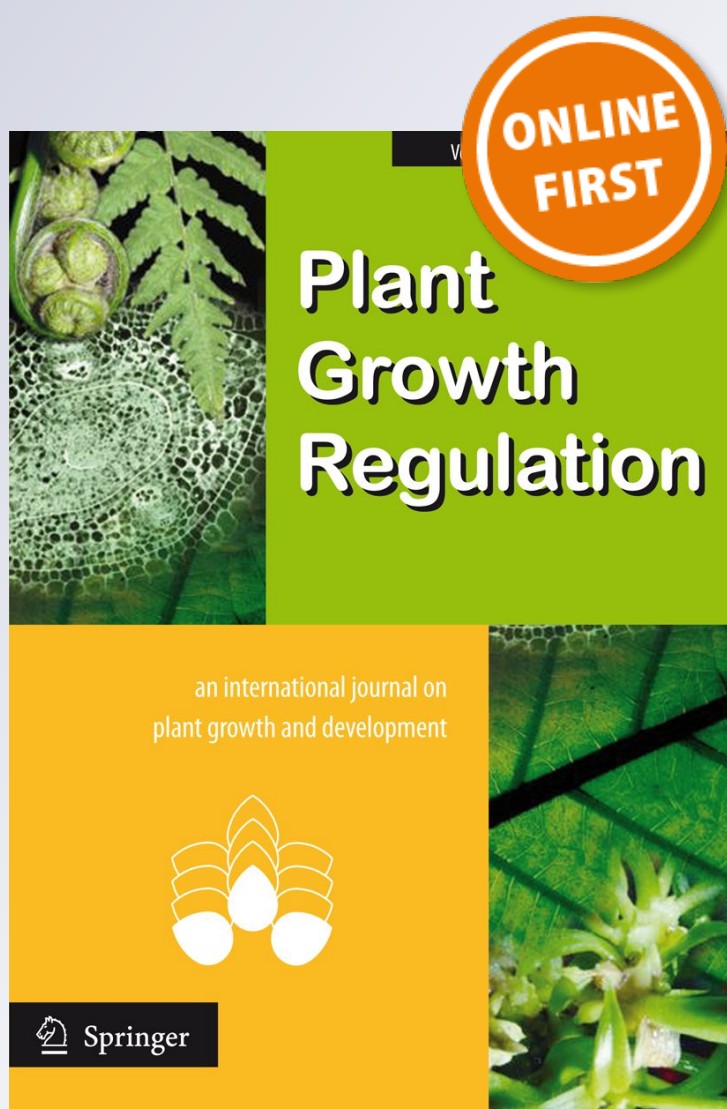
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Interactions between plant hormones and thiol-related heavy metal chelators

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Abstract

Upon toxic metal stress numerous defence mechanisms have been induced, including the synthesis of metal-binding ligands and plant hormones or plant growth regulators in plants. As several elements in the promoter region of the heavy metal-responsive genes can be activated by plant hormones and growth regulators, understanding and revealing possible and special relationships between these regulator compounds and the metal chelator phytochelatin, which are in the first line of heavy metal defence mechanism is of great important. Phytochelatin are synthesized from glutathione and have a structure of $[(\gamma\text{-Glu-Cys})_n]\text{-Gly}$, where n is the number of repetition of the $(\gamma\text{-Glu-Cys})$ units. Evidences for the role of PCs in heavy metal tolerance are very strong; however, little information is available on how plant growth regulators influence the phytochelatin synthesis at molecular or even gene expression level. In the present review we provide an overview of the role and synthesis of phytochelatin in metal-tolerance mechanism from a new point of view, i.e. their relation to the plant growth regulator molecules, with special regard also on those cases, when close direct relationship exists because of the partly overlapped synthesis pathways of plant growth regulators and glutathione/phytochelatin.

Keywords Heavy metal · Phytochelatin · Plant growth regulators · Plant hormones · Thiols

Abbreviations

ABA	Absciscic acid
ACC	1-Aminocyclopropane-1-carboxylic acid
ACO	ACC-oxidase
ACS	ACC-synthase
BRs	Brassinosteroids
CKs	Cytokinins
dcSAM	Decarboxylated <i>S</i> -adenosyl-methionine
γ -GCS	γ -Glutamyl-cysteine synthase
GA	Gibberelins
GSH	Glutathione
GSS	Glutathione synthase
JA	Jasmonic acid
MeJA	Methyl jasmonic acid
NaSA	Sodium salicylate
PA	Polyamine
PC	Phytochelatin
PCS	Phytochelatin synthase

PUT	Putrescine
SA	Salicylic acid
SAM	<i>S</i> -adenosyl-methionine
SPD	Spermidine
SPDS	Spermidine synthase
SPM	Spermine
SPMS	Spermine synthase

Introduction

Heavy metals induce numerous physiological changes in plants, such as growth inhibition, imbalance of water and ion homeostasis, inhibition of photosynthesis, changes in enzyme activities, and formation of free radicals, that are all well-studied (Di Toppi and Gabbrielli 1999). Mechanism leading to the heavy metal tolerance can be divided into two groups: avoidance and tolerance strategies. The first one limits the uptake of the heavy metal, thus excluding them from plant tissue, includes modification of rhizosphere pH, exudation of organic acids, development of mucous barrier on root tips or immobilization in the cell walls (Hall 2002). While plants with tolerance strategies are capable of accumulating, storing and immobilizing heavy metals by binding them to amino acids,

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proteins or peptides (Pál et al. 2006). The nitrate and sulphate assimilation pathways activated during heavy metal stress play an important role in the increase of phytochelatin (PC) production (Astolfi et al. 2004; Wu et al. 2015); furthermore, priority of phytochelatin synthesis during cadmium exposure even under reduced sulfate uptake has been also reported (Yamaguchi et al. 2017).

A growing body of evidence suggests that growth regulators or plant hormones, such as abscisic acid (ABA), ethylene, jasmonic acid (JA), polyamines (PA) and SA, are involved in the heavy metal stress signalling of plants (Groppa et al. 2007; Pál et al. 2013; Chmielowska-Bąk et al. 2014; Yan et al. 2015; Keunen et al. 2016; Singh et al. 2016). Acting as signalling molecules in a highly complex relationship, these molecules allow plants to retain growth plasticity during development and are probably the main means by which plants respond to abiotic and biotic stresses (Bücker-Neto et al. 2017). Both their increased endogenous levels under heavy metal stress and the relationship between their modified endogenous content (resulted from genetic modification or exogenous treatment) and the level of heavy metal tolerance have been reported in various plant species (Xiang and Oliver 1998; Groppa et al. 2001; Maksymiec and Krupa 2002; Hsu and Kao 2003; Maksymiec et al. 2005; Wen et al. 2010a; Tao et al. 2013; Chmielowska-Bąk et al. 2013; Kovács et al. 2014; Gondor et al. 2016; Pál et al. 2017). Majority of the experimental data focuses on the changes in growth and photosynthesis parameters, the induction of antioxidant system or the relationship between the observed protective effect and the hormonal changes.

Details of structures, biosynthesis, analytical methods, related genes and other aspects of PCs are also available in previous reviews (Cobbett 2000; Inouhe 2005; Yadav 2010; Merlos Rodrigo et al. 2014). However, little information is available on how plant growth regulators influence the PC synthesis at molecular or even gene expression level. The synthesis and subsequent vacuolar compartmentalization of PCs, which are capable to form complexes with metal ions, are induced shortly after the exposure to heavy metal, while other defence mechanisms, involving the action of stress proteins, antioxidant enzymes or salicylic acid (SA) also play an important role (Pál et al. 2006). Finding novel mechanism participating in the regulation of PC synthesis during toxic metal stress conditions will be of great importance. In the present review, we provide an overview of the role and synthesis of PCs for the better understanding of the metal-tolerance mechanism from a new point of view, i.e. their relation to the plant growth regulator molecules.

Synthesis and role of PCs

When Cd enters the cytosol, numerous metal-binding ligands, e.g. the system linked to the sulphur metabolism, become activated in plants, resulting in the development of complex-forming agents. PCs have a structure of $[(\gamma\text{-Glu-Cys})_n\text{-Gly}]$, where n is the number of repetition of the $(\gamma\text{-Glu-Cys})$ units, which is generally in the range of 2–11. The structural model of the Cd–PC complex is $[\text{Cd}_3(\text{PC}_3)_4]$, which contains discrete $\text{Cd}(\text{SCys})_4$ units (Cobbett 2000). Different PC families are well-characterised (Cobbett 2000). PCs form low-molecular-weight (LMW) complexes with metal ions. These complexes then form high-molecular-weight (HMW) complexes with acid-labile sulphur, which are more stable and have greater affinity for heavy metal. HMW complex contains mostly longer peptides [higher number of repetition of the $(\gamma\text{-Glu-Cys})$ units], while LMW complex contains shorter peptides (Cobbett 2000).

The enzyme catalysing the synthesis of PCs is γ -glutamylcysteine dipeptidyl transpeptidase (phytochelatin synthase: PCS) (Chen et al. 1997). PCS gene was expressed constitutively in *Arabidopsis*, but in wheat increased level of PCS transcriptome was found during Cd exposure suggesting that, in some species, PCS activity may be regulated at both the transcriptional and post-translational levels (Cobbett 2000). Furthermore, the biosynthesis of PCs is self-regulated, as the product of the reaction (PC) upon binding the activator metal ions, inhibit the enzyme. As PC accumulation is usually in parallel with the decline of GSH level, cells exposed to heavy metal need to replace the utilized GSH. According to these, the γ -glutamylcysteine synthetase and glutathione synthetase activities co-limit GSH production under heavy metal stress (Zhu et al. 1999) and metal tolerance is related to the ability of plants to produce PCs and to prevent associated GSH depletion (Jozefczak et al. 2012).

The literature on the relationship between heavy metal tolerance and PC synthesis is interspersed with many contradictions. On one hand, evidences for the role of PCs in heavy metal tolerance are very strong (Zagorchev et al. 2013). The *cad1* mutant of *Arabidopsis* plants were deficient in their ability to accumulate PCs and the amount of accumulated PCs correlated with the level of Cd tolerance (Howden et al. 1995). However, higher gene expression level of PCS in *Arabidopsis*, due to its transgenic modification, resulted in increased PC production, but did not lead to increased Cd tolerance (Lee et al. 2003). When comparing the Cd tolerance of maize, rice and wheat plants, maize proved to be the most sensitive to stress (Wójcik and Tukendorf 1999) despite having accumulated far less Cd than the other species and synthesised sufficient amount of PCs in the most intensive mode for the efficient detoxification of Cd.

In general, it can be declared that the results on PCs differ depending on the metal ion, its concentration, the duration of the treatment and the plant species. PCs may form the first line of defence with a decisive role being played by the quantity of PCs, the rate of PC formation, the number of (γ -Glu-Cys) units and the rate of HMW complex formation. The second line is ensured by other systems, such as exclusion and immobilisation, antioxidant system, stress proteins and hormones (Pál et al. 2006). However, PCs have dual role: they can bind the heavy metals to inactivate and detoxify in the vacuole, and they can transfer the essential metals to newly synthesised apoenzymes (Zenk 1996). In addition, PCs are found to be part of the defence mechanism not only against metal-related stresses but also in response to other stressors such as drought, cold, heat, salt, UV-B and herbicide (Emamverdian et al. 2015).

Plant growth regulators

Investigations on naturally occurring compounds capable of reducing the stress sensitivity of plants are of great importance in the twenty-first century, where certain human activities have been identified as primary causes of ongoing climate change and contamination of the environment with heavy metals is a serious problem. Plant hormones and PAs are ubiquitously found in the plant kingdom. Like other abiotic stresses, heavy metal stress also induces the alteration in the metabolism of plant hormones or plant growth regulators. These changes are involved in the perception and responses to heavy metal stress (Chmielowska-Bąk et al. 2013). Table 1 shows exemplary studies on the relationship between plant growth regulators and thiol-related peptide synthesis. In this chapter, connection of PC synthesis to these plant growth regulators is discussed.

ABA

ABA is a multifaceted plant hormone. Besides controlling many developmental and growth processes in plants, ABA is commonly known as a stress hormone (Vishwakarma et al. 2017). Several stress factors including toxic metals induce ABA synthesis. Arsenic stress resulted in increased expression of ABA biosynthesis genes, as well as in the up-regulation of ABA signalling genes in rice (Huang et al. 2012). Exogenous ABA decreased Cd content and enhanced Cd tolerance in rice, while application of ABA biosynthesis inhibitor reduced ABA accumulation, increased the Cd content and decreased Cd tolerance (Hsu and Kao 2003). Despite the lack of exact knowledge regarding the way in which the ABA signalling pathway is involved in the response to heavy metal exposure, strong correlation has been assumed between ABA content and the level of heavy

metal tolerance (Bücker-Neto et al. 2017). Cd treatments led to increased ABA levels in roots of *Phragmites* and *Typha* plants and this increase indicated the involvement of ABA in early Cd stress responses. In the same experiment it was also found that ABA has a role in an activation of *O*-acetylserine (thiol) lyase, the enzyme responsible for cysteine biosynthesis (Fediuc et al. 2005). Cd treatment also increased the ABA content of potato tuber discs and, in parallel with this elevated StPCS1 transcript level, PCS activity and total PC content was detected. Similar changes in PC synthesis were found after ABA treatment (Stroiński et al. 2010). Early peak in ABA content occurs in response to heavy metal suggesting that ABA is involved in Cd detoxification by increasing acting as a stress signal triggering downstream reactions, such as synthesis of PCs in *Deschampsia cespitosa* L. (Hayward et al. 2013). Using inhibitor of ABA synthesis also revealed that ABA is required in Cd signal transduction and therefore, in the regulation of PC synthesis as well (Stroiński et al. 2013). ABA differentially affected the GSH content, GSH:GSSG ratio, GR activity and γ -ECS transcript level in two maize genotypes differing in their stress tolerance (Kellős et al. 2008), vice versa GSH treatment also enhance ABA synthesis and accumulation (Cheng et al. 2015) suggesting crosstalk between ABA and GSH. In spite of considerable progress in the understanding of ABA signalling pathways, its relationship with the detoxification system is still poorly known.

Auxins

Auxin, as a plant growth hormone, is able to stimulate plant growth and development in response to gravity or light stimuli (Zhao 2010). Its homeostasis (synthesis, distribution, metabolism and transport) within the plant is regulated by various environmental factors and may be disturbed by heavy metals (Hu et al. 2013). However, application of tryptophan, a precursor of auxin to the roots of rice seedlings, enhanced plant growth and yield under Cd stress (Farooq et al. 2015). It has been also revealed that under optimum growth conditions, root growth dependence on glutathione is linked to auxin transport in *Arabidopsis* (Koprivova et al. 2010). In addition, the level of endogenous GSH has been reported to affect the expression of auxin transport gene (Eckardt 2010), and increased auxin level has a crucial role in the increment of the glutathione-S-transferase activity in the roots of Cd-treated barley (Bočová et al. 2013). Nevertheless, the mode of action, i.e. how increase in endogenous auxin level prevents growth inhibition in plant exposed to heavy metal stress, is still poorly understood, and only a few studies are available on the relationship between auxin and PCs. Investigation on correlation between hormonal homeostasis and physiological responses in *Arabidopsis thaliana* exposed to Cd, Cu, Zn and their combined treatments suggested that no

Table 1 Selected studies on the effects of plant growth regulators on thiol and/or phytochelatin levels in plants

Plant growth regulator	Heavy metal	Changes related to thiol metabolism	Plant species	References
ABA+	Cd	Increased activity of <i>O</i> -acetylserine (thiol) lyase	<i>Phragmites</i> and <i>Typha</i>	Fediuc et al. (2005)
	Cd	Increased gene expression and activity of PCS increased PC content	Potato	Stroiński et al. (2010, 2013)
Auxin–	Zn	Decreased cysteine, glutathione and PCs content	Rice	Begum et al. (2016)
CKs+	Cd	Increased non-protein tiol content	<i>Solanum melongena</i> L.	Singh and Prasad (2014)
	Ni	No changes in γ -glutamylcysteine, GSH and PC contents	<i>Alyssum murale</i>	Cassina et al. (2011)
CKs–	As	Increased gene expression level of γ -glutamylcysteine synthetase, GSH synthetase and PCS increased GSH and PC contents	<i>Arabidopsis</i> , tobacco	Mohan et al. (2016)
Ethylene+	Cd	Increased GSH content	<i>B. juncea</i>	Masood et al. (2012)
	Cd	Increased gene expression level of GSH synthesis enzymes	tobacco	Guan et al. (2015)
Ethylene–	Ni, Zn	Increased GSH content	<i>B. juncea</i>	Khan and Khan (2014)
	Pb	Decreased GSH content	<i>Arabidopsis</i>	Cao et al. (2009)
	Cd	Decreased activity of PCS activity decreased the total SH groups	Carrot	Di Toppi et al. (1998)
	Cd	Decreased gene expression level of GSH synthesis enzymes	<i>Arabidopsis</i>	Schellingen et al. (2015)
GA+	Cd, Pb, Cu	Increased GSH content	<i>Chlorella vulgaris</i>	Piotrowska-Niczyporuk et al. (2012)
BRs+	Cr	Increased GSH content	Radish	Choudhary et al. (2011)
	Cd, Pb	Increased GSH content	Tomato	Rady and Osman (2012)
	Cu, Pb, Cd	Increased GSH content	<i>Chlorella vulgaris</i>	Bajguz (2010)
	Pb	Increased PC content	<i>Chlorella vulgaris</i>	Bajguz (2002)
	Cd, Cu	Increased gene expression level and activity for GSH synthesis	<i>Arabidopsis thaliana</i>	Xiang and Oliver (1998)
MeJA+	Pb	Increased GSH content	<i>Wolffia arrhizal</i>	Piotrowska et al. (2009)
	Cd	Increased GSH content	Rice	Singh and Shah (2014)
PUT+	Cd	Increased GSH and PC contents	Mung bean	Nahar et al. (2016)
	Cd	Decreased gene expression level and activity of PCS decreased GSH and PC contents	Rice	Pál et al. (2017)
SPD+	Cd, Pb, Zn	Decreased GSH content	European pear	Wen et al. (2010a, b)
	Cr	Increased GSH and PC contents	Radish	Choudhary et al. (2012)
SPM+	Cd	Decreased GSH content	Wheat	Groppa et al. (2007)
	Cd	Increased GSH content	Rice	Hsu and Kao (2007)
	Cd	Decreased GSH and PC contents	<i>Canavalia lineata</i>	Yun et al. (1997)
SA+	Ni	Increased activity of serine acetyltransferase increased GSH content	<i>Arabidopsis</i>	Freeman et al. (2005)
	Cd	Increased GSH content	Rice	Guo et al. (2009)
	Cd	Changes in PC pattern	Maize	Szalai et al. (2013)
	Cd	No changes in gene expression level of PCS no changes in GSH content	Barley	Metwally et al. (2003)
	Cd	Increased activity of PCS increased PC content	Maize	Gondor et al. (2016)
NaSA+	Cd	Increased PC content	Maize	Gondor et al. (2016)
Melatonin	Cd	Increased GSH and PC contents	Tomato	Hasan et al. (2015)

Plants overexpressing the synthesis enzymes related to the plant growth regulators or treated with plant growth regulators are indicated with +, while changes found in plants deficient in the synthesis or signalling of plant growth regulator or treated with inhibitors of the plant growth regulators are indicated with: – (*ABA* abscisic acid, *CKs* cytokinins, *GA* gibberelins, *BRs* brassinosteroids, *JA* jasmonic acid, *MeJA* methyl jasmonic acid, *PUT* putrescine, *SPD* spermidine, *SPM* spermine, *SA* salicylic acid, *NaSA* sodium salicylate)

direct relationship exists between the level of GSH/PCs and auxin (Sofo et al. 2013). However, significant decreases in the amount of cysteine, glutathione and total PCs was found after treatment with auxin inhibitor in roots of rice plants (Begum et al. 2016). Recently it was also demonstrated that Cd inhibits root meristem growth by NO-mediated repression of auxin accumulation and signalling in *Arabidopsis* (Yuan and Huang 2016), in addition, that the knockout mutation of PCS1 in *Arabidopsis* may decrease the auxin content and distribution (De Benedictis et al. 2018). According to these PCs/PCS may have putative role in the root meristem maintenance and growth recovery under heavy metal stress, due their direct or indirect relationship with auxin.

Cytokinins (CKs)

Since their discovery, the roles of CKs were documented in almost all aspects of plant growth and development. In addition, CK-dependent modulation of stress responses has also been studied at various levels (Raines et al. 2016). The decrease in CK content was found in wheat plants after Cd treatment, which was in parallel with elevated CK oxidase activity (Veselov et al. 2003). Cd-induced CK oxidation/degradation has also been reported in durum wheat (Kaminek et al. 1997). According to these, application of CK antagonist or an inhibitor of CK degradation can improve plant growth of Cd-treated *Bulbine natalensis* and *Rumex crispus* (Gemrotová et al. 2013). On the other hand, it has been noted that exogenous CKs were able to reverse heavy metal induced toxicity in various plant species (Al-Hakimi 2007; Piotrowska-Niczyporuk et al. 2012), which was related to enhanced antioxidant capacity (increased activity of antioxidant enzymes and the contents of non-enzymatic antioxidants, such as non-protein thiols) (Singh and Prasad 2014). In contrast, CK signalling mutants and transgenic *Arabidopsis* and tobacco plants with reduced endogenous CK levels showed higher accumulation of thiol compounds, such as GSH and PCs (PC2, PC3 and PC4) leading to higher As tolerance compared to the wild type. Furthermore, plants overexpressing cytokinin oxidase/dehydrogenase 1 showed increased expression of genes related to PC biosynthesis (γ -glutamylcysteine synthetase, GSH synthetase and PCS) compared to wild-type controls (Mohan et al. 2016). However CK treatments produced a significant increase in plant biomass and transpiration rate of the Ni hyper-accumulator, *Alyssum murale*, whereas no significant variation in Ni accumulation or the concentration of non-protein thiols (γ -glutamylcysteine, GSH and total PC) was observed (Cassina et al. 2011). Recently it has also been demonstrated that Cd-induced inhibition of root growth is related to an altered homeostasis of auxin/CK signalling, which in turn influence meristem size and stem cell niche activity (Bruno et al. 2017). These findings are suggesting the existence of

an antagonistic interaction between auxin and CK in their relationship with PCs.

Ethylene

This volatile molecule mediates many complex aspects of plant growth and development and also mediates adaptive responses to a various stresses (Chang 2016), such as heavy metal stress (Keunen et al. 2016). Several toxic metal treatments have been reported to induce ethylene synthesis and signalling in various plant species (Keunen et al. 2016). These data suggested that the heavy metal-induced ethylene production is plant-specific and also depends on the type and concentration of the heavy metal. Generally, the induction of ethylene by metals may cause unbeneficial symptoms in plants and have role in Cd-induced cell death. Application of the ethylene inhibitor, silver thiosulphate, completely ameliorated the Cd-induced negative effects (Maksymiec 2011). In *acs2-1 acs6-1* (ACS: 1-aminocyclopropane-1-carboxylic acid synthase), double knockout *Arabidopsis* mutants showed higher leaf biomass in parallel with decreased ethylene production under short-term, low concentration Cd-stress (Schellingen et al. 2014). However, it has also been reported that both *acs2-1acs6-1* double knockout mutant and wild type of *Arabidopsis* were sensitive to prolonged, severe Cd-stress, and lower transcript levels of genes encoding GSH biosynthesis enzymes were found in *acs2-1 acs6-1* double knockout *Arabidopsis* mutant (Schellingen et al. 2015) indicating that ethylene was necessary to maintain the GSH level. It was also found that ethylene insensitive *ein2-1* mutants were more sensitive to Pb, which was related to the decreased GSH-content and it also suggests that crosstalk exists between ethylene and GSH (Cao et al. 2009) (Keunen et al. 2016). Since GSH synthesis is affected by S-availability and ethylene induces the activity of ATP sulphurylase leading to the accumulation of sulphur (Iqbal et al. 2013), ethylene seems to have a modulator role in making up the demand for GSH during metal stress in order to alleviate metal toxicity. According to these, a certain accumulation of ethylene, which was lower than the heavy metal-induced ethylene level, but still higher than the ethylene level of control plants, could lead to beneficial plant responses, such as increased sulphur metabolism and GSH synthesis (Masood et al. 2012; Thao et al. 2015). Transgenic tobacco plants overexpressing ethylene responsive factor 1 (ERF1) showed greater tolerance to Cd stress than the wild, which was related to an enhanced expression level of GSH biosynthesis genes (Guan et al. 2015). It has also been mentioned that this positive crosstalk exists despite the fact that Cys is a common precursor for both GSH and ethylene synthesis (Keunen et al. 2016) (Fig. 1). Nevertheless, there is only a few studies on the direct relationship between ethylene and PC synthesis under heavy metal stress. In cell

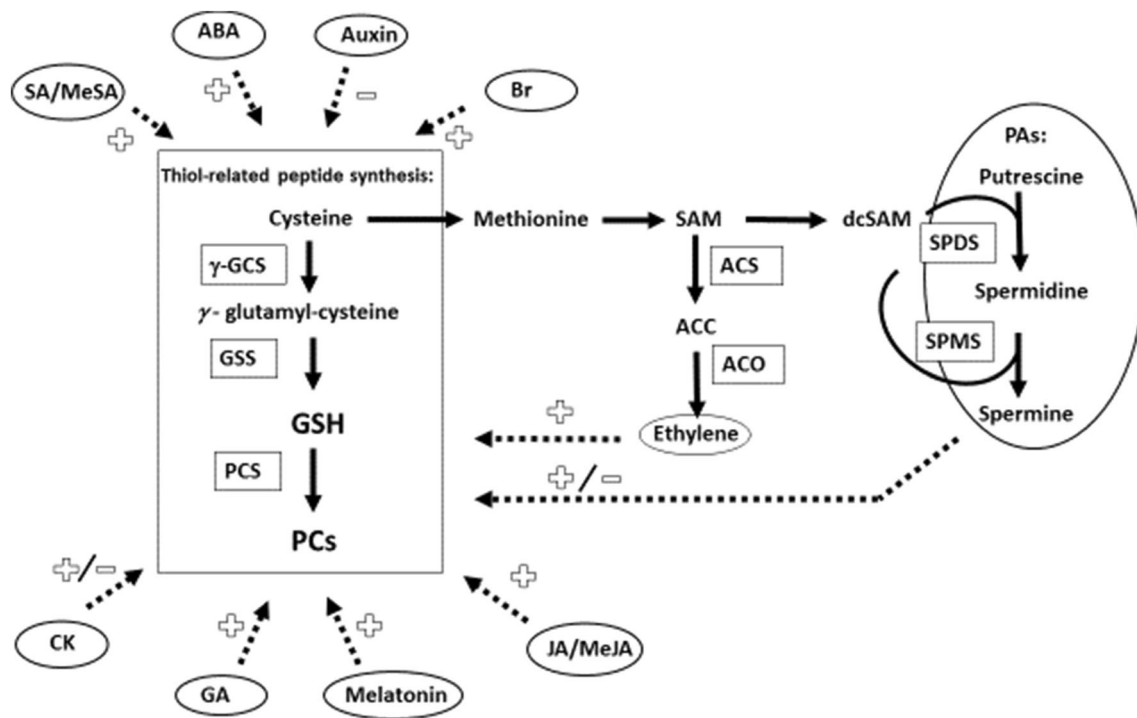


Fig. 1 Possible relationship between plant growth regulators and thiol-related peptide synthesis. Black arrows indicate the synthesis pathways of phytochelatin, ethylene and polyamines with the com-

mon precursor, cysteine. Dotted arrows show the positive (+) or negative (–) effects of the plant growth regulators on the phytochelatin synthesis pathway (for details see text)

suspension cultures of carrot, the absence of ethylene (after treatment with an inhibitor of ethylene-biosynthesis, or use of ethylene traps) caused both a decrease in the PCS activity and a strong decrease in the amount of total SH groups in plants. However, the 1-aminocyclopropane-1-carboxylic acid (precursor of ethylene) supply did not increase them (Di Toppi et al. 1998).

Gibberelins (GAs)

GAs are plant hormones that are essential for many developmental processes in plants but their central role in the response to abiotic stress is becoming increasingly evident (Colebrook et al. 2014). The role of GAs in the protection against Cd stress has been extensively reported (Asgher et al. 2015) and it has been demonstrated that the expression of adenosine 5'-phosphosulphate reductase, the key enzyme of sulphate assimilation, was increased by GA in *Arabidopsis* (Koprivova et al. 2008). Furthermore, increased GSH content has also been detected upon Cd, Pb and Cu exposure in combination with GA in *Chlorella vulgaris* (Piotrowska-Niczyporuk et al. 2012). Although, increased level of GA3 levels has been detected in the root of *Arabidopsis* plant after Cd, Cu and Zn treatments, this increased hormone level was not in direct correlation with the induction of GSH or PC synthesis both at molecular and gene expression level (Sofa

et al. 2013). According to these, possible interaction between GAs and PCs still needs extensive further research.

Brassinosteroids (BRs)

BRs are steroidal plant hormones that play role in the regulation of various plant growth and development processes. Moreover, BRs regulate the expression of hundreds of genes affecting numerous metabolic pathways (Fariduddin et al. 2014). Besides reducing the accumulation of heavy metals, BRs stimulate the growth and development of plants exposed to heavy metal stress (Rajewska et al. 2016). In most of the cases, the protective effect of BRs were related to enhanced antioxidant enzyme activities (Bajguz 2010; Choudhary et al. 2011) or increased GSH pool in various plant species under heavy metal stress (Bajguz 2010; Choudhary et al. 2011; Rady and Osman 2012). However, BRs have also been demonstrated to stimulate the syntheses of PC in *C. vulgaris* treated with Pb, and the different BRs had various stimulatory effects (Bajguz 2002). Increase in total PC content was also observed in radish treated with epibrassinolide alone or in combination with Cu (Choudhary et al. 2010). However, investigation on *Arabidopsis* for the better understanding of the relationship between Cd stress responses and BR signalling has suggested that the synthesis

of PCs is BR-independent in response to Cd stress (Villiers et al. 2012).

Jasmonates

Jasmonates (jasmonic acid: JA and methyl jasmonates: MeJA) are known to take part in various physiological processes. JA also has role as a signalling molecule in plants under different environmental stresses (Ahmad et al. 2016). The involvement of JA in the early response to Cd has been described in *Phaseolus coccineus* (Maksymiec 2011). Cu^{2+} and Cd^{2+} induced accumulation of JA in the leaves of *Arabidopsis thaliana* and *P. coccineus* plants, and the relation of JA to the mechanism of toxic action of both heavy metals have been also reported (Maksymiec et al. 2005). In addition, Cd-induced growth inhibition of *Phaseolus coccineus* leaves was alleviated by most inhibitors of the jasmonate pathway (Maksymiec 2011). However, MeJA alleviates cadmium toxicity in *Solanum nigrum* by regulating metal uptake and antioxidant capacity (Yan et al. 2015), while JA alleviates negative impacts of Cd by modifying osmolytes and antioxidants in faba bean (Ahmad et al. 2017). Treatment with JA at the higher concentration (100 μM) also resulted in the enhancement of heavy metal toxicity in *Wolffia arrhizal*, while at lower concentration (0.1 μM) it inhibited heavy metal accumulation, restored plant growth and increased the GSH content (Piotrowska et al. 2009). Seed priming with JA also help in ameliorating toxic effect of Cu in *Cajanus cajan* (Poonam et al. 2013) and that of Ni in *Glycine max* (Sirhindi et al. 2016).

MeJA treatment accelerated the Cd-induced GSH accumulation in rice (Singh and Shah 2014). JA pre-treatment also potentiated and enhanced GSH synthesis before copper treatment in *Arabidopsis* (Xiang and Oliver 1998). In contrast, the lack of induction of further accumulation of GSH was found after combined JA and heavy metal treatment in *C. vulgaris* compared to heavy metal stress alone (Piotrowska-Niczyporuk et al. 2012). It has been revealed that glutathione accumulation is necessary for the up-regulation of the JA signalling pathway, implicating glutathione as a factor, which determines basal JA gene expression (Han et al. 2013). In addition, among others, genes related to JA biosynthesis were activated during GSH treatment in *Arabidopsis* (Cheng et al. 2015). Although there are a few studies, which investigate how JA influence GSH content especially under heavy metal stress and, to our knowledge, none of them focuses on PC synthesis.

PAs

Heavy metals affect various processes in plants including synthesis of protective compounds, such as PAs, which are present in almost all living organisms. However, PAs should

no longer be considered simply as protective molecules but rather as compounds that are involved in a complex signalling system with a key role in the regulation of stress tolerance (Chmielowska-Bak et al. 2014; Pál et al. 2015). Cd stress has been reported to increase the putrescine (PUT) content and to induce the enzymes involved in its synthesis in wheat plants, while Cd-induced oxidative stress was alleviated by PA treatment in the same plant species (Groppa et al. 2007). Exogenous PAs have been reported to reverse heavy metal-induced oxidative stress mainly due to the activation of the antioxidant system (Groppa et al. 2001; Tang et al. 2005; Zhao and Yang 2008). Although changes in the content of PAs and GSH and/or PC contents under heavy metal stress have been studied for a long time, for example, in the case of Hg (Agrawal et al. 1992), Cd (Pal et al. 2006) and Zn (D'Souza and Devaraj 2012), only a few controversial studies have been found in the literature on the effect of PAs on PC synthesis under heavy metal stress; in particular, regarding the special aspect of their interaction, as the synthesis of PCs and the triamine spermidine (SPD) and the tetramine spermine (SPM) (also called higher PAs) are linked with each other due to their common precursor, Cys (Fig. 1). SPM-treatment in combination with Cd resulted in decreased GSH and PC levels in *Canavalia lineata* and in the roots an additional PC form with lower affinity for Cd was found (Yun et al. 1997). In a transgenic European pear, the overexpression of SPD synthase resulted in increased SPD content and significantly depleted GSH level under heavy metal stress conditions compared to the wild type (Wen et al. 2010b). Comparative study on poplar and willow plants revealed that Cd enhanced the PC content especially in the leaves of poplar but not in willow. However, Cd increased PA contents in both the roots and leaves of willow, but only that of putrescine in the roots of poplar, which suggests antagonistic relationship between PA and PC (Zacchini et al. 2011). SPM was also found to provide protection against Cd-induced oxidative damage in wheat, but failed to reverse the depletion in GSH content (Groppa et al. 2007). However, there may be a difference in the effect of PUT and higher PA treatments as SPD and SPM, as with the exception of PUT, PAs reduced Cd toxicity and reversed the Cd-induced decrease in GSH in rice (Hsu and Kao 2007). Exogenous application of SPD and SPM does not require additional decarboxylated S-adenosyl methionine for the synthesis of higher PAs, while after PUT treatment, the PUT taken up is metabolised to higher PAs in the PA cycle (Pál et al. 2015), thereby using up large quantities of Cys (Fig. 1). However, PUT reduced Cd uptake and increased GSH/PC content in mung bean (Nahar et al. 2016) and in another case, SPD increased the GSH and PC content, but did not alleviated Cr-induced growth inhibition in radish (Choudhary et al. 2012). In a recent study, inhibition of PUT synthesis, but not PUT application was proved

to be protective against Cd stress in rice. PUT pre-treatment decreased the content of thiols and PCs, the activity of PCS and even on the gene expression level of PCS (Pál et al. 2017). The decrease in PC may be resulted from the depletion of GSH content due to the increased PA metabolism. In addition, as the role of PAs as metal chelators has been also suggested (Lomozik et al. 2005), PUT treatment and the subsequent PA accumulation and PA–Cd binding may have reduced the amount of free Cd ions required for the induction of PCS activity and also for PCS gene expression (Pál et al. 2017).

SA

Phenolic compounds, such as SA are also involved in many important physiological processes (Dempsey and Klessig 2017). This endogenous plant growth regulator was first demonstrated to play a role in biotic stress responses. Soon afterwards, however, it became increasingly clear that SA plays a role during the plant response to abiotic stresses, such as heavy metal toxicity (Janda et al. 2007). On one hand, SA pre-treatment was reported to alleviate Cd toxicity, while on the other hand, Cd induced SA accumulation in several cases (Metwally et al. 2003; Pál et al. 2005; Krantev et al. 2008; Chao et al. 2010; Kovács et al. 2014; Bai et al. 2015). One of the major role of SA in modulating plant responses to various stresses is the induction of antioxidant capacity, involving increase in GSH content and GSH:GSSG ratio to combat environmental stress in plants (Ghanta et al. 2014). Constitutively elevated SA level enhances the activity of serine acetyltransferase, leading to elevated GSH content and increased Ni resistance in *Arabidopsis* (Freeman et al. 2005) and SA treatment increased GSH level in rice under Cd stress (Guo et al. 2009). Despite the numerous studies on the protective role of SA against various heavy metal stresses, only a few experiments are focused on the effect of SA on PC synthesis. Although SA alleviates Cd toxicity in barley, the protection was not related to the metal chelator system, as the amount of GSH and the transcription level of PCS was indistinguishable between the Cd treatments with or without SA (Metwally et al. 2003). Cd stress-induced increase in the root SA level has been reported to be related to the enhancement of the internal GSH cycle, thus inducing not only the antioxidant system but also the metal chelator PC synthesis, which promotes Cd stress tolerance in wheat seedlings (Kovács et al. 2014). SA treatment as seed soaking before Cd exposure of maize plants reduced the heavy metal injury and influenced PC composition. SA pre-treatment followed by lower concentration of Cd decreased the total PC content in the leaves of maize, while in the case of higher Cd concentration (after SA pre-treatment), the total PC content increased even in comparison to the solely Cd-treated plants (Szalai et al. 2013). Along with these, the combination of

SA with higher Cd concentration also caused a shift towards the occurrence of PC with higher number of repetition of the (γ -Glu-Cys) units. Comparison study on the effect of SA and Na-SA has been revealed that both of them induce various defence mechanisms during Cd stress in maize. However, SA mainly influences PC content and PCS activity in the leaves, while NaSA increased the PC level in the roots. These differences between SA and NaSA were manifested in the distribution of the Cd and in the changes of the antioxidant activities in maize plants (Gondor et al. 2016). Induced PCS transcripts has also been reported due to SA supplementation in Cr-stressed rice plants in parallel with increased PC and thiol contents compared to the control or Cr-treated ones (Huda et al. 2016).

Melatonin

Although the role of melatonin as a signal molecule is well-characterized in animals, the knowledge about their function in plants is still fragmentary. Melatonin has been accepted as a new plant growth regulator rather than plant hormone (Arnao and Hernández-Ruiz 2007; Li et al. 2017). Cd stress has been found to increase melatonin content in rice (Byeon et al. 2015); in addition, melatonin treatment mitigates Cd stress through the induction of PC synthesis in tomato (Hasan et al. 2015). In this latter study, melatonin treatment resulted in higher GSH, PC₂, PC₃ and PC₄ content in the leaves of tomato under Cd stress.

Conclusion and further perspectives

Investigations on naturally occurring compounds capable of reducing the stress sensitivity of plants are of great importance in the ever changing environment. Recently, more and more attention has been given to find and/or develop strategies in order to alleviate the adverse effects of heavy metal stress in plants, in addition to expand our understanding of defence mechanisms involved in the metal detoxification of plants. Involvement of several plant hormones and plant growth regulators was found to be associated with heavy metal stress responses. However, the clear link between hormonal pathways and metal-binding ligands in plants, either due to certain signalling pathway or common synthesis pathway, still needs to be explained (Fig. 1.). As there are numerous elements in the promoter region of the Cd-responsive genes that become activated in plant hormone signalling and as these plant growth regulators become induced simultaneously upon exposure to heavy metals and act in a coherent way, understanding and revealing possible and special relationships are even more difficult. GSH, the precursor of PCs, has several roles—among them the most important are primary metabolism, redox signalling

and detoxification—and its synthesis is affected by many factors (Noctor et al. 2012). Accordingly, revealing direct relationship between plant growth regulators and GSH/PCs at molecular level is only possible if their synthesis pathway is linked to each other, as in the case of ET or PAs. However, there is also a gap in the knowledge of the hormonal regulation of PC synthesis at gene expression level. It should be also take into consideration that the hormone mediated responses of plants may overlap, for example NO acts as an intermediate signalling molecule in CK, ABA, auxin, and ET signalling, in addition several *S*-nitrosylated proteins were identified as being polyamine-regulated (Tanou et al. 2014). There is some evidence that phytochelatins may also undergo *S*-nitrosylation, which could reduce the ability to chelate metals (Arasimowicz-Jelonek et al. 2011). It has been also shown that mitogen activated protein kinases (MAPKs) play a role in the signalling of plant hormones. In addition, the effect of cadmium on plant MAPKs in rice suggests that a MAPK cascade may also function in the cadmium-signalling pathways (Chmielowska-Bąk et al. 2014). Analysis of the promoter sequences of Cd-inducible genes in soybean seedlings revealed that their promoters possess several regulative motifs associated with plant response to ABA and ethylene (Chmielowska-Bąk et al. 2013).

Nevertheless, plant tolerance proven by phytohormones can not only be promoted directly but also indirectly, e.g. ABA-induced stomatal closure may decrease the root-to-shoot translocation of heavy metals in *Arabidopsis* (Perfus-Barbeoch et al. 2002); in case of polyamines, SPD has been reported to stimulate root Cd uptake, but not translocation in wheat (Tajti et al. 2018) maybe in relation with the heavy metal binding property of polyamines; or auxin-induced increased hemicellulose level and subsequent metal fixation in the roots, also decrease the translocation in *Arabidopsis* (Zhu et al. 2013).

Overall, improving our knowledge of hormone signalling in plants is critical for the development of new biotechnologies in order to mitigate heavy metal toxicity. Further investigations on hormone synthesis mutants or transgenic plants may help to identify clear interrelations. The gathered information may open the way for future experiments exploiting these relationships in phytoremediation strategies. According to these plant hormones may have promising role in improving the phytoextraction capacity of plants.

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