Role of Phytochelatins in Heavy Metal Stress and Detoxification Mechanisms in Plants

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Abstract Being sessile in nature, plants respond to heavy metal stress in the environment in different ways. The responses include immobilization, exclusion, chelation, and compartmentalization of the metal ions. Simultaneously, plants have general stress response mechanisms within their system especially through the expression of stress molecules like metallothionein and phytochelatins (PCs). PCs are the best-characterized heavy metal chelators especially in the context of cadmium (Cd) tolerance in plants; they were first discovered as Cd-binding "Cadystins A and B" in a fission yeast and then found in plants, fungi, and all groups of algae including cyanobacteria. PCs are non-protein cysteine-rich oligopeptides having the general structure of (γ-glutamyl-cysteinyl) n-glycine ($n = 2$–11) and produced by the enzyme phytochelatin synthase. They are capable of binding to various metals including Cd, As Cu, or Zn via sulfhydryl and carboxyl residues, but the biosyntheses are preferentially controlled by the metal Cd or metalloid As. The fundamental roles of PCs in metal detoxification by plant cells are now well known and tolerance of Cd increases in yeast and bacteria with the overexpression of PC synthase genes. Sequestration of PC-metal complex in both plant and yeast cells occurs at the vacuole, where PCs are involved in the accumulation of the metal as complexes, particularly in response to Cd by forming high molecular weight compounds after incorporation of sulfur (S^{2-}). The role of PCs may further be explored to improve the metal detoxification activities and tolerance characteristics of higher plants under various conditions.

Keywords Phytochelatin · Cadmium · Arsenic · Glutathione · Tonoplast · Metal transporters

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1 Introduction

Unprecedented anthropogenic activities lead to rapid changes in the environment causing pollution and degradation in the quality of air, water, and soil, and increasing the harmful ultraviolet radiation, acid rain, salinity, etc. Heavy metal pollution in the environment is of universal concern due to dispersion of untreated industrial and municipal wastes, which creates instability in the natural equilibrium. Constituting a diverse group of elements, heavy metals vary in their chemical characteristics, biological functions, and toxicity (Chatterjee et al. 2007). Heavy metals are frequently classified as metals having density equal or greater than 5.0 g/cm^3. Although many of the metals are important for plant nutrition and growth at low concentrations, all of them are toxic at high concentrations, leading to interference with metabolism via generation of free radicals and disruption of function of essential cellular enzymes (Prasad and Freitas 2003). The Agency for Toxic Substances and Disease Registry of The Centers for Disease Control and Prevention, USA (http://www.atsdr.cdc.gov) published a list of these metals, according to their toxicity (2007 CERCLA Priority List of Hazardous Substances), where, among the top 10 toxic substances in the list, are arsenic (ranked first), lead (ranked second), mercury (ranked third), and cadmium (ranked seventh) (CERCLA 2007).

Reports suggest that heavy metals like cadmium (Cd), arsenic (As), lead (Pb), and mercury (Hg) do not have any known biological functions (Duruibe et al. 2007; Chetia et al. 2011). Cadmium (Cd), a relatively rare transition metal with a density of 8.6 g/cm^3, is a significant pollutant due to its high toxicity and greater solubility. This metal is in fact extremely toxic even at low concentrations and often interferes with other essential metal (zinc, iron, copper, manganese, magnesium, and calcium) containing enzymes by displacing these elements (Wagner 1993). Prolonged exposure to Cd through inhalation or ingestion, in humans, leads to several diseases including pulmonary irritation and increased lung cancer risks, kidney and liver disease, and the itai–itai disease in which patients suffer from severe osteoporosis and osteomalacia, in addition to kidney damage (Inaba et al. 2005). In plants, Cd primarily damages different enzyme systems and photosystems.

In natural non-polluted soils concentrations of cadmium may be up to 40–300 nM, although it may increase with clay concentration up to 1 $\mu g\ g^{-1}$ dry soil (Wagner 1993; Mengel et al. 2001; Inaba et al. 2005). Availability of Cd to plants is greater in acid soils and its solubility increases with exudates of roots (Zhu et al. 1999; Mengel et al. 2001; Kirkham 2006; Lux et al. 2011). Delivery of Cd^{2+} to plant roots is dominated by a transpiration-driven mass-flow process of the soil solution (Sterckeman et al. 2004). Reports suggest that Cd accumulation by plants grown in soil is directly related to transpiration (Ingwersen and Streck 2005).

Cd contamination in soil may take place either naturally or through anthropogenic activities. Weathering of Cd-rich rocks can enrich natural mineral outcrops which in turn pollute the environment. Major anthropogenic sources of Cd include burning of fossil fuels such as coal or oil and the incineration of municipal wastes, cement factories, and as a by-product of phosphate fertilizers (Mengel et al. 2001; Chen 2005; Kirkby and Johnson 2008; Lux et al. 2011). Plant roots have the ability either to exclude and/or chelate or sequester Cd from the plant tissues. Chelation of Cd leads to production of a non-toxic compound that may be sequestered within a non-vital cellular compartment (Lux et al. 2011).

Arsenic (As) has been known as a poison for years. Though rarely present in its elemental state it is more common in sulfides and sulfosalts such as Arsenopyrite, Orpiment, Realgar, Lollingite, and Tennantite (Elangovan and Chalakh 2006). Arsenic is used in several industries like paints, dyes, metals, soaps, insecticides, and semi-conductors and is also released into the environment through burning fossil fuels, paper production, cement manufacturing, and mining activities. It can exist in several forms with arsenite (+3) and arsenate (+5) is the most prevalent toxic form of inorganic arsenic. Arsenic has several deleterious effects on human health, viz., cardiovascular, gastrointestinal problems, anemia and leucopoenia, liver and kidney damage, mental confusion, skin disorder, hyperkeratosis and carcinogenic effects, etc. (Chakraborti et al. 2002; Elangovan and Chalakh 2006; Mondal and Polya 2008).

To reduce the deleterious effect of As, plants convert methylate arsenic into volatile (e.g., methylarsines) or nonvolatile (e.g., methylarsonic acid and dimethylarsinic acid [DMAA]) (Bentley and Chasteen 2002), that help the plant to sequester the same within their tissue system. It is also reported that a few aquatic plants like *Ceratophyllum demersum* and *Elatine triandra* synthesize lipid-soluble As compounds to alleviate the toxicity of arsenic (Tamaki and Frankenberger 1992; Zheng et al. 2003). Roots were found to be the major sites of accumulation for inorganic arsenicals (Carbonell-Barrachina et al. 1998).

2 Heavy Metal Uptake and Accumulation by Plants

Being sessile in nature, plants cannot migrate from one place to other to avoid the natural or edaphic stress factors that may influence their growth, development, or productivity due to the shift in inner concentrations of bio-reactive metals (Schützendübel and Polle 2002; Chatterjee et al. 2011). Root systems of plants acquire micronutrients, like iron (Fe), zinc (Zn), copper (Cu), etc., from the surrounding terrestrial or aquatic environments. Non-essential metals like cadmium (Cd), arsenic (As), lead (Pb), and mercury (Hg) may also be present at low to high concentrations in these supplies. Plants follow diverse tactics in response to heavy metal toxicity, which include immobilization, exclusion, chelation, and compartmentalization of the metal ions, and expression of the general stress responses (Cobbett 2000). Several plants have been identified that possess the unique capability to live under toxic conditions at heavy metal contaminated sites and have also been found to accumulate a considerable amount of such metals within their biomass (hyperaccumulators). Studies have shown that natural hyperaccumulators like the As hyperaccumlating fern species *Pteris vittata* (Gumaelius et al. 2004) and the Ni hyperaccumulating species *Thlaspi caerulescens* (Freeman et al. 2004) can withstand higher amounts of metal accumulation without having significant damage within their system.

Physiological transport of nutrients like Ca, Fe, Mg, Mn, Co, and Zn is unique in plants. Cd competes with these essential nutrients during transportation through occupying transmembrane nutrient transporters (Clemens et al. 1998; Curie et al. 2000; Thomine et al. 2000; Papoyan and Kochian 2004). Cd enters the root through the cortical tissue, and is likely to be accumulated in the roots. However, it reaches the xylem through an apoplastic and/or symplastic pathway for further transportation to shoots, and may be complexed by several ligands such as organic acids and/or PCs (Cataldo and Wildung 1983; Salt et al. 1995). Once it has entered into the root, Cd damages cells, especially nucleoli, and hampers several enzymatic processes such as nitrate reductase and ribonuclease activities (Shah and Dubey 1995; Hernandez et al. 1997). Cd leads to Fe(II) deficiency in shoot tissues of plants, and hence affects photosynthesis by damaging the light harvesting complex II and photosystems I and II and by increasing the non-photochemical quenching (Krupa 1988; Alcantara et al. 1994; Siedlecka and Krupa 1996; Larsson et al. 1998).

Cd has no known positive biological activities and is toxic to plant cells even at low concentrations. It is reported that, approximately 5–10 μg of Cd concentration per gram of dry mass in leaf is toxic to most plants (White and Brown 2010; Lux et al. 2011). However, there are also reports indicating roots of the ecotypes of a few genera of plants that proliferate in Cd-enriched soils (Whiting et al. 2000; Liu et al. 2010). Lux et al. (2011) provided a list of Cd-hyperaccumulator plants along with their ecotypes like *Noccaea caerulescens* (J&C Presl.) FK Mey, and *Arabidopsis halleri* (L.) O'Kane and Al-Shehbazs, etc. These plants also have a defensive mechanism that restricts the entry of Cd to the xylem and prevents metal accumulation in shoot tissues, especially through the production of Cd-chelators at the root zone (Liu et al. 2010; Lux et al. 2011). As mentioned, chelators like PC synthesis can also take place in cells that are exposed to various metals and metalloids such as Cu^{2+}, Zn^{2+}, As^{2+}, Pb^{2+}, and Ag^{+} (Gekeler et al. 1989; Cobbet 2000; Inouhe 2005; Tennstedt et al. 2009).

Cd concentrations in shoot of plants vary widely in nature, for which, environmental factors and phylogenetic variation are responsible (Watanabe et al. 2007). Species of the Caryophyllales and Lamiales group accumulate Cd in shoots at much higher concentrations than other species (Broadley et al. 2001). However, Cd concentrations are mostly higher in roots than in shoots, signifying that transportion of Cd to the xylem and phloem is limited in most plants, and lowest in seeds, fruits, and tubers (Seregin and Kozhevnikova 2008; Conn and Gilliham 2010; Lux et al. 2011).

Selection and uptake at optimum level of heavy metals essential for growth, and rejection of those metals that are not essential is the most important activity in the strategy of plants (Cobbett and Goldsbrough 2002; Perales-Vela et al. 2006). In plants, stress due to heavy metals involves an intricate system of signal transduction, which is basically a two-step process, where the activation of the process starts by sensing of the heavy metal(s). Reduction in essential nutrients will decrease the plant vitality and its ability to cope with (metal) stress (Huang et al. 2008). Once the plant senses the presence of metals, synthesis of proteins and signaling molecules related to stress takes place, leading to the explicit activation of metal-responsive genes to counteract the stress (Maksymiec 2007). Thus the generation of legends specific for the metal (chelation) and subsequent compartmentalization of the ligand–metal complexes in the cells can be the typical defense mechanisms for detoxification of heavy metal within plants and other organisms (Cobbett 2000).

3 Root System, Transporters and Heavy Metals

Heavy metal absorption in higher plants is a critical issue, where especially the rhizosphere region interacts with heavy metals (Wenzel et al. 2003). Heavy metals are usually co-transported in the form of cations across the plasma membrane (Manara 2012). Reports suggest that, plant roots primarily secrete exudates in the surrounding soil matrix that helps in the chelation of unwanted metals to prevent transportation inside the cell (Marschner 1995). For example, histidine (His) and citrate are secreted as root exudates to prevent Ni uptake from the soil (Salt et al. 2000). Pectic sites and a number of extracellular carbohydrate molecules present on the cell wall play an important role in immobilization of toxic heavy metal ions (Manara 2012).

However, heavy metal homeostasis is mainly maintained by transporters present on the plasma membrane (Fig. 1). These transporters are heavy metal P1B–ATPase, the NRAMP, the CDF (Williams et al. 2000), and the ZIP families

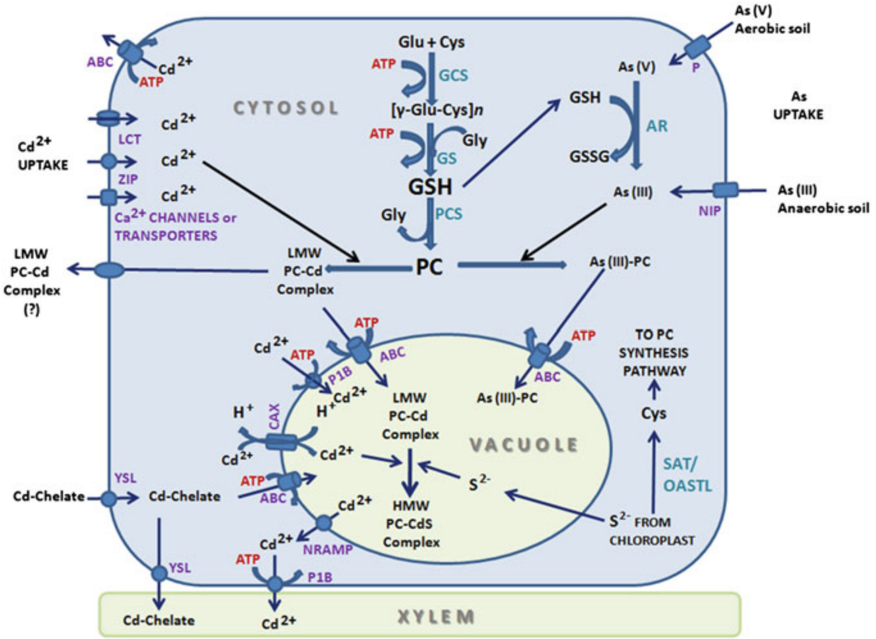

Fig. 1 Schematic diagram showing the main events involved in uptake of cadmium (Cd^{2+}) and arsenic (As, principally As III or As V) by plant cells and their detoxification by phytochelatin (PC). The figure shows the synthesis of glutathione (GSH) from glutamate (Glu) and cysteine (Cys) and subsequently to PC through the coordinated orchestration of γ-glutamylcysteine synthetase (GCS), GSH synthetase (GS) and phytochelatin synthase (PCS), respectively. Cd^{2+} uptake by the plant cells is mediated through different transporter proteins such as ABC, Ca^{2+} channel transporters, Zinc Regulated Transporter/Iron Regulated Transporters like Protein (ZIP), and Low Affinity Cation Transporters (LCT). In the cytosol, Cd^{2+} interacts with PC, which is responsible for chelation of Cd^{2+} and formation of low molecular weight (LMW) PC-Cd complexes. These LMW PC-Cd complexes are sequestered into the vacuoles by means of transporter molecules present in the tonoplast. Following compartmentalization, LMW complexes further integrate Cd^{2+} (sequestered from cytosol by antiporter molecules) and sulfide (S^{2-}, generated by the chloroplasts) to finally form high molecular weight (HMW) PC-CdS complexes. Alternatively, Cd-chelate complexes may enter cytosol through *yellow*-strip 1-like (YSL) protein and get localized inside the vacuoles by transporters. Cd^{2+} and Cd-chelate complexes may also leave cytosol through the activity of different transporters, shown in the figure. Similarly, uptake of As III or As V is also mediated by phosphate transporters (P) and nodulin 26 like intrinsic protein (NIP), a plant aquaporin, and then is followed by reduction of As V to As III, catalyzed by an arsenic reductase (AR). As III is subsequently chelated by PC to form As III-PC complex, which is finally transported into the vacuoles by the ABC transporters. [abbreviations: *ABC* ATP-Binding Cassette Transporters, *AR* Arsenic Reductase, *As* Arsenic, *ATP* Adenosine Triphosphate, Ca^{2+} Calcium Ion, *CAX* Calcium Exchangers (Transporter proteins), *Cd* Cadmium, *Cys* Cysteine, *GCS* γ-Glutamyl-Cysteine Synthetase, *Glu* Glutamate, *Gly* Glycine, *GS* GSH Synthetase, *GSH* Glutathione, *GSSG* Glutathione disulfide (L(-)Glutathione), H^+ Hydrogen Ion, *HMW* High Molecular Weight, *LCT* Low Affinity Cation Transporters, *LMW* Low Molecular Weight, *NIP* Nodulin 26 Like Intrinsic Protein (Plant Aquaporin), *NRAMP* Natural Resistance -Associated Macrophage Protein, *OASTL* OAS (Thiol)-Lyase, *P* Phosphate Transporters, *P1B* (P_{1B} ATPase), *PC* Phytochelatin, *PCS* Phytochelatin Synthase, S^{2-} Sulfide, *SAT* Ser Acetyl Transferase, *YSL Yellow*-Strip 1-Like Protein, *ZIP* Zinc Regulated Transporter/ Iron Regulated Transporters Like Protein] (Adopted from Cobbett 2000; Saito 2004; Nocito et al. 2007; Lux et al. 2011)

(Guerinot 2000). The ZIP family transporters are well characterized for divalent metal uptake, which consists of eight transmembrane domains with similar topology at N- and C- termini exposed to apoplast, also containing a histidine-rich domain supposed to involve in specific metal binding (Guerinot 2000; Nishida et al. 2008). ZIP protein gets activated in response to Fe or Zn loading. In *Arabidopsis thaliana*, IRT1 was the first reported transporter in root cells and has an important role in Fe^{2+} uptake from the soil (Vert et al. 2002). IRT1 can also transport Mn^{2+}, Zn^{2+}, and Cd^{2+} (Korshunova et al. 1999). AtIRT1 in yeast enhances the Ni^{2+}-uptake activity (Nishida et al. 2011). Furthermore, AtZIP4 proteins, expressed in roots and shoots, are involved in Zn transport and also help in Cd uptake from soil into the root cells and Cd transport from root to shoot (Krämer et al. 2007).

The HMAs family transporters (P1B-type ATPases) that belong to P-type ATPase superfamily and use energy from ATP hydrolysis to efflux various metal cations across biological membranes (Axelsen and Palmgren 2001) are basically internal transporters to load Cd and Zn metals into the xylem from the surrounding tissues and act as an efflux pump. The HMAs was categorized as both monovalent and divalent cation transporter (Baxter et al. 2003; Krämer et al. 2007). In *A. thaliana*, AtHMA3 transporter helps in sequestration of a wider range of heavy metals and its overexpression increases the tolerance to heavy metals like Cd, Pb, Co, and Zn (Morel et al. 2009; Manara 2012). In ABC transporter family, AtPDR8 was discovered in the plasma membrane of *A. thaliana* root hairs and epidermal cells that help in effluxion of Cd and Pb from plasma membrane (Kobae et al. 2006; Kim et al. 2007). Oligopeptide transporters (OPTs) are a group of membrane-localized proteins. The OPT proteins belong to a small gene family in plants, named as the YSL subfamily taking its name from the maize Yellow stripe 1 protein (ZmYS1), and are involved in uptake of Fe by transporting Fe(III)–phytosiderophore complexes (Curie et al. 2001). Heavy metal ions like Fe, Zn, Cu, Ni, and, to a lesser extent, Mn and Cd are transported by ZmYS1 transporter (Schaaf et al. 2004). Based on sequence similarity with maize gene, eight presumed YSL transporters have been identified in *A. thaliana* (Colangelo and Guerinot 2006). AtYSL1 is expressed in the leaf xylem parenchyma, in pollen, and in young siliques, whereas AtYSL2 is expressed in shoot and root vascular tissues and is present in the lateral plasma membrane, steady with a role in the lateral movement of metals into the veins (DiDonato et al. 2004; Schaaf et al. 2005).

Metal Tolerance Proteins (MTPs) are metal efflux transporters in plants that belong to the CDF transporter family involved in pumping divalent metal cations like Zn, Cd, Co, Fe, Ni, and Mn and transportation from the cytoplasm to the vacuole (Nies 1992; Krämer et al. 2007; Peiter et al. 2007; Montanini et al. 2007; Manara 2012). CDF transporters consist of six transmembrane domains, a C-terminal cation efflux domain, and a histidine-rich region between transmembrane domains IV and V (Mäser et al. 2001) which probably act as a sensor for heavy metal concentration (Kawachi et al. 2008). NRAMP transporters, for example, AtNRAMP3 and AtNRAMP4 are localized in the tonoplast and help in the transportation of Fe from the vacuole (Thomine et al. 2003; Lanquar et al.

2005). Cd sensitivity increases on overexpression of AtNRAMP3 (Thomine et al. 2000) and prevents accumulation of Mn (Thomine et al. 2003), indicating a possible role in the homeostasis of metals other than Fe.

Tonoplast is the vacuolar membrane mainly involved in regulating the movements of ions and metals around the cell, as the vacuole mainly acts as storage for metal ions (Vögeli-Lange and Wagner 1990). The tonoplast contains many membrane transporters, for example, ABC transporter that helps in the transportation of xenobiotic compound and heavy metal. The ABC transporter subfamily (MRP and PDR) is mainly actively involved in sequestration of heavy metals (Manara 2012). MRPs are concerned with mediating PC-Cd complex transport across the tonoplast in plants (Vögeli-Lange and Wagner 1990).

4 Sequestration of Metals into Vacuole

Once a heavy metal enters the plant cell, strategies for sequestration of the metal into the vacuole is activated so that the metal may be removed from the active cellular compartments and from cytosol, where sensitive metabolic activities take place (Dalcorso et al. 2010; Hossain et al. 2012). Thus, the major vacuole in a plant cell seems to be a suitable storage reservoir for heavy metal accumulation. Reports suggest that, proton pumps in the vacuole, especially vacuolar proton-ATPase (V-ATPase) and vacuolar proton-pyrophosphatase (V-PPase), help in vacuolar uptake of most solutes. Some well-characterized heavy metal transporter proteins are zinc-regulated transporter (ZRT), iron-regulated transporter (IRT) like protein ZIP family, the P-type metal ATPases, ABC transporters of the mitochondria (ATM), ATP-binding cassette (ABC) transporters, multidrug resistance-associated proteins (MRP), the natural resistance-associated macrophage protein (NRAMP) family, copper transporter (COPT) family proteins, cation diffusion facilitator (CDF) family of proteins, yellow-stripe-like (YSL) transporter and Ca^{2+}: cation antiporter (CAX), pleiotropic drug resistance (PDR) transporters, etc. (Lee et al. 2005; Chiang et al. 2006; Krämer et al. 2007; Dubey 2011; Huang et al. 2012). Vacuole isolation or compartmental flux analysis on heavy metal accumulation, specifically on Cd-exposed tobacco (*Nicotiana rustica* var Pavonii) seedlings, revealed vacuoles that contained virtually all of the Cd-binding peptides and Cd found in protoplasts (Vögeli-Lange and Wagner 1990; Huang et al. 2012).

Sequestration of PC-metal complex, in both plant and yeast, occurs at the vacuole (Fig. 1). PCs, particularly in response to Cd and As involve with the accumulation of the metal as complexes in the vacuole by forming high molecular weight (HMW) compound after incorporation of sulfur (S^{2-}) (Salt et al. 1998). Cd/H^+ antiporters and ATP-dependent ABC transporters in the tonoplast help the transportation of PC-Cd complexes into the vacuole (Salt and Wagner 1993; Salt and Rauser 1995). It was reported by Ortiz et al. (1992) that a Cd-sensitive mutant of *Schizosaccharomyces pombe* (mutation in the gene *hmt1* that encodes for an

ABC-type transporter) can synthesize PCs but not accumulate the Cd-PC-sulfide complexes. Root-to-shoot transport of Cd is another important activity of PCs. Wheat gene TaPCS1 when expressed in transgenic *A. thaliana* increased transport efficiency of Cd from root to shoot (Gong et al. 2003).

PCs chelate Cd^{2+} with higher affinity than glutathione (GSH) molecules and both the complexes between PCs and GSH may be sequestered into vacuoles (Kneer and Zenk 1992; Howden et al. 1995; Li et al. 1996; Pal and Rai 2010; Huang et al. 2012). This process has been most clearly demonstrated through studies on Cd-sensitive mutants in *S. pombe*. Ortiz et al. (1992, 1995) first discovered hmt1, in Cd^{2+} -sensitive mutant of *S. pombe* that confers the inability to accumulate vacuolar PC-Cd. HMT1 is a half-molecule ATP-binding cassette (ABC) transporter that recognizes PCs and PC-Cd. Similarly, YCF1, discovered from Cd^{2+}-sensitive mutant of *Saccharomyces cerevisiae*, which was a full molecule ABC transporter that helps in sequestration of a GS2-Cd complex into vacuoles (Li et al. 1996, 1997; Huang et al. 2012). Preveral et al. (2009) reported SpHMT1 for transport of GS2-Cd conjugates in overexpressed *S. pombe* mutants that lack PCs for substrate. Though, HMT1 has also been reported from *Caenorhabditis elegans* (CeHMT1) and *Drosophila melanogaster*, HMT1 ortholog has not yet been recognized in higher plants (Vatamaniuk et al. 2005; Sooksa-Nguan et al. 2009).

Recently, two ABCC subfamily members of ABC transporters in *Arabidopsis*, were discovered in higher plants, which facilitates vacuolar PC-As (III) transport (Song et al. 2010). Again, in the atabcc1 atabcc2 mutant, considerable reduction in vacuolar Cd^{2+} was reported signifying that AtABCC1 and AtABCC2 play a significant role in vacuolar Cd^{2+} sequestration (Park et al. 2012; Huang et al. 2012). However, ectopically expressed SpHMT1 in *Arabidopsis* has shown that PCs play a primary role in vacuolar Cd^{2+} sequestration, and PC-deficient mutant cad1-3 has shown no effect on this process, suggesting the SpHMT1 function that requires PCs (Huang et al. 2012).

5 Role of Metal-Binding Ligands

Metal-binding ligands play an important role in plants by maintaining concentrations of essential biometals at optimum level and reducing the toxicity thresholds of non-essential metals. A number of metal-binding ligands have now been recognized which help plants to sustain in hostile environments (Rauser 1999; Callahan et al. 2006). For example, organic acids, like citrate and malate help in extracellular chelation for aluminum (Al) and are reported to be correlated with tolerance of Al in plants (Delhaize and Ryan 1995), which are also evident in Al-resistant mutants of *Arabidopsis* (Larsen et al. 1998). Further, amino acids like histidine (His) help to chelate metal ions in xylem sap and within cells (Rauser 1999).

Peptide ligands are also present in plants. Phytochelatins (PCs) and metallothioneins (MTs) are among the most common peptide ligands. These cysteine-rich polypeptides provide thiols to bind different kinds of metals that help in cellular metal homeostasis and detoxification (Rauser 1995; Cobett 2000; Inouhe et al. 2012). However, synthesis of PCs to alleviate toxic effects of non-essential heavy metals such as As, or Cd are arguably the most sophisticated enzyme-catalyzed mechanisms known to protect plants and some algae, fungi, and invertebrates (Rea 2012).

6 Phytochelatins: The Heavy Metal Chelator

In plants, Phytochelatins (PCs) are the best-characterized heavy metal chelators especially in the context of Cadmium Cd tolerance (Cobbett 2000; Manara 2012). Phytochelatins, found in plants, fungi, and all groups of algae including cyanobacteria, are non-protein cysteine-rich oligopeptides, produced by the enzyme phytochelatin synthase. The peptide was first discovered by Hayashi and his group as the Cd-binding complexes in fission yeast, *S. pombe*, exposed to Cd^{2+} and was named as "cadystins" (Murasugi et al. 1981). Again, in 1984, Hayashi and his team identified Cadystins, A and B (γ-Glu-Cys) n-Gly with $n = 2$ and 3; however, Grill et al. (1985) reported a ubiquitous presence of the same peptides and those with higher degrees of polymerization and termed them as "phytochelatins" ($n = 2$–11) (Kondo et al. 1984; Grill et al. 1985; Inouhe 2005). A lot of work related to this peptide came out signifying the importance of the PC peptides in toxic ion sequestering of borderline class of metals in plants, yeast, and microorganisms and Cd was reported to be the preeminent activator of the enzyme PC synthase (Robinson 1989; Rauser 1990; Steffens 1990). Reports appeared that cite the evidence of activation PC synthase enzyme for the synthesis of the peptide from GSH (Grill et al. 1989; Hayashi et al. 1991). Heavy metal tolerance and its relationship to PCs were also examined using chemicals for inhibitor GSH biosynthesis like Buthionine sulfoximine (BSO) (Grill et al. 1987) and PC-deficient Cd-hypersensitive mutants were also obtained from *S. pombe* and *A. thaliana* (Mutoh and Hayashi 1988; Howden et al. 1995). Plants exposed to As substantially increase the synthesis of GSH and PCs (Gupta et al. 2004; Grill et al. 2006) and augmented PC synthesis has been observed in non-tolerant and non-accumulator, as well as in hypertolerant and hyperaccumulator plants (Grill et al. 2006; Gupta et al. 2008).

Reports suggest that PCs are also involved in the chelation of essential metal ions when present in excess. An essential metal like Zn is the structural component of approximately 10 % of different Zn-dependent proteins (Tennstedt et al. 2009). When threshold limit of Zn in plants (between 100 and 300 $\mu g\ g^{-1}$ dry weight, depending on plant species and physiological state) exceeds, unusual binding of Zn ions to thiols or other functional groups leads to the disruption of many essential proteins (Marschner 1995; Kramer and Clemens 2005). Likewise, to alleviate the problem of metal toxicity PCs take a major role in buffering for a wide range of metal ions, whose synthesis is activated both in vivo and in vitro by forming complexes with a molecular weight of 2.5–3.6 kDa (Grill et al. 1987; Cobbet 2000; Vatamaniuk et al. 2000; Oven et al. 2002; Tennstedt et al. 2009).

7 Phytochelatins Biosynthesis

Phytochelatin biosynthesis involves common precursors like, glutamine (Glu, E), cysteine (Cys, C), and glycine (Gly, E) and the associated glutathione that makes the peptides similar to ubiquitous tripeptide glutathione, γ-Glu-Cys-Gly with the general structure (γ-Glu-Cys) n-Gly. However, in some plant species, serine, glutamine, glutamate, or alanine replace the glycine of C-terminal end leading to the generation of iso-phytochelatins (iso-PCs). Distribution and quantity of iso-PCs may vary in different plant species, as cells of *A. thaliana* are capable of synthesizing different PC-related peptides (PCs and iso-PCs) (Ducruix et al. 2006). Again, synthesis of iso-PCs mostly depends upon the availability of Gly or glutathione synthetase in the cells and may switch over to synthesize iso-PCs (as for example, synthesis of dipeptide γ-glutamylcysteine (γ-EC) when the plant comes under stress (Ducruix et al. 2006; Rea 2012). Similar iso-PCs are also reported, as, (γ-Glu-Cys) n-β-Ala, (γ-Glu-Cys) n-Ser, and (γ-Glu-Cys) n-Glu (Cobbett 2000; Rea 2012).

The pathway of PCs biosynthesis is mostly related with that of GSH biosynthesis, because GSH is the direct substrate for the generation of PCs (Noctor and Foyer 1998). The reactions for biosynthesis of GSH require ATP as substrate. It consists of two successive reactions mediated by γ-EC synthetase (EC 6.3.2.2) and GSH synthetase (EC 6.3.2.3). The γ-EC synthetase is a rate-limiting enzyme for GSH synthesis (Noctor and Foyer 1998) whose activity is enhanced by metal ions like Cd^{2+} ions and suppressed by treatment with BSO (Grill et al. 1987; Scheller et al. 1987; Inouhe 2005). Whereas, PC synthase for the synthesis of PCs from GSH was categorized as the γ-EC dipeptidyl transpeptidase (EC 2.3.2.15) that adds a γ-EC-unit of GSH to PCs or another GSH in vitro (Grill et al. 1989; Loeffler et al. 1989).

Identification of the fundamental mechanisms of PCs activity toward heavy metal detoxification was a challenge for researchers in the 1980s after its discovery. A few model organisms, like *A. thaliana*, *S. pombe*, and *Candida glabrata*, were chosen to analyze the molecular genetic mechanisms in plants related to Cd-sensitive mutants. Along with the identification of metal-sensitive varieties, isolation and characterization of mutants, and the expression of the cDNAs contributed significantly in understanding the genes involved in GSH and PC biosynthesis and the biological role of PCs (Howden and Cobbett 1992; Murphy and Taiz 1995; Cobbett 2000a).

Three groups working on the Cd-sensitive mutants in different plants, in the same year, reported the gene primarily involved in the process. Roots of various dicotyledonous plants that lack the ability to produce the Cd-binding complex are generally Cd-sensitive compared with roots of monocotyledonous plants

(Inouhe et al. 1994). Such a Cd-binding complex was lacking in chickpea roots exposed to Cd, although the root tissues produced a substantial level of PCs (Gupta et al. 2002b). Ha et al. (1999) also reported that mutants of *Arabidopsis* with Cd-sensitive cad1 have wild-type levels of GSH but are PC-deficient and lack PC synthase activity in vitro, where CAD1 was predicted as the structural gene for PC synthase. However, the same CAD1 gene which has been isolated using a positional cloning by other two groups referred to as AtPCS1 (in *Arabidopsis*), SpPCS (in *S. pombe*) and TaPCS1 (in wheat) showed similar ability to confer resistance to Cd (Vatamaniuk et al. 1999; Clemens et al. 1999). In addition, PC synthases purified from recombinant *A. thaliana* and *S. pombe* catalyze the production of PCs from GSH in vitro (Vatamaniuk et al. 1999; Clemens et al. 1999). Similar genes were also identified in *Caenorhabditis elegans*, *Dictyostelium discoideum*, Chironomus and earthworm species, suggesting that PCs play a wider role in heavy metal detoxification (Cobbett 2000b).

8 Role of Phytochelatins in Heavy Metal Stress and Detoxification

Plants respond to heavy metal stress within by chelation and subsequent sequestration of the ions. Since immobilized metals are less toxic than free ions, PCs are considered to be part of the mechanism detoxifying heavy metals in higher plants (Cobbett and Goldsbrough 2002). Appearance of such metal-binding peptides in plants could be an important biochemical indication of heavy metal contamination under various environments (Gupta et al. 2002a, b).

Reports demonstrate that plants form PC-metal complexes for detoxification not only for cadmium, but also for a wide range of metal ions like Cd, Pb, As, Ag, Hg or Zn, Cu, and Ni (Maitani et al. 1996; Mehra et al. 1996; Rauser 1999; Ha et al. 1999; Manara 2012). Stress response of plants in respect to the role of phytochelatins and with isolated cad1 mutants were analyzed for different heavy metals. More sensitivity was found toward Cd and arsenate for the cad1–3 mutant of *A. thaliana* than wild-type plants; however, no considerable difference was found for metals like Zn, selenite, and Ni ions (Ha et al. 1999). PCs (PCS-deficient) mutant of cad1–3 to *S. pombe* was found modestly sensitive to Cu and Hg and showed intermediate sensitivity to Ag (Maitani et al. 1996; Ha et al. 1999; Manara 2012). Studies on the role of PCs in Cu tolerance and detoxification have uncertainty. Activation of PC biosynthesis both in vivo and in vitro by Cu is evidenced in different reports. PC-deficient mutants showed comparatively low sensitivity to Cu. Salt et al. (1989), in their study on copper-tolerant plant *Mimulus guttatus*, corroborated the role for PCs in Cu tolerance. In contrast, when the root tips were exposed to Cu, both the Cu-tolerant and Cu-sensitive ecotypes of *S. vulgaris* produced comparable quantity of PCs. It is also known that PC–Cu complexes are comparatively transient and relatively poorly sequestered to the vacuole.

This phenomenon indicates a differential tolerance that may occur due to some other mechanisms (Schat and Kalff 1992; De Knecht et al. 1994; Cobbett and Goldsbrough 2002). In another study with plant *Rubia tinctorum*, it was found that PC-metal complexes are formed in the roots when they are subjected to exposure to different heavy metals. Ag, arsenate, Cd, Cu, Hg, and Pb ions appeared most effective in induction of PCs; however, PC complexes identified in vivo were with Cd, Ag, and Cu ions (Maitani et al. 1996; Cobbett and Goldsbrough 2002). It was again found that Pb and arsenate-induced PC complexes contained Cu ions and not the metal ion used for induction of synthesis. The phenomenon again appeared in disagreement with general PC synthase activity, where a metal-GSH thiolate is the substrate for PC-metal biosynthesis; however, this may indicate exchange of some metal in complexes with PCs (Cobbett and Goldsbrough 2002). Nevertheless, much evidence suggests a strong role of PCs in plant response and detoxification of different heavy metals. Apart from the well-recognized roles for PCs in the cell, like homeostasis of metals, antioxidant property and sulfur metabolism (Dietz et al. 1999; Cobbett 2000), additionally, PCs are also responsible for developing hypersensitivity to heavy metals. Reports advocate that in transgenic plants excessive PC levels help the plant to accumulate greater amounts of heavy metals without enhancing tolerance conferring hypersensitivity to heavy metals (Lee et al. 2003; Pomponi et al. 2006; Manara 2012).

9 Phytochelatins on Sulfur Metabolism During Heavy Metal Stress

Sulfur (S), an essential and ubiquitous element for all living organisms, is involved in a large number of vital biochemical and physiological processes. It is the component of two important amino acids in plants, cysteine (Cys) and methionine (Met), and is the essential component of proteins, hetero-polysaccharides and lipids, iron-sulfur clusters, and a variety of biomolecules such as vitamins (biotin and thiamine), cofactors (CoA and S-adenosyl Met), peptides (glutathione and phytochelatins), and secondary products (allyl Cys sulfoxides and glucosinolates) (Leustek et al. 2000; Rausch and Wachter 2005; Nocito et al. 2007). Although in biological systems, sulfur does not take part in any precise structural formations, it is responsible for their catalytic or electrochemical properties when present in different biomolecules. As for example, many thiols have the capacity to react with a broad spectrum of agents, like cytotoxic electrophilic organic xenobiotics, heavy metals, free radicals, etc., due to their extreme nucleophilicity of the sulfhydryl group of Cys residues (Leustek et al. 2000; Nocito et al. 2007). Two thiols in disulfide can also be involved in redox cycles. Such cycle represents the chemical base making GSH a powerful cell redox buffer and its capability to readily react with a wide range of electrophilic compounds to form covalent bound glutathione S-conjugates (Leustek et al. 2000; Nocito et al. 2007). Thus, GSH is directly involved with the regulation of sulfur metabolism and inter-organ sulfur distribution (Lappartient and Touraine 1996). GSH is also the precursor of PCs and hence biosynthesis of both GSH and PCs is highly regulated and orchestrated in such a fashion that meet the demand for Cys-consuming activities, which largely contribute to delineate the overall sulfur demand by plants. The requirement of sulfur usually varies under diverse environmental conditions, biotic and abiotic stresses, including heavy metals (Rausch and Wachter 2005). The direct interrelationships may be ascertained when withdrawal of sulfate from the growing medium dramatically decreases the levels of sulfate, Cys, and GSH in plant tissues (Lappartient and Touraine 1996; Lappartient et al. 1999; Saito 2004; Nocito et al. 2007). Recently, Gupta et al. (2010) reported the role of GSH in lead (Pb) detoxification in *Sedum alfredii*, although this was accomplished without any induction of PC; this suggests that GSH may play an important role in detoxifying Pb, under stress conditions where PCs are absent and also reported that chelated Pb, in conjunction with PCs synthesis and complexation, reduces stress in Pb-tolerant plants (Gupta et al. 2013).

10 Concluding Remarks

In recent years, intensive research on plants, stressing on different biotic and abiotic factors, have led to the support of phytoremediation of contaminated soils. The understanding of the processes of different heavy-metal detoxification mechanisms will help to select plant species capable of hyper-accumulation of metal(s). Further, the selected genetic maneuvering of those plants may also enhance their usefulness for this purpose. Stress proteins play a major role in plants for sequestration of essential or non-essential heavy metals. The isolation and identification of phytochelatins and its biosynthetic pathway from a number of plants species allows the understanding of the mechanism of metal chelation and partitioning within the plant body. Further development of techniques, such as miniaturization and quantitation of specific complexes in various plant tissues, will enhance progress toward evaluating the actual functional import of the γ-Glu-Cys peptides in cellular metal sequestration. Further elucidation of the genes involved in differential metal tolerance is important that may help additionally for amelioration of heavy metal impacts on food production and bioremediation of contaminated soils.

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