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MOLECULAR ASPECTS OF HEAVY METAL STRESS- AN OVERVIEW

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ABSTRACT

Anthropogenic activities like contemporary agricultural methods, and the effects of industrialization on our environment are long-term and harmful. As a result of all of these variables, heavy metal levels in soil, water and air rise. Metals have the potential to be hazardous to plants only when, they are found in bioavailable forms in large quantities. Cadmium, zinc, mercury, lead, chromium, copper, cobalt, nickel, manganese, arsenic and iron are among the elements. Hence, plants have also evolved some of the detoxifying mechanisms to mitigate the harmful effects of heavy metal exposure, its build-up and the studies regarding these have been covered in this overview.

KEY WORDS : **Heavy metals, Heavy metals stress, Molecular aspects of heavy metal stress**

INTRODUCTION

Anthropogenic interferences evidenced in an ample range of global phenomena for instance rapid industrialization (Shahid *et al.,* 2015), heavy mining activities, and intensive agricultural production have not only impacted negatively on natural available resources, but have also created huge contamination of environment and heavy metals build up in the environment is one of the consequences of it and is of major concern at present scenario. A heavy metal is a metal that has a density more than 5 g $cm^{-3}$and in general are hazardous to plant, but in small concentration they could be beneficial micronutrients in small (Duffus, 2002, Salla *et al.,* 2011; Shahid *et al.,* 2015). Heavy metal buildup hinders plant growthindirectly by interfering with numerous molecularand physiological functions (Panuccio *et al*., 2009; Hassan *et al*., 2017). Heavy metal damage is caused by a variety of mechanisms, including reactive oxygen species (ROS)-induced oxidative stress (Mithofer *et al*., 2004). Plants, just like all the other organisms, have a unique ability to adapt and have several mechanisms to keep a physiological balance of essential metal ions while minimising heavy metal exposure which is not required by them (Shahid *et al.,* 2015). These systems have been widely activesince they are necessary for metal balance in general, and they also help to attenuate the impacts of excessive heavy metal concentrations by detoxifying them in plants, allowing plants to survive from the effects of heavy metals.

Signalling Induced by Heavy Metals

Proteins related with stress and signalling moleculesare produced due to stress produced by heavy metals, followed by several metal-responsive genes which are transcriptionally activated to combat the stress. A multitude of kinases and

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phosphatases transmit stress signals, which results in creation of many transcription and metal-detoxifying peptides (Islam *et al*., 2015; Kumar and Trivedi, 2016).

The Ca-calmodulin approach, ROS signalling, hormones, and MAPK phosphorylation cascade are all relevant signal transduction pathway which converge by inducing the expression of genes associated with stress (Conde *et al.,* 2011; Lin and Aarts, 2012; Dubey *et al.,* 2014; Steinhorst and Kudla, 2014; Tiwari *et al.,* 2017a). Distinct heavy metals may activate different signalling pathways(DalCorso *et al*. 2010).

The Ca-Calmodulin approach

Heavy metals in excess alter the integrity of Ca channel, this leads the calcium flux into the cell to increase. Intracellular calcium and calmodulin interact to spread the signal and, in turn, affect genes that are downstream associated with the heavy metal transportation, biosynthesis, and tolerance (Yang and Poovaiah, 2003).

Plants that have been exposed to Cd have increased levels of calcium in their cells, which trigger adaptive mechanisms that reduce the heavy metal's harmful effects. Heavy metal toxicity likePb and Ni, is mediated by the Ca-calmodulin system.

The Role of Reactive Oxygen Species

The development of reactive oxygen species (ROS), which can cause broad damage as well as serve as signalling molecules, is among the most damaging consequences of heavy metal absorption. Heavy metals like Cd can create ROS both directly and indirectly by blocking antioxidant enzymes by the Fenton and Haber–Weiss reactions (Schutzendubel and Polle, 2002; Romero-Puertas *et al*. 2007).

Heavy metals and other pressures elicit some reactions, H_2O_2 in particular, functions as a signal molecule (Dat *et al*. 2000) and H_2O_2 level rises, which happens in response to,

1. Treatment of *Arabidosis thaliana* with Cu and Cd
2. Hg exposure in tomato
3. Toxicity of Mn in Barley (Cho and Park, 2000).

This surge in H_2O_2 levels, build-up the cell's redox level, causing antioxidant synthesis and activation.

Hormonal activities for heavy metal stress

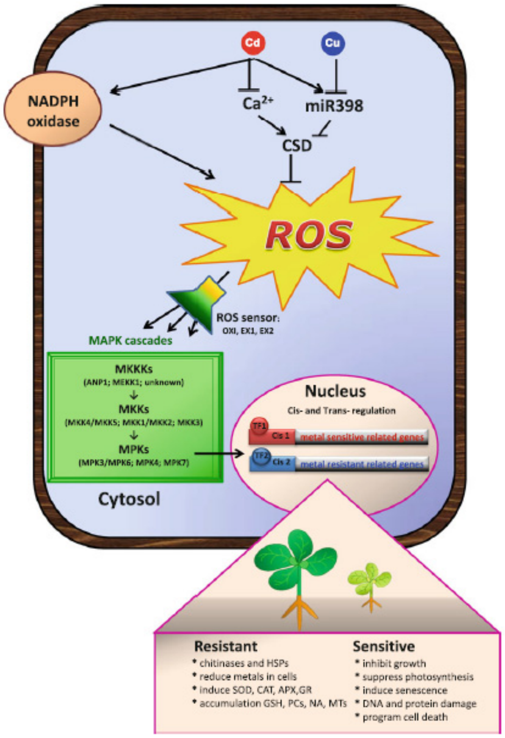
Abiotic stress adaptability is aided by plant hormones, as evidenced by the control productionof hormone under the effect of these heavy metals.Cu and Cd stimulate the production of ethylene by upgrading the ACC synthase expression and activity.

Cu and Cd have also been demonstrated to cause the rapid build-up of jasmonic acid (JA) in *Phaseolus coccineus* (Tiwari *et al*., 2016, 2017b), and in rice and *Arabidopsis thaliana*, Cu has been demonstrated to have a similar impact. (Tiwari *et al*., 2016, 2017b) (Maksymiec *et al*., 2005). The presence of Cd causes increase in Salicylic acid (SA) levels in barley roots, which shows the involvement of Salicylic acid in heavy metal response to stress.

The MAPK Cascade

The MAPK cascade consisted of three types of phosphorylation-activated kinases: MAPK kinase (MAPKK),MAPK kinase(MAPKKK), and MAPK. MAPKs phosphorylate several substrates in various transcription factors are also included, cellular compartments in the nucleus, towards conclusion of this phosphorylation cascade (Tiwari *et al.,* 2017a; Lin and Aarts, 2012).

All of these signalling pathways are frequently associated with the modification of transcriptional regulators which trigger genes linked to stress tolerance, although in the case of heavy metals includes genes associated in metal transporter activation and the synthesis of chelating chemicals.



Mode of action and plant responses of toxic heavy metals

Plants have evolved biochemical pathways that may be involved in detoxification, allowing them to withstand heavy metal stress. Immobilization, restriction of metal absorption and translocation, heavy metal chelation, exclusion from plasma membrane, formationof particular transporters of heavy metal and sequestration by ligands such as metal lothioneins and phytochelatins, introduction of mechanisms that contrast the effects of Reactive Oxygen Species and MG (up-regulation of antioxidant and glyoxalase systems), induction of stress protein, and proline synthesisare among the few developmental mechanisms that tolerant plants have developed.

Restriction of heavy metal transport and uptake

Interception by roots, entrance into it and translocation into the shoot are all part of the heavy metal absorption process by plants which depends on the ions that enter the plantvia symplast or apoplast (Bubb and Lester, 1991).

Heavy metal exposure affects the majority of plant cells *via* different pathways *viz*. heavy metal ion carriers or channels, either specific or generic, which is an energy-dependent process. Excess heavy metals are kept out of the plant as one mechanism. A plant can achieve this in one of two ways: by precipitating metals in the root environment or by forming complexes ions in the root environment (Delhaize *et al*., 1993). Plants can cause the rhizosphere's pH to change or precipitate heavy metals by excreting anions like phosphate.

Exclusion of heavy metals from cells

Heavy metal exclusion from cells is a way of tolerance for heavy metal stress. Plant roots in the apoplastic region contain a sizable amount of metals. The movement of hazardous metal ions from the symplast to the apoplast may be significantly aided by heavy metal transporter proteins (Tice *et al.*, 1992).

Formation of heavy metal complex at the cell wall-plasma membrane interface

Heavy metal complexation and the role of heavy metal transporters in vacuole compartmentalization

A plant employs a range of strategies to combat its damaging effects whenever heavy metal enter into a plant cell. One technique is to transport heavy metals from the cell to outside or sequester them in the vesicles, so removing them from the cell, which hosts complex metabolic processes (DalCorso *et al*., 2008; DalCorso *et al*., 2010). As a result, the central vacuole appears to be an appropriate store house for heavy metals that have accumulated excessively. Most solutes are energised by a vacuolar proton-ATPase (VATPase), two intracellular proton pumps, and a vacuolar proton pyrophosphatase (VPPase). Either channels or transporters can catalyse uptake.

The zinc-regulated transporter (ZRT), which is similar to the iron-regulated transporter (IRT), P-type metal AT Pases, the multidrug resistance-associated proteins (MRP), the protein ZIP family, the natural resistance-associated macrophage protein (NRAMP) family, the cation diffusion facilitator (CDF) family of proteins,ATP-binding cassette (ABC) transporters, copper transporter (COPT) transporters, and ABC transporters of the mitochondria are some well- (ATM).

Phytochelatins in heavy metal complexation

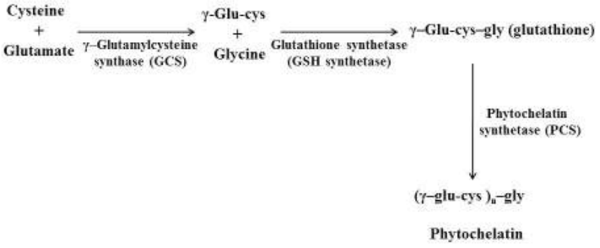
Due to the heavy metals, plants are capable to extract as well as to tolerate the metals in part by employing high affinity ligands that chelate the metals in the cytosol (MTs), such as phytochelatins and metallothioneins.

Table 1. Overexpression of some of the heavy metal transporters

Transporter	Effect of overexpression	Reference
NtCBP4	Enhanced Ni tolerance and reduced Ni accumulation, Reduced Pb tolerance and enhanced Pb accumulation	Arazi *et al.*, 1999
ZnTI	Increased Zn influx in roots	Pence *et al.*, 2000
ZAT	Enhanced Zn resistance and accumulation	van der Zaal *et al.*, 1999
CAX2	Accumulation of Ca, Cd, Mn and Mn tolerance	Hirschi *et al.*, 2000
AtMHX	Reduced tolerance to Mg and Zn, but it did not show altered accumulation of these elements	Shaul *et al.*, 1999
AtNramp1	Increase in Fe tolerance	Curie *et al.*, 2000
AtNramp3	Reduced Cd tolerance but no difference in Cd accumulation	Thomine *et al.*, 2000
AtABCC1 and AtABCC2	Cd and Hg tolerance	Park *et al.*, 2012

Biosynthesis of Phytochelatin

By combining with Cd^{2+} ions to create PC-Cd complexes, the thiolic group (-SH) of cysteine in PCs blocks the free Cd^{2+} from migrating around the cytoplasm. These complexes are then transported by ABC transporters and deposited in the vacuole. Furthermore, plants are unable to metabolise or excrete Cd. Instead, they adopt a technique called Cd-GSH and Cd-PC complex formation to efficiently sequester Cd within vacuoles and transport Cd across the vast distance through vascular tissues.



Role of metal lothioneins in heavy metal complexation

Cysteine-rich proteins with a low molecular weight (4–8 kDa) called metallothioneins (MTs) can bind heavy metals because their cysteine residues have thiol groups (Hamer, 1986). Although the precise physiological roles of MTs are still not entirely understood, it is thought that they serve a variety of purposes.

(a) engagement in crucial transition heavy metal homeostasis,

(b) toxic heavy metal sequestration, and

(c) shielding against cellular oxidation.

MT genes are variably controlled in different tissues and according to developmental stages, as well as in reaction to a variety of stimuli, such as heavy metals.

Heavy metal chelation with organic acids, amino acids and phosphate derivatives

External exclusion and internal tolerance are two types of heavy metal tolerance and detoxification mechanisms in the plant system.

The organic acids from root system may eventually release heterocyclic metal-ligand complexes containing heavy metal ions in the external detoxifying process, changing their movement and availability and thereby restricting heavy metal ions from reaching plants and their build-up in sensitive root locations.

By chelating with heavy metals, organic acids can create complexes in the cytoplasm during intracellular heavy metal detoxification, enabling the ions to be changed into a safer or less toxic form (Hall, 2002).

There are a variety of ligands that are produced by plants, including carboxylic and amino acids like oxalate, citrate, malate, phosphate derivatives, nicotianamine (NA), and histidine (His) like phytate, which have all been linked to the tolerance and detoxification of heavy metals.

Metal chelation by phytate

Phytate, also known as myo-inositol-1,2,3,4,5,6-hexakis, is an inositol phosphate salt of phytic acid (dihydrogen phosphate). The phosphate groups have the capacity to release 12 hydrogens into water, resulting in the creation of six negatively charged sites on phytic acid, which can bind the cations of both macronutrients (Mg^{2+}, P^{2+}, Ca^{2+}, K^{+}) and micronutrients (Fe^{2}, Cu^{2+}, Co^{2+}, Mn^{2+}, Zn^{2+}, Ni^{2+})in plants in a mineral-like manner. Although it is a potent cation-chelating agent, phytic acid is not selective.

Heat shock proteins' (HSPs') function in promoting metal stress tolerance

The molecular chaperones known as heat shock proteins (HSPs) aid in the folding of proteinsand assemble normally, but they may also help repair and protect under stressful situations(Lewis *et al.*, 2001). Heavy metals are evident in inducing HSPs such as large HSP (HSP70), small HSP (sHSP), and HSP25. They may be able to prevent permanent protein denaturation caused by oxidative stress from heavy metal exposure.

Micro RNA's (miRNAs)in metal stress

MicroRNAs contribute in a number of processes that are involved in plant development(miR156, miR169, and miR172), sulphur absorption, transportation, and accumulation (miR395, miR838 and miR854), and hormone synthesis (miR395, miR838 and miR854) (miR319, miR167, miR164 and miR159). They control the target genes either by post-transcriptional cleavage or translational suppression of target mRNA, or by methylation of target DNA.

In relation to metal stress, miRNAs change how they are expressedin time and organ dependent ways to control (1) metal complexation, (2) oxidative stress defence, and (3) signal transduction for regulating wide range of biological reactions

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(Sudhakar *et al*., 2013).

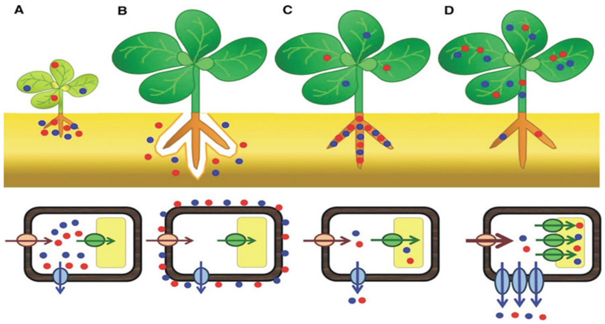
Response of plant molecular system to heavy metal stress

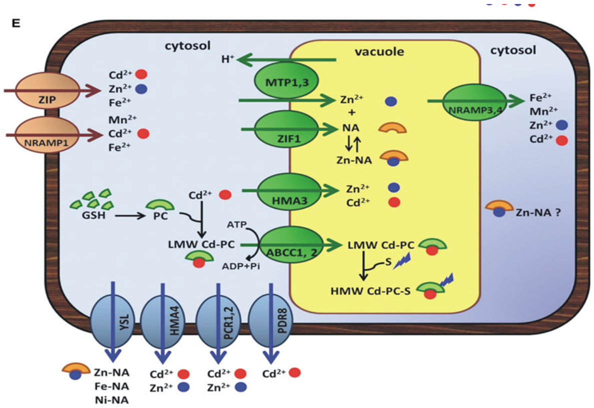
Plants can be classified into four categories based on their sensitivity to high metal levels (in this case Cd, red dots and, Zn, blue dots).

A. Heavy metal-sensitive plants, which are unable to retain metals from out their roots or block their transportation to the shoot, and will succumb as a result of metal toxicity in root and shoot cells.

B. Heavy metal-resistant excluder plants,that can keep hazardous metals out of root cells or handle rapid efflux if they do get in.

C. Heavy metal-tolerant non-hyperaccumulator plants, which allow metals to enter root cells and be sequestered in root vacuoles, inhibiting transfer to shoots.





D. Heavy metal-hypertolerant hyperaccumulator plants, Metals are quickly absorbed through the root and are mostly placed into the vascular tissue for movement from the root to the shoot. During the shot, metals are safely kept in vacuoles.

E. The molecular mechanism in plant cells that maintains metal homeostasis

F. Metal tonoplast located transporters (green) are directly in charge of metal (Zn or Cd), chelator (NA),

Table 4. Transgenic plants overexpressing phytochelatin synthase (PCS) and metallothionein (MT) genes

Gene	Plant	Effect	Reference
AtPCS1	Arabidopsis	Cd tolerance and accumulation	Lee *et al*., 2003
		As tolerance and Cd hypersensitivity	Li *et al*., 2004
		Cd tolerance	Brunetti et al., 2011
AtPCS1	Mustard	As and Cd tolerance	Gasic and Korban, 2007
AtPCS1	Tobacco	Cd tolerance and accumulation	Pomponi *et al*., 2006
AtPCS1/ CePCS1	Tobacco	As tolerance and accumulation	Wojas *et al*., 2010
CdPCS1	Tobacco	Accumulation of As and Cd	Shukla *et al*., 2013
NaPCS1	Arabidopsis	Accumulation of Cd	Liu *et al*., 2012
TaPCS1	Poplar	Accumulation of Pb and Zn	Couselo
PtPCS1	Poplar	Zn accumulation	Adams *et al*., 2012
TaPCS1	Rice	Cd hypersensitivity	Wang *et al*., 2012
TcPCS1	Tobacco	Accumulation of Cd	Liu *et al*., 2011
Mouse MT	Ralstonia	Enhanced Cd immobilizing ability	Valls *et al*., 2000
Mammalian MT	Tobacco	Reduced root-shoot Cd transport	Kramer and Chardonmens 2000
YeastMT (CUPI)	*Brassica oleraceae*	Cd accumulation	Kramer and Chardonnens, 2000
CUPl	Sunflower	Cd tolerance	Watanable *et al*., 2005
AtMT2a	Broad bean	Cd tolerance	Lee *et al*., 2004
AtMT3	Broad bean	Cd tolerance	Lee *et al*., 2004
BjMT2	Arabidopsis	Cd and Cu tolerance	Zhingang *et al*., 2006
CcMT1	Arabidopsis	Cd and Cu tolerance	Sekhar *et al*., 2011
OsMT1	Rice	Zn accumulation	Yang *et al*., 2009
TcMT2a	Arabidopsis	Cd and Zn hypersensitivity	Schiller *et al*., 2013
		No change in tolerance	Hassinen *et al*., 2009
TcMT3	Arabidopsis	No change in tolerance	Hassinen *et al*., 2009
NnMT2a/NnMT3	Arabidopsis	Increased seed germination vigour	Zhou *et al*., 2012

or metal-chelator complex (Cd-PC) sequestration into the vacuole, or absorption from the vacuole (NRAMP3/4), while metal efflux transporters (blue) are in charge of metal efflux from the vacuole. Metal influx transporters (dark purple) are in charge of metal uptake into the cytosol. The vacuole is yellow and the cell wall is brown, with the cytoplasm being grey.

CONCLUSION

Plants transgenic for phytochelatin synthase (pcs) and metallothionein (mt) genes and heavy metal tolerance

- The plants that could be employed for phytoremediation may be aided by the overexpression of genes associated in accumulation of heavy metals, translocation, and its sequestration.
- By increasing the expression of the genes for phytochelatin synthase and metallothionein, heavy metal stress resistance in plants can be achieved.

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