Chapter 13
Genetic Control of Metal Sequestration in Hyper-Accumulator Plants

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Abstract Heavy metal contamination is an emergent environmental dilemma all over the world, posing serious threat to environment as well as human being by disturbing the ecological balance. There are a number of physical, chemical, and biological techniques applicable worldwide for wastewater treatment, but the phytoremediation techniques are the green, sustainable, and promising solutions to problem of environmental contamination. Studies revealed that there are certain hyper-accumulator genes present in plants, which make them more metal tolerant than non-hyper-accumulator plants species where those genes are absent. In addition, hyper-accumulator plants tackle with heavy metals by activating their responsive genes for chelation, trafficking, and sequestration. Therefore, studying such hyper-accumulator genes opens a gateway for the thorough understanding of phytoremediation techniques.

Keywords Hyper-accumulator • Non-hyper-accumulator • Phytoremediation • Contamination • Tolerant

Abbreviations

Al	Aluminum
BjMT	*Brassica juncea* metallothioneins
Ca	Calcium
CaM	Calmodulin
CBL	Calcineurin B-like protein
CIPK	Calcium-interacting protein kinase
CRKs	Cysteine-rich receptor-like kinases
DHAR	Dehydroascorbate reductase
DNA	Deoxyribonucleic acid
GR	Glutathione reductase

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GSH	Glutathione
H_2O_2	Hydrogen peroxide
K	Kalium (potassium)
MAPK	Mitogen-activated protein kinase
MDHAR	Monodehydroascorbate reductase
MV	Methyl viologen
MTs	Metallothioneins
OSMT	Oryza sativa metallothioneins
PCs	Phytochelatins
RLKs	Receptor-like kinases
ROS	Reactive oxygen species
SOD	Superoxide dismutase
tApx	Tobacco ascorbate peroxidase

13.1 Introduction

Wastewater released from industries makes human lives easier but brings heavy metals menace, which is disturbing the ecological balance. Heavy metals are non-biodegradable chemical species which may accumulate in different plants parts and therefore cause threats to plants and human health [1]. There are a number of techniques developed over times for remediation of heavy metals, but natural treatment systems are more effective compared to a conventional treatment system. Phytoremediation or the use of living plants to remove heavy metals from soils and water bodies and is proposed as a cost-effective and environment-friendly way to clean up the contaminants [2, 3].

In nature, plants are tolerant towards some heavy metals to some extent and assimilate these as essential nutrients. Green plants can be categorized on the basis of plant-metal interaction as hyper-accumulating and non-accumulating plants. On the basis of adaptations against heavy metals exposure, plants are divided into four main categories, metal-tolerant species, metal-resistant species, metal-tolerant non-hyper-accumulator species, metal hyper-tolerant hyper-accumulator plants species [4]. Hyper-accumulators are plant species which are able to uptake, translocate, and accumulate metals in aboveground plant tissues. A hyper-accumulator should have an intensive root uptake system and faster root-to-shoot translocation. Roots uptake metal from the soil and transport them to the stems and into the leaves. As low concentration of trace metals are present in soil so high affinity transport system is used to accumulate metal ions. A number of transporter genes are involved in this process of metal transport [5].

In most of the plants, heavy metals interaction produces oxidative stress in the chloroplast and mitochondrial membranes. This oxidative stress produce of ROS species causes disruption of intercellular and extracellular membranous organelles, ion leakage, lipid peroxidation, and DNA strand cleavage [6–8]. Most of the heavy metals are recalcitrant in nature thus causing serious damage to the environment. These are nonbiodegradable in nature but biologically can be transformed from more toxic to less toxic condition by their transformation of oxidation state and their conversion from more complex to simplest forms [9].

In nature, plants are capable of self-protection by the production of less toxic reactive compounds or by controlling metals transportation, accumulation, and metal binding with cell wall and vacuole [10, 11]. Many plants when expose to toxic concentration of metal ions try to avoid or decrease its uptake into root cells by limiting the metal ions to the apoplast, binding them to the cell wall or cellular exudates, or by reducing their long distance transportation. If this does not happened, then metals already in the cell adopted storage and detoxification strategies, along with metal transportation, chelation, trafficking, and sequestration into the vacuole. When these actions were completed, then plants trigger oxidative stress defense mechanism and synthesis of stress-related proteins and signaling molecules, such as heat-shock proteins, hormones, and reactive oxygen species [12]. This review has attempted a comprehensive description of plants mechanisms against heavy metals avoidance, transportation, accumulation, and detoxification of heavy metals contamination, and exploring the genetically based defense strategies adopted by plants against trace element excess.

13.2 Avoidance Strategy in Plants

13.2.1 Extracellular Defense Strategy of Plants Against Heavy Metals

Plants possess different intrinsic and extrinsic defense strategies for tolerance or detoxification whenever faces the stressful conditions due to the high concentrations of heavy metals. Initially, regarding metal intoxication, plants implement avoidance strategy to prevent the arrival of stress via restricting metal removal from soil or eliminating it, and control metal entry into plant roots [13]. This can be attained by some mechanisms such as restriction of metals by mycorrhizal association, metal sequestration, or complication by releasing organic compound from root [14, 15].

For heavy metals prevention or reduction of its toxicity impacts, plants develop avoidance approach against HMs entrance. Plants adapted two main pathways by taking part in enhancing its complexity in roots vicinity. For the reduction of heavy metal toxicity, plants enhance the pH of rhizosphere which released anions of phosphate. Studies revealed that South American maize variety 3 released phosphate ions without toxicity while sensitive maize variety 5 showed toxicity symptoms under Al stress [16]. Studies revealed that under Cd stress, malate is secreted from sorghum (*Sorghum bicolor* L.) roots, and citrate is secreted from maize roots [17]. Studies accomplished the fact that root exudates in plants rhizosphere decrease the level of toxicity by activating HM-binding proteins which inhibit the HM uptake [18].

Similarly, oxalate released from the root apex facilitates the prevention of Cd from entering into tomato (*Lycopersicon esculentum* L.) roots, thus in the Cd-resistant tomato cultivar (Micro-Tom) these exudates promote Cd resistance. Genetic studies revealed that under Al exposure Al-tolerant higher plants produces more malic acid than sensitive genotypic plants species [19]. Thus, it is concluded that the tolerant plant species may have adopted precipitation as an avoidance mechanism for the prohibition of the HM.

13.3 Signaling Strategy in Plant

13.3.1 Signals Transduction in Plants

In all plants, reaction towards heavy metal stress involves a complex signal transduction system that is trigger by sensing the heavy metal and is characterize by the production of stress-related proteins and signaling molecules, and finally the transcriptional activation of particular metal-responsive genes to neutralize the stress [20].

The most significant signal transduction processes consist of the Cacalmodulin system, hormones, ROS signaling, and the mitogen-activated protein kinase (MAPK) phosphorylation flow, which activates stress-related genes [21]. There are two main types of plants signaling, i.e., extracellular signaling and intracellular signaling.

13.3.2 Signaling Networks

The ROS network is highly dynamic for plants growth, development, and stress states thus producing ROS-signaling response effectively by ROS-scavenging and ROS-producing protein [22]. The production of ROS physiologically occurs as a by-product of biological reactions. During ROS production, P-450 and other cellular elements are released as a by-product [23]. Under chemical toxicity, ROS genes network is being regulated by cytochromes P-450 which slow down the ROS level in plants cells. For instance, the ROS gene network of *Arabidopsis thaliana* contains more than 150 genes for the maintenance of ROS level in plants [24]. Calcium-signaling network regulates the transmission of calcium signals through channels, pumps, and carriers that between cellular, subcellular, and extracellular parts of plants. Ca2+-binding proteins decoded and transmitted the information provided by calcium signaling for transcription by Ca2+-responsive promoter elements that ultimately regulate proteins phosphorylation [25].

13.3.3 Extracellular Signaling

When plants exposed to multiple abiotic stress stimuli, it rapidly activates signaling proteins MAPKs. An extracellular signaling ROS system has been named as "the ROS wave" that covers about 8 cm/min distance. The concept of "ROS wave" is concerned with the perception or signaling of ROS produced in the plants [26]. In many signaling network, the most important thing is the presence of transmembrane proteins that act as receptor-like kinases (RLKs) and recognize signals with their extracellular kinase containing parts then transmit them through the intracellular kinase containing parts. RLKs manage developmental and hormone responses, stomata closing and opening and stress response, and resistance against bacterial and fungal pathogens [27, 28].

About 600 members of RLK gene family has been reported in Arabidopsis. In extracellular parts of plants, the RLK groups like CYSTEINE-RICH RECEPTOR-LIKE KINASES (CRKs) have two preserved cysteine domains (C-2x-C-8x-C; DUF26 domain). Various studies revealed that on the basis of transcriptional and phenotypic analysis of CRK mutants like their extracellular domain structure, phenotype, and genotypic expression, these could be concerned in apoplastic ROS signaling [29–32].

13.3.4 Intercellular Signaling

During intercellular signaling plants, information is transmitted in the form of mobile signals, including transcription factors and membrane-associated proteins. Generally, membrane-associated proteins are significant in transcription, as small RNAs and revealed intercellular movement through mobile peptides [33]. ROS are important mediators of developmental procedures in different organisms like prokaryotes, fungi, plants, and animals through redox-sensitive transcriptional regulator genes expression. In plants, regulation of peroxidase genes is possible by novel ROS-sensitive transcription factor, UPB1 [34]. Various organelles within the cell like chloroplasts, peroxisome, and mitochondria can generate reactive oxygen species under stress situations and donate to plant stress tolerance [35]. In case of calcium intercellular signaling, calcium signatures transformed the cellular levels of calcium [36]. Cell organelles like vacuoles, endoplasmic reticulum, mitochondria, and cell wall are the store houses of Ca2+ from where these are released when it is necessary by the plant cells [37]. Similarly, cell organelles surrounded by double membrane (e.g., mitochondria, chloroplasts, and nuclei) can generate Ca^{2+} signals whenever posed to stress conditions [38].

13.3.5 Calcium Signaling in Plants

When plants come across a number of physiological stimuli or stress, like low temperature, drought, salinity stress, and pathogen or herbivorous attacks, then free calcium ions of cytoplasm enormously activated [39]. The Ca^{2+} are later on transcripted by intercellular reactions, mainly by Ca^{2+} sensor proteins which have been preserved in all eukaryotic organisms, so that by activating complex downstream signals in reaction of developmental and environmental stimuli. The physical changes of Ca^{2+} binding is measured by structural changes of sensor proteins in Ca^{2+} relying proteins [40, 41]. In response to abiotic stress, calcium signaling is produced with the regulation of cell cycle. The equilibrium of Ca^{2+} ions depends on the Ca^{2+} deficiency, Ca^{2+} transporters, efflux pumps, Ca^{2+}/H^+ antiporters, Ca^{2+} signatures, Ca^{2+} memory, Ca^{2+} sensor, and transducer proteins [42] (Fig. 13.1).

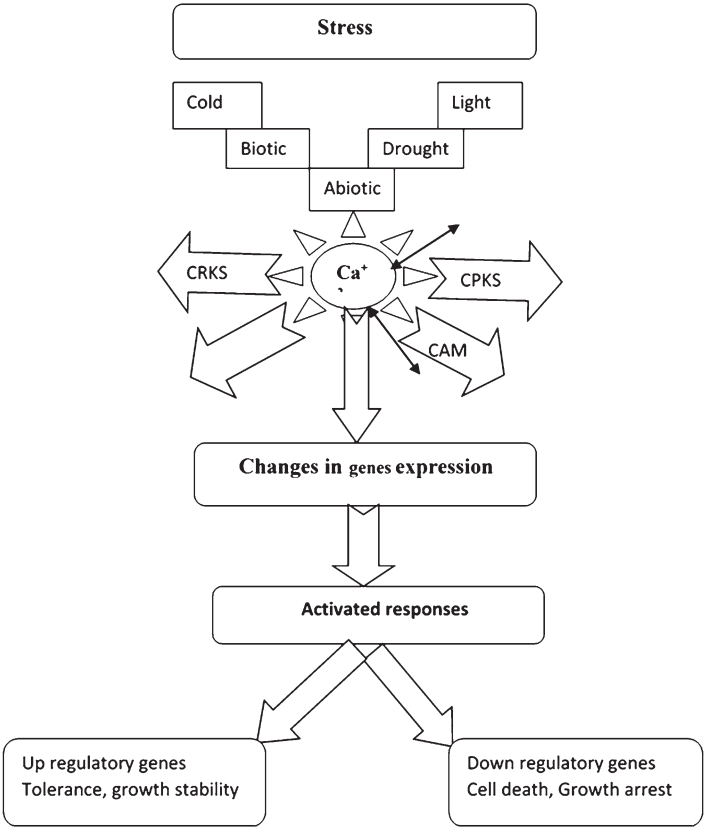

Fig. 13.1 Representation of Ca2+ signals under diverse abiotic stresses regulated by CAM, CRKS, CPKS, and CCaMK for the activation of regulatory genes

13.3.6 Genes Involved in Calcium Signaling

Calcium and protein kinases significantly take part in signaling pathways against environmental stress in plants. The first calcium-dependent, calmodulin-independent protein kinase activities were reported in pea (*Pisum sativum*) extracts 20 years ago [43]. Various studies have shown that Ca-regulated proteins and kinases [44]. Such as CaM protein [45] neurin B-like 218 (CBL) 8 proteins, CDPK genes [46], and CBL-interacting protein kinase (CIPK) [47] are associated to abiotic stress response in plants.

By the activation of protein kinesis, calcium sensors or Ca^{2+}-binding proteins detect the high concentration of Ca^{2+}. In response to the genes, expression of regulatory proteins produced by protein kinesis can enhance transcription factors by phosphorylation and by changing the metabolism that ultimately results in producing phenotypic responses for enhancing stress tolerance [37]. Because of the rise of calcium level two types of genes, specifically up regulating or down regulating are overexpressed and produces stunt growth or death of plant cells. These consist of known quick stress-responsive genes in addition to genes of nonspecific function [48]. Recent studies show that calcium signaling plays a significant role in some pathways, for example, in *Arabidopsis*, Ca^{2+}-signaling pathway can also control a K^+ channel for low-K response in the presence of a blue light receptor phototropins, which successfully promotes growth and plant development [49]. Calcium signaling is correlated with the sucrose-signaling pathway that is an essential source of fructan synthesis [50]. Under abiotic stress, calcium signaling controls the cell cycle progression.

13.3.7 ROS Signaling in Plants

Plants can sense, transduce, and translate ROS signal into suitable cellular response with the assistance of redox-sensitive proteins, calcium mobilization, protein phosphorylation, and gene expression. ROS can be sense directly by some important signaling proteins such as a tyrosine phosphatase through oxidation of conserved cysteine residues [51]. ROS can also regulate many enzymes in signaling, such as protein phosphatases, protein kinases, and transcription factors [52], and transmit to other signal molecules and the ways forming part of the signaling network that regulate the response downstream of ROS [53]. Usually, the power, lifetime, and size of the ROS-signaling pool rely on balance between oxidant production and removal by the antioxidant. By using mutants that lack in key ROS-scavenging enzymes, Miller and coworkers determined a signaling pathway that is operated in cells in response to ROS accumulation [54]. In tomato leaves, ROS produced in cell walls of vascular bundle cells, as a result of wounding and produce H_2O_2 from wound-inducible polygalacturonase which is acted as a second messenger for the activation of defense genes in mesophyll cells, but not for signaling pathway genes in vascular bundle cells [55]. Tracing or detoxification of the unnecessary ROS is achieved by well-organized antioxidative system that contains the nonenzymatic as well as enzymatic antioxidants [56].

13.3.8 Genes Involved in ROS Signaling

ROS are a sort of free radicals, reactive molecules, and ions that are obtained from O_2. It has been evaluated that about 1% of O_2 used by plants is transformed to ROS [57]. The most frequently existing ROS are O_2, $O_2^{\bullet -}$, H_2O_2, and $^{\bullet}OH$. As O_2 is a completely nontoxic molecule as in its ground state it has two unpaired electrons with equidistant spin which form it paramagnetic and, therefore not likely to take part in reactions with organic molecules unless it is activated [58]. Naturally, in living organisms the production of ROS is responsible for the intracellular communication system that regulated the response to environmental stresses [59]. When the plants are under salinity stress, the target of the ROS is regulated by vesicle trafficking complexes [60].

At low concentration, ROS have been concerned as second messengers in intracellular signaling cascades that mediate several plant responses in plant cells with closing of stomata [53, 61, 62], automatic cell death [63, 64], gravitropism [65], and achievement of tolerance to both biotic and abiotic stresses [54, 66] (Fig. 13.2).

13.3.9 Genetic Control of ROS Production

Studies revealed that under abiotic conditions signaling is produced due to oxidative stress, which result in activation of defense genes giving out specific adaptive responses [67]. The system involved in up regulation of mRNA due to ROS production can occur by redox-sensitive second messenger systems (e.g., MAP kinase activation) [68]. Plants genetics analysis showed that ROS signaling in Arabidopsis plants enhances the antioxidative defense by rising the antioxidative genes expression and activation of the genes of inducible stress proteins [69]. The specific effect of ROS-mediated signaling is related with the confirmation of definite genes expression. Some specific promoters and transcription factors have been recognized as a producer of oxidative stress-responsive elements [70]. Under chilling and salt stress, overexpression of a cytosolic APX-gene taken from pea (*Pisum sativum* L.) in transgenic tomato plants (*Lycopersicon esculentum* L.) improves the oxidative injury [71]. Likewise, tolerance against oxidative stress can also be improved by overexpression of the tApx genes in tobacco or in Arabidopsis [72]. Several studies revealed those in plants, under environmental stresses MDHAR show overexpression [73]. Gene expression analysis of wheat showed two varieties of tolerant wheat bHLHs (bHLH2: CA599618 and bHLH3: CJ685625) that have been affected by salinity [74]. Another gene family WRKY plays effective regulatory role in plants under biotic

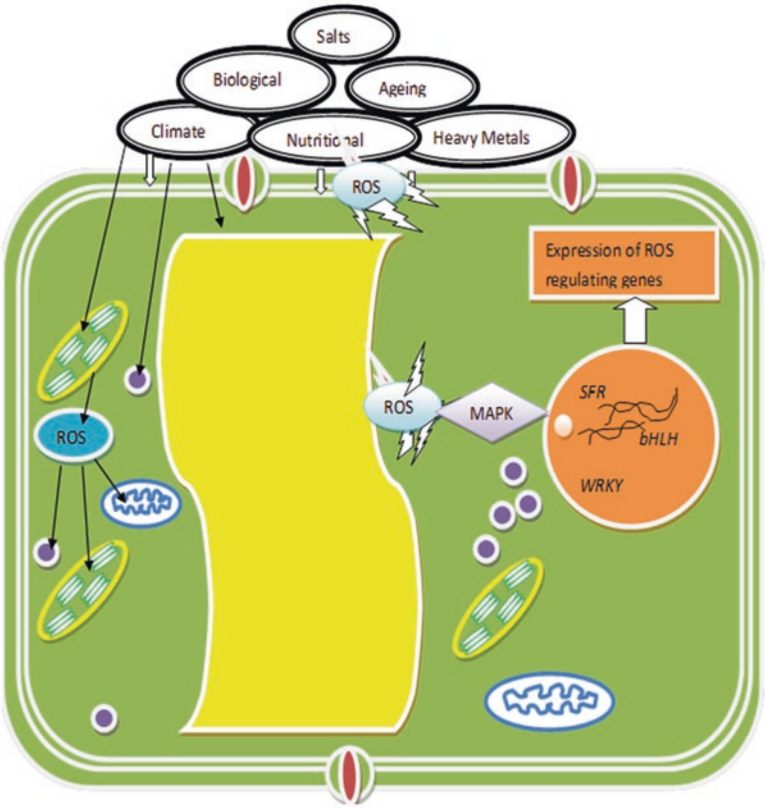

Fig. 13.2 Representation of the transduction pathways involved in ROS signaling in response to abiotic stresses. External stimuli produce ROS in chloroplast, mitochondria, and peroxisomes; these activated MAPK cascade. These mitogen-activated protein kinesis that regulate transcription factor and synthesis of genes (SFR, WRKY, bHLH, etc.) to overcome the negative effects of stress

and abiotic stress conditions like cold, drought, wounding, salinity, UV, H_2O_2, salicylic acid (SA), viral and bacterial attack [75, 76].

Overexpression of Arabidopsis MDHAR gene in tobacco enhanced salt tolerance and mitigated polyethylene glycol stress [77]. Tomato chloroplastic MDHAR overexpressed in transgenic Arabidopsis improved its tolerance to temperature and methyl viologen-related oxidative stresses [78]. Likewise, regulation of the gene-encoding cytosolic DHAR was observed in *L. japonicas*, which was proved to be more tolerant to salt stress than other legumes. This increase of DHAR was associated with its action in AsA recovery in the apoplast [79]. Transgenic potato overexpressing Arabidopsis cytosolic AtDHAR1 proved higher tolerance to herbicide, drought, and salt stresses [80].

Kwon et al. [81] verified that simultaneous expression of Cu/Zn-SOD and APX genes in tobacco chloroplasts increased tolerance to methyl viologen (MV) stress relatively to expression of either of these genes alone. Similarly, improved tolerance to multiple environmental stresses has been produced by instantaneous overexpression of the genes of SOD and APX in the chloroplasts [82–84] SOD and CAT in cytosol [85] and SOD and GR in cytosol [86]. Moreover, the instantaneous expression of numerous antioxidant enzymes, such as Cu/Zn-SOD, APX, and DHAR, in chloroplasts has shown to be more efficient than single or double expression for developing transgenic plants with improved tolerance to various environmental stresses [87]. So, in order to attain tolerance to multiple environmental stresses, increased importance is now given to produce transgenic plants overexpressing multiple antioxidants.

13.4 Detoxification Strategy in Plants

13.4.1 Detoxification Mechanism in Plants

Main detoxification mechanisms in plants are

1. Transportation to storage parts
2. Chelate formation
3. Compartmentalization in subcellular parts
4. Removal from the plant body [88].

13.4.2 Metal Transporters

Transport system of metal ions is very complex and miscellaneous. Metal transport system is involved in uptake of metal, its translocation to various plant organs and metal liberation in subcellular parts together with metal storage in vacuoles [89]. For long distance or intercellular transport in plants and subcellular compartmentalization of metals, low-molecular-weight chelators, such as glutathione, phytochelatins, histidine, or citrate, play a crucial role. By selective metal chelation and trafficking or by internal transporter selectivity, there is a requirement to make a distinction between metal cations of different elements.

A wide variety of transport proteins occurs that belongs to different families including

- The zinc-regulated transporter, iron-regulated transporter protein (ZIP) family
- The cation diffusion facilitator (CDF) family
- The P1B-type subfamily of P-type ATPases
- The natural resistance-associated macrophage protein (NRAMP) family
- The yellow-stripe 1-like (YSL) subfamily of the oligopeptide transporter (OPT) superfamily
- The copper transporter (COPT) family
- The Ca2+-sensitive cross complementer 1 (CCC1) family
- The iron-regulated protein (IREG) family

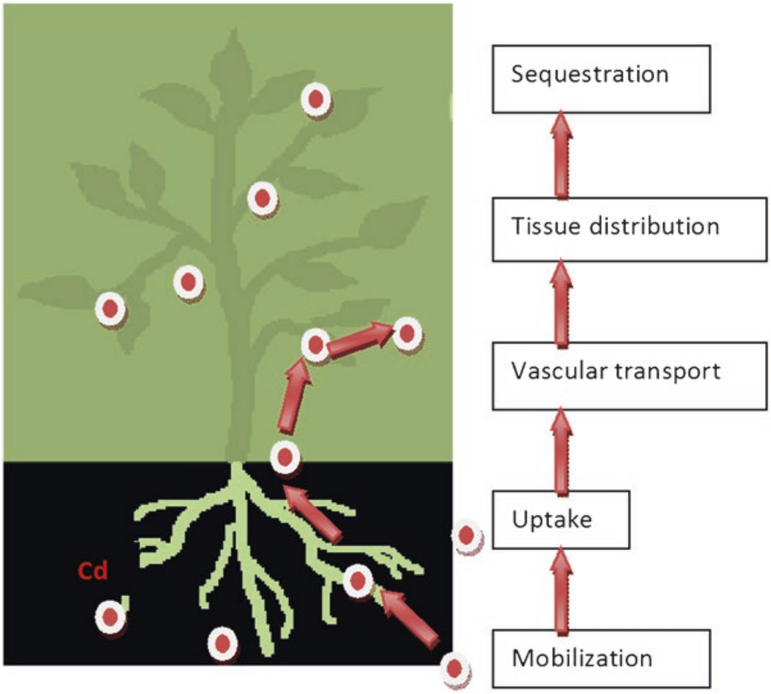

Fig. 13.3 Mechanisms involved in heavy metal transport

Other membrane protein families that have been found to involve in transition metal transport are the cation exchanger (CAX) family and three subfamilies of ATP-binding cassette (ABC) transporters, the multidrug resistance-associated proteins (MRP), the ABC transporters of the mitochondria (ATM), and the pleiotropic drug resistance (PDR) transporters [90, 91]. A metal transporter is involved in metal detoxification and metal hyper-accumulation [89].

Metal transporters are involved in:

1. Uptake from the soil to root
2. Translocation from the root to the shoot
3. Detoxification by storage in the vacuoles (Fig. 13.3)

13.4.3 Uptake from the Soil to Root

Transporter genes involved in cellular uptake of metals from soil have been identified by researchers**.** A number of ZIP transporters are found to be involved in Zn uptake across plasma membrane [89]**.** Fifteen potential ZIP genes may be identified in the *Arabidopsis thaliana* genome [92].

Transport properties of plant metal transporters that mediate metal entry into the cytoplasm, for example, the Zn transporter AtZIP1 have been analyzed upon heterologous expression in yeast by measuring metal uptake into yeast cells [93]. IRT1 is one of the most important members of ZIP family, involved in iron uptake from the soil [93]. Puig et al. [94] found that AtZIP2 and AtZIP4 are involved in cellular accumulation of zinc and copper. OsIRT1 and OsIRT2 have been proposed to contribute in Cd uptake [95]. Milner et al. [96] found that expression of *AtZIP1* is localized to the root stele and is a vacuolar transporter while AtZIP1 expression was also found in the leaf vasculature and is localized to the plasma membrane. Functional studies with *Arabidopsis AtZIP1* and *AtZIP2* T-DNA knockout lines suggest that both transporters play a role in Mn (and possibly Zn) translocation from the root to the shoot. AtZIP1 may play a role in remobilizing Mn from the vacuole to the cytoplasm in root stellar cells and may contribute to radial movement to the xylem parenchyma. AtZIP2, on the other hand, may mediate Mn (and possibly Zn) uptake into root stellar cells, and thus also may contribute to Mn/Zn movement in the stele to the xylem parenchyma, for subsequent xylem loading and transport to the shoot [96].

13.4.4 Translocation from the Root to the Shoot

In phytoextraction, transport of metals from root to shoot is of utmost important. The study of Zn and Cd hyper-accumulators provides the evidence of involving P-ATPase also called HMA (Heavy Metal transporting ATPase) as an important factor in their transport from the cystol of root cells into vascular tissues [97]. The HMAs divide into two groups: those transporting monovalent cations (Cu, Ag) and those transporting divalent cations (Pb, Cd) group [98]. Eight HMAs has been identified in Arabidopsis and *Oryza sativa* [99]. Analysis of the complete genomic sequence in Arabidopsis shows the division of these eight HMAs in two groups: HMA1–4 for the transport of Zn/Co/Cd/Pb and HMA5–8 for the transport of Cu/Ag [100], while HMA2 and HMA4, are involved, in the transport of Zn and Cd. HMA4 confers increased Cd tolerance when expressed in yeast [99].

Transport of cadmium from root to shoot is a control process and most of the Cd is stored in roots. To increase root-to-shoot translocation of Cd, transformation with the genes of high biomass responsible for high root-to-shoot translocation such as HMA4 can be done. In *A. thaliana,* HMA2 and HMA4 genes are involved in the transport of Zn and also nonessential Cd to the shoots [101, 102]. Studies show that both proteins encoding for Cd loading in xylem and are plasma membrane contained proteins [101]. The P1B-type ATPases (also known as HMAs) have a major role in translocation of metal ions against their electrochemical gradient by using ATP as energy. All living organisms including humans, yeast, and plants contain HMAs [100]. In Arabidopsis, HMA4 is found to be more expressed in vascular tissues of root, stem, and leaves. It has been characterized and its role in Cd detoxification has been confirmed in Arabidopsis [100]. HMA4 plays a role in xylem loading of Zn and Cd, and hence in the control of translocation to shoots in *Arabidopsis halleri* and *Thlaspi caerulescens*. *A. halleri* plants (from a Cd-hyper-tolerant accession) with a lowered expression of HMA4 translocated less Zn from the root to the shoot and were more sensitive to Cd and Zn treatments [103].

Work of Courbot et al. [100] leads to conclusion that elevated expression of HMA4 P_{1B}-type ATPase is an efficient mechanism for improving Cd/Zn tolerance in plants under conditions of Cd/Zn excess by maintaining low cellular Cd^{2+} and Zn^{2+} concentrations in the cytoplasm. HMA2 and HMA4 are the only P1B ATPases identified so far, which are predicted to have a long C-terminal domain. It is interesting that the CC dipeptides and the His-rich domains are found in the prolonged C termini of HMA2 and HMA4 and not in the N-terminal domain, where HMA domains are always found. The N-terminal end of HMA1 also harbors a poly-His domain [104] (Table 13.1).

13.4.5 Detoxification by Storage in the Vacuoles

In the hyper-accumulation of Zn, Ni, and Cd, an enhanced capacity of metal storage in leaf vacuoles seems to play an important role [98]. ABC transporter is involved in many physiological processes. Several members of ABC transporter are involved in vacuolar sequestration of metals. Hmt1 is found to be involved in transport of PC-Cd complexes in the vacuoles of *S. pombe* [89]. Some members of the MATE family were shown to function as cation antiporters that remove toxic compounds from the cytosol by exporting them out of the cell or sequestering them to vacuole [105].

Members of CDF family involved in the cytoplasmic efflux of metal cations from cytoplasm to organelles like Zn^{2+}, Cd^{2+}, and have been named MTP (metal tolerance protein). CDFs are highly expressed in *A. halleri* and *T. caerulescens*: MTP1, MTP, and MTP11. AtMTP1 suggested being involved in Zn tolerance and basal Zn accumulation in leaves. MTP11 and especially MTP8 are close homologues of ShMTP8 that give Mn tolerance when expressed in yeast and when ectopically overexpressed in *A. thaliana* [93] (Fig. 13.4).

Mechanism of metal transport in plant cell. Heavy metals enter into cytosol through metal transporter from cytosol into vacuole via metal transporters.

HM—High Metal
LM—Low Metal
ZIP—Zinc-regulated transporter, iron-regulated transporter protein
NRAMP—Natural resistance-associated macrophage protein
CAX—Cation exchanger
ABC—ATP-binding cassette
MT—Metallothioneins
PC—Phytochelatins

Table 13.1 Gene expression of various abiotic stress conditions in plants

Type of stress	Genes involved	Plant species	References
Heat	AtCaM3	*A. thaliana*	Xuan et al. [145]
Heat	AtCaM7	*A. thaliana*	Lu et al. [146]
Heat	OsCAM1-1	*O. sativa*	Wu et al. [147]
Salt	GmCaM4/5	*Glycine max*	Park et al. [148]
Heat	TaCaM1-2	*Triticum aestivum*	Liu et al. [149]
Salt	AtCML8	*A. thaliana*	Park et al. [150]
ABA, droughts, salt	AtCML9	*A. thaliana*	Magnan et al. [151]
Salt	AtCML18/CaM15	*A. thaliana*	Yamaguchi et al. [152]
Heat, cold, ABA	AtCML24/TCH2	*A. thaliana*	Delk et al. [153]
ABA, salt	AtCML37/38/39	*A. thaliana*	Vanderbeld and Snedden [154]
ABA, drought	AtCML42	*A. thaliana*	Vadassery et al. [155]
Cold, heat, drought, Salt.ABA	OsMSR2	*O. sativa*	Xu et al. [156]
Heat	AtPP7	*A. thaliana*	Liu et al. [157]
Heat	AtCBK3/CRK1	*A. thaliana*	Liu et al. [158]
Cold, heat, Salt.ABA, H_2O_2	AtCRCK1	*A. thaliana*	Yang et al. [159]
Cold	AtCRLK1	*A. thaliana*	Yang et al. [160, 161]
ABA, H_2O_2, ROS, dehydration	OsCCaMK/DMI3	*O. sativa*	Shi et al. [162]
ABA, ROS	ZmCCaMK	*Z. mays*	Ma et al. [163]
ABA, salt	TaCCaMK	*Pisumsativum*	Pandey et al. [164]
Salinity	DHAR	*Oryza sativa*	Chen and Gallie [165]
Drought, ozone	DHAR	*A. thaliana*	Ushimaru et al. [166]
Cu, Zn	AtZIP2, AtZIP4	*A. thaliana*	Puig et al. [94]
Cd	OsIRT1, OsIRT2	*O. sativa*	Clemens et al. [95]
Mn	ShMTP	*A. thaliana*	Delhaize et al. [167]
Cu/Ag	HMA5–8	*A. halleri*	Courbot et al. [100]
Zn/Co/Cd/Pb	HMA1–4	*A. halleri*	Courbot et al. [100]

13.4.6 Metal-Binding Genes

Plants have been authentically proved to minimize the harmful effects of metal toxicity, by pathways or methods relating to the binding of heavy metals to cell wall and its transporation [106, 107]. Generally, the synthesis of metal-binding peptides based on the production of metallothioneins and phytochelatins [108]. Usually, chelation is the most common intracellular system for the maintenance of low concentrations and detoxification of free metals in plant cytoplasm that can be achieved by thiol compounds (which contain sulfhydryl/thiol groups; such as a tripeptide glutathione, GSH, γ-Glu-Cys-Gly; phytochelatins, PCs; metallothioneins, MTs), and

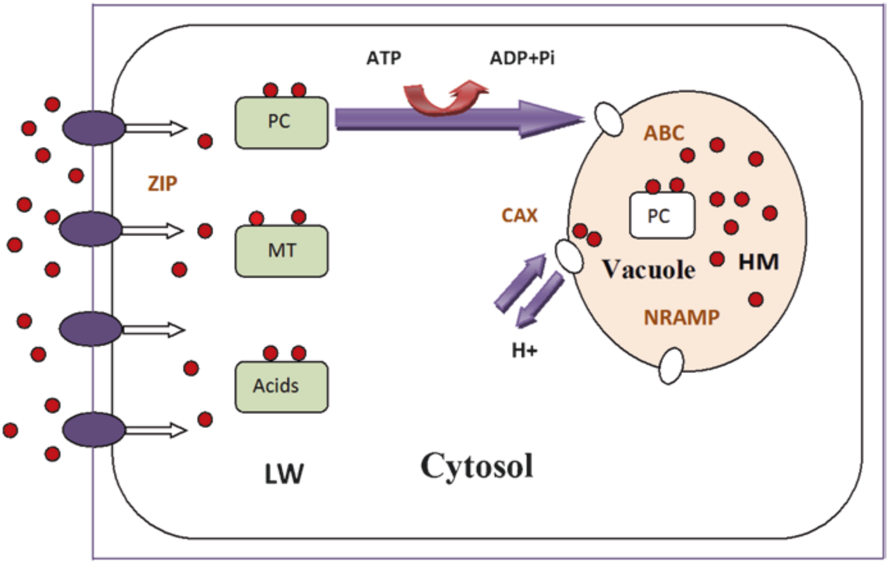

Fig. 13.4 Mechanism of metal transport in plant cell. Heavy metals enter into cytosol through metal transporter from cytosol into vacuole via metal transporters

also by non-thiol compounds (such as organic acids and amino acids) [109–118]. Studies revealed that peptides that have either histidines (GHHPHG) 2 (HP) or cysteines (GCGCPCGCG) (CP) be engineered to Lam B and expressed on the surface of *E. coli*. Surface demonstrated that CP and HP enhanced the bioaccumulation fourfold and twofold [119].

13.4.7 Phytochelatins

Phytochelatins are a family of cysteine-rich, thiol-reactive peptides that attach many toxic metals and metalloids, producing good messengers for genetically better phytoremediation system [120]. The general structure of PCs is (g-Glu-Cys) n-Gly, where n differs from 2 to 11 [121]. Practical descriptions of an unusual phytochelatin synthase, LjPCS3, of *Lotus japonicus*, have been acknowledged in an extensive variety of plant species and some microorganisms [122].

Phytochelatins plays an important role in biosynthesis and detoxification of heavy metals [122]. After production, PCs combine with heavy metal ions and make possible their transportation as complex into the vacuole, where they finally produce complexes of high molecular mass, which is the key method that utilizes to bind heavy metal ions in both plants and yeasts [122]. Genes concerned in the production of PCs are phytochelatin synthases, such as g-glutamyl cysteine trans peptidase [121].

Overexpression of phytochelatin synthase in *Arabidopsis thaliana* seedlings causes tolerance of arsenic, but hypersensitivity to cadmium and zinc [123]. The same fact was observed in other transgenic plants with diverse PCS genes show diverse phenotypes, including heavy metal tolerance (by or lacking accumulation) and hypersensitivity to heavy metal ions [124]. Overexpression of pytochelatin synthase in tobacco: distinctive effects of AtPCS1 and CePCS genes on plant response to cadmium [125]. Phytochelatin synthase of *Thlaspi caerulescens* increases tolerance and accretion of heavy metals when expressed in yeast and tobacco. PCs are a group of cysteine-rich, thiol-reactive peptides that combine many toxic metals and metalloids, thus producing best messenger for genetically improved phytoremediation pathways [120].

13.4.8 Metallothioneins (MTs)

Apart from PCs that are the product of enzymatically formulated peptides, MTs are formulated resultantly by mRNA translation [126]. While PCs in plants may primarily deal with Cd detoxification, MTs appear to elaborate the attraction with a larger series of metals such as Cu, Zn, Cd, and As [127]. MTs demonstrated unusual properties and performance that depends on their presence in a type of plants and are extremely mottled in terms of their molecular characteristics and structural qualities [128]; they probably contain a number of various activities in plants than a few other living creatures. In plants, these ligands are concerned to negate the toxicity of HMs by cellular sequestration, homeostasis of intracellular metal ions, and metal transport modifications [129–131].

Additionally, MTs play an important role in HM detoxification, actively involved in cellular-related events including ROS scavenger [132], maintaining of the redox level [133], repair of plasma membrane [134], cell proliferation, and its growth and repair of damaged DNA [135]. There are numerous endogenous and exogenous agents other than HMs that are able to bring the synthesis and expression of MTs. Of these, osmotic stress, drought, intense temperatures, nutrient deficiency, release of different hormones, natural and dark-induced tissue decay, injuries, and viral infections can be mentioned [12, 127, 136].

Ectopically expressed MTs in transgenic plants are proved to increase their tolerance towards metal intoxication. Kumar et al. [137] showed that OSMT1e-p, a type 1 MT extracted from a salt-tolerant rice genotype (*Oryza sativa* L. cv. Pokkali), participated in tolerance for copper and zinc toxicity when ectopically expressed in transgenic tobacco. They evaluated that tobacco plants in which gene have been inserted possessed to hold more quantity of Cu^{2+} and Zn^{2+} in their roots or lower leaves, considerably decreasing the HMs ions transportation and quantity in leaves and harvestable plant parts. Zhigang et al. [138] accomplished that the ectopic expression of BjMT2, a metallothionein type 2 from *Brassica juncea*, in *Arabidopsis thaliana* enhanced copper and cadmium tolerance at the seedling phase but intensely decreased root growth when there was no heavy metal treatment. This tendency may propose that ectopic expression of MTs in transgenic plants may proceed in host plant in a nonspecific method and in a different way effect the organ growth.

13.5 Conclusion and Future Prospects

The present review outlines the impact of abiotic stresses on plants. Most of the investigations done so far mainly described the genetic investigation of plants against abiotic stress; this review involved in genetically based defense and detoxification pathways mainly Ca and ROS signaling, transportation, chelation, and detoxification has been discussed in detail. Under stress conditions, plants activate specific molecules which enhance plants tolerance and the development of defense mechanisms in it. It has been observed that the activation of defense genes cascade transmit various signals in cell organelles under various biotic stress conditions [40].

As abiotic condition produces oxidation stresses that overexpressed a number of stress-induced proteins, this review could provide fundamental information about antioxidant and regulatory genes production. As ROS have regulatory function as signaling molecules, this feature may open a gateway to physiological, molecular, and evolutionary research perspectives. Due to the importance of ROS, it is central to modern plant biology to obtain a comprehensive understanding of the processes where ROS have regulatory roles. Studies revealed that ROS signaling with ozone as a tool is significant for the transmission of distinct from of ROS signals to chromatin reformation and transcriptional regulation [140].

Therefore, the elaboration of transcriptomics and proteomics analysis will be more helpful in understanding the bioinformatics and mutant studies. It has been observed that ROS signals play an important role in intercellular Ca2+ signals, Ca2+ influx which can be regulated by various PAs and Spm4+ proteins [141]. There is need to highlight the mechanism and interrelation of Ca2+-efflux systems with ROS and AtMPK6-signaling under biotic and abiotic stresses along with the overexpression of regulatory genes against stress. Xing et al. [142] observed various proteins kinesis like MKK2-MPK4/MPK6 and MEKK1 activation in salt, cold, drought, and wounding stress can phosphorylate MPK4 which is also significant in abiotic stress signaling. Studies also revealed that ABA is a key hormone in inducing abiotic stress responses in plants like barley showed pronounced effect of ROS and PAs in salt-sensitive variety then salt-tolerant plants [143]. Likewise, ABA is an important hormone under biotic and abiotic stress in plants [144]. But the links between ABA and MAPKs under biotic and abiotic stresses has not yet been properly studied at cellular and subcellular levels in plants. Similarly, little work has been done on K+/Na+ -signaling pathways under biotic and abiotic stresses like Ca^{2+} homeostasis. Therefore, plants responses towards multiple abiotic stresses would be another interesting area of future transgenic hyper-accumulator plants production.

In future, it is needed to identify molecular interaction of chelators with metal transporters. Further investigation is needed to determine more about functional significance and biological role of transporter genes, especially, overexpression in plants with greater biomass to increase their potential use in phytoremediation processes. Furthermore, these genes can provide better understanding in the analysis of gene regulation in metal-rich environment as well as metal-deficient environment. Similarly, silencing of transporter genes in edible crops may decrease metal bioaccumulation in food chain. Likewise, there is a greater area of exploration in terms of 3D structures of protein and functional analysis of the candidate genes. In future, gene cloning and plant transformation can be done to determine efficiency of metal transporter genes in transgenic plants. Thus, the application of powerful genetic and molecular techniques may surely be helpful in designing of hyper-accumulator transgenic plants for bioremediation.

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