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 molecules are 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
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 like Pb 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, H₂O₂ in particular, functions as a signal molecule (Dat et al. 2000) and H₂O₂ level rises, which happens in response to,

1. Treatment of Arabidopsis thaliana with Cu and Cd
2. Hg exposure in tomato

This surge in H₂O₂ 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 production of 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, formation of particular transporters of heavy metal and sequestration by ligands such as metallothioneins 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 synthesis are 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 plant via 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 vacular proton-ATPase (VATPase), two intracellular proton pumps, and a vacular 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-

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

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Effect of overexpression</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NiCBP4</td>
<td>Enhanced Ni tolerance and reduced Ni accumulation, Reduced Pb tolerance and enhanced Pb accumulation</td>
<td>Arazi et al., 1999</td>
</tr>
<tr>
<td>ZnTI</td>
<td>Increased Zn influx in roots</td>
<td>Pence et al., 2000</td>
</tr>
<tr>
<td>ZAT</td>
<td>Enhanced Zn resistance and accumulation</td>
<td>van der Zaal et al., 1999</td>
</tr>
<tr>
<td>CA2X</td>
<td>Accumulation of Ca, Cd, Mn and Mn tolerance</td>
<td>Hirschi et al., 2000</td>
</tr>
<tr>
<td>AtMHX</td>
<td>Reduced tolerance to Mg and Zn, but it did not show altered accumulation of these elements</td>
<td>Shaul et al., 1999</td>
</tr>
<tr>
<td>AtNramp1</td>
<td>Increase in Fe tolerance</td>
<td>Curie et al., 2000</td>
</tr>
<tr>
<td>AtNramp3</td>
<td>Reduced Cd tolerance but no difference in Cd accumulation</td>
<td>Thomine et al., 2000</td>
</tr>
<tr>
<td>AtABCC1 and AtABCC2</td>
<td>Cd and Hg tolerance</td>
<td>Park et al., 2012</td>
</tr>
</tbody>
</table>
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.

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 proteins and 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 expressed in 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.
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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Plant</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AtPCS1</td>
<td>Arabidopsis</td>
<td>Cd tolerance and accumulation</td>
<td>Lee et al., 2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>As tolerance and Cd hypersensitivity</td>
<td>Li et al., 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cd tolerance</td>
<td>Brunetti et al., 2011</td>
</tr>
<tr>
<td>AtPCS1</td>
<td>Mustard</td>
<td>As and Cd tolerance</td>
<td>Gasic and Korban, 2007</td>
</tr>
<tr>
<td>AtPCS1</td>
<td>Tobacco</td>
<td>Cd tolerance and accumulation</td>
<td>Pomponi et al., 2006</td>
</tr>
<tr>
<td>AtPCS1/CePCS1</td>
<td>Tobacco</td>
<td>As tolerance and accumulation</td>
<td>Wojas et al., 2010</td>
</tr>
<tr>
<td>CdPCS1</td>
<td>Tobacco</td>
<td>Accumulation of As and Cd</td>
<td>Shukla et al., 2013</td>
</tr>
<tr>
<td>NaPCS1</td>
<td>Arabidopsis</td>
<td>Accumulation of Cd</td>
<td>Liu et al., 2012</td>
</tr>
<tr>
<td>TaPCS1</td>
<td>Poplar</td>
<td>Accumulation of Pb and Zn</td>
<td>Couselo</td>
</tr>
<tr>
<td>PtPCS1</td>
<td>Poplar</td>
<td>Zn accumulation</td>
<td>Adams et al., 2012</td>
</tr>
<tr>
<td>TaPCS1</td>
<td>Rice</td>
<td>Cd hypersensitivity</td>
<td>Wang et al., 2012</td>
</tr>
<tr>
<td>TePCS1</td>
<td>Tobacco</td>
<td>Accumulation of Cd</td>
<td>Liu et al., 2011</td>
</tr>
<tr>
<td>Mouse MT</td>
<td>Ralstonia</td>
<td>Enhanced Cd immobilizing ability</td>
<td>Valls et al., 2000</td>
</tr>
<tr>
<td>Mammalian MT</td>
<td>Tobacco</td>
<td>Reduced root-shoot Cd transport</td>
<td>Kramer and Chardonmens 2000</td>
</tr>
<tr>
<td>YeastMT (CUP1)</td>
<td>Brassica</td>
<td>Cd accumulation</td>
<td>Kramer and Chardonmens, 2000</td>
</tr>
<tr>
<td>CUPI</td>
<td>Sunflower</td>
<td>Cd tolerance</td>
<td>Lee et al., 2004</td>
</tr>
<tr>
<td>A1MTT2a</td>
<td>Broad bean</td>
<td>Cd tolerance</td>
<td>Lee et al., 2004</td>
</tr>
<tr>
<td>A1MT3</td>
<td>Broad bean</td>
<td>Cd tolerance</td>
<td>Lee et al., 2004</td>
</tr>
<tr>
<td>BjMT2</td>
<td>Arabidopsis</td>
<td>Cd and Cu tolerance</td>
<td>Zhingang et al., 2006</td>
</tr>
<tr>
<td>CcMT1</td>
<td>Arabidopsis</td>
<td>Cd and Cu tolerance</td>
<td>Sekhar et al., 2011</td>
</tr>
<tr>
<td>OsMT1</td>
<td>Rice</td>
<td>Zn accumulation</td>
<td>Yang et al., 2009</td>
</tr>
<tr>
<td>TeMT2a</td>
<td>Arabidopsis</td>
<td>Cd and Zn hypersensitivity</td>
<td>Schiller et al., 2013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No change in tolerance</td>
<td>Hassinen et al., 2009</td>
</tr>
<tr>
<td>TcMT3</td>
<td>Arabidopsis</td>
<td>No change in tolerance</td>
<td>Hassinen et al., 2009</td>
</tr>
<tr>
<td>NnMT2a/NnMT3</td>
<td>Arabidopsis</td>
<td>Increased seed germination vigour</td>
<td>Zhou et al., 2012</td>
</tr>
</tbody>
</table>
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.

REFERENCES


