Chapter 6
System Biology of Metal Tolerance in Plants: An Integrated View of Genomics, Transcriptomics, Metabolomics, and Phenomics

Noreen Khalid, Muhammad Aqeel, and Ali Noman

6.1 Introduction

For the successful plant life, mineral elements absorption by roots from rhizosphere is indispensable. The water and mineral uptake are, therefore, major drivers in developmental plasticity and physiological activities in plant systems. In the process of mineral and nutrient absorption, heavy metals (HMs) can also be taken up and make their way to plant tissues. Some metals are functional in very small amounts for various biochemical and physiological activities such as photosynthesis, chlorophyll biosynthesis, DNA synthesis, sugar metabolism, redox reactions, and protein modifications. Some are toxic to plants in excess amounts, while others are toxic even in traces (Garzón et al. 2011; Shahid et al. 2012; Gill et al. 2013). The toxic metals are cadmium, lead, aluminum, zinc, cobalt, arsenic, chromium, nickel, copper, iron, and molybdenum (Sharma and Agrawal 2005; Hossain and Komatsu 2013; Noman and Aqeel 2017; Noman et al. 2018a).

Many heavy metals naturally occur at various levels in the earth's crust, but their excess amounts are released into the environment mainly by anthropogenic activities (Singh et al. 2011a, b; Khalid et al. 2018a, b). Heavy metal release from industries, mines, vehicle emissions and municipal wastes have contaminated outsized areas with HMs (Yang et al. 2005). Plants growing in these areas of localized high metal ions concentrations get exposed to HMs (Khalid et al. 2018e). The HMs

N. Khalid (✉)
Department of Botany, Government College Women University, Sialkot, Pakistan

M. Aqeel
State Key laboratory of Grassland and Agro-Ecosystems, School of Life Science, Lanzhou University, Lanzhou, China

A. Noman (✉)
Department of Botany, Government College University, Faisalabad, Pakistan
e-mail: alinoman@gcuf.edu.pk

toxicity in plants alters physiological activities and cause activation of plant's defense systems and other adaptations. In response to the harmful impacts of heavy metals, plants keep HM utilization and uptake under control (Singh et al. 2012; Farias et al. 2013). The common HM toxicity symptoms in plants include chlorosis, senescence, stunted growth, low biomass accumulation, poor yield, and sometimes even death (Vassilev et al. 2005; Malar et al. 2016; Khalid et al. 2017, 2018c). In addition to having toxic effects on plants, they also pose a great threat to human health. For example, cadmium is principal human carcinogen (ATSDR 2015; Luckett et al. 2012).

Abiotic stresses have markedly reduced the crop production throughout the world (Jewell et al. 2010; Noman et al. 2018a, b). To ensure food security for ever-increasing human population, improvement in plant adaptations under various abiotic stresses is very essential to understand. Productivity of our major food crops is expected to decrease in the next decade due to increased occurrence of floods, storm, drought, and global warming. Hence, for improving crop production quality and quantity, set of modern technologies are required to unravel the plants responses to abiotic stresses especially HMs. Measuring the expression of genes in HM stress conditions in tolerant plant species can give better insight in understanding the mechanisms these plants follow to curtail the toxicity of metals. Recent advances in various biotechnology approaches made possible to understand plant's responses to heavy metal stress (Lowe et al. 2017). Current studies mainly focus on various approaches which take into consideration the role of metabolomics (metabolites), transcriptomics (RNA transcripts), genes (genomics), and phenotypes (phenomics) to address the heavy metal stress in plants (Fig. 6.1). The data gathered in this chapter will aid in increased understanding to heavy metal stress tolerance and can be employed in engineering programs using desired traits for developing new plants especially HMs tolerant crops.

6.2   Plant Responses to Heavy Metal Stress

Being sessile, plants are the most sensitive victims of the unwanted changes in the environment. The most known plants response to heavy metal toxicity is reduced growth. Heavy metal toxicity triggers a series of biochemical and physiological perturbations in the plants. Toxic metal ions interrupt the normal metabolic activities in several ways. The metal ions are similar in structure with other nutrient cations. Hence, they compete with other cations at root absorptive surfaces. Due to this similarity and competition, they also quench essential cations inside vital biomolecules making them inactive. This interaction culminates in the destruction of functional proteins after displacing their sulfhydryl groups (-SH). Another important consequence of HMs toxicity is the production of reactive oxygen species (ROS), that have detrimental effects on biomolecules leading to disrupted metabolism (Sharma and Dietz 2009; DalCorso et al. 2013).
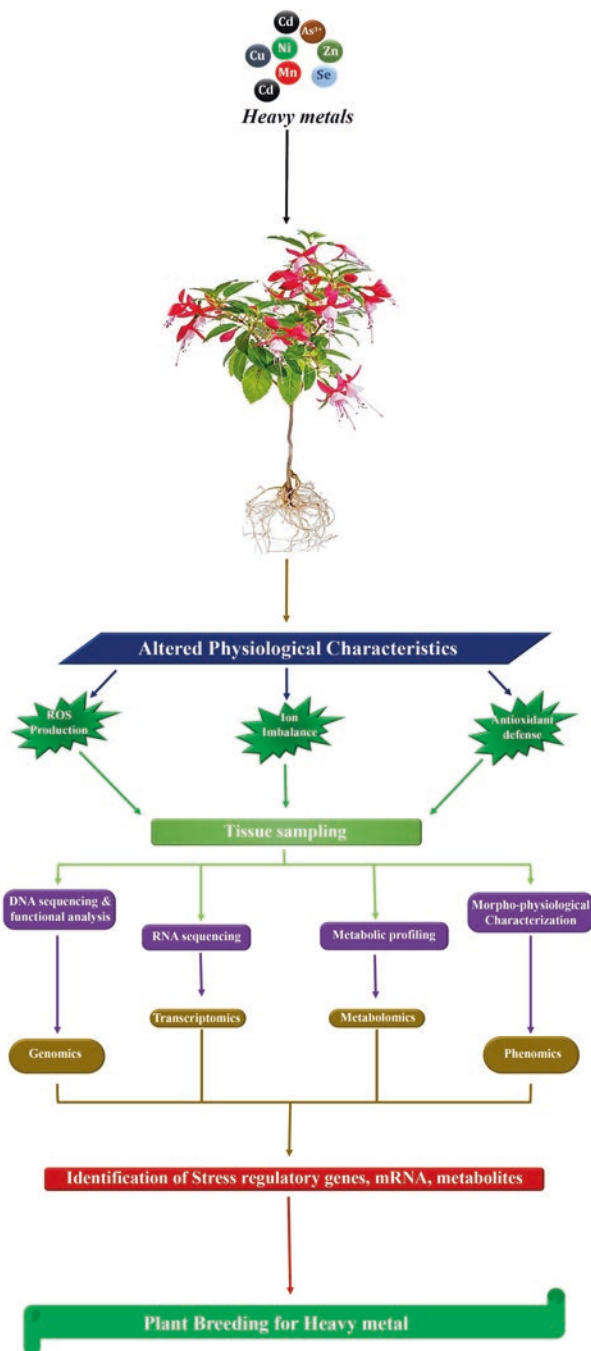
Fig. 6.1 Omics approaches used for identification of stress response regulatory genes in plants

To cope with the negative impacts of HM toxicity, plants use different approaches. Plants respond to toxicity by early sensing of external metal stress ending at intracellular signal transduction. Then by modulating molecular, biochemical, and physiological activities inside the cell, plants counteract the adversaries of heavy metal toxicity. For their defense, plants facing toxicity reduce or prevent metal ions uptake by restricting them to the apoplast in root cell walls. However, if the metal ions somewhat make their way to the cytoplasm, then plants adapt other detoxification and storage strategies to get rid of noxious metal ions, for example, sequestration into the vacuole, chelation, or their trafficking and transport (Manara 2012). Hence, plants can react in several ways to survive by sensing the external stress signals. But after exposure to metal toxicity, it is very difficult to measure changes in signal transduction at the whole plant level; however, responses of plants to toxic effects caused by metals can be monitored at earlier stages. The responses which can be measured at early stages to sense the signal transduction in plants include metabolites accumulation, proteomic changes, compromised efficiency of transcription factors, and the accumulation of ROS.

The toxic effects of various HMs on plants have been reported by many researchers. For example, Singh et al. (2011a, b) and Mohamed (2011) have reported the effects of lead (Pb) toxicity in *Brassica campestris* and *Vigna sinensis*. The high Pb levels retarded the radical emergence through increased carbohydrates and protein contents. Thus, it badly affected the activities of enzymes, that is, β-amylases, α-amylases, phosphatases, and invertases in *B. campestris* and caused alterations in the genome of *V. sinensis*. Similarly, high arsenic (As) levels can lead to severe metabolic disorders, for example, disruption of respiratory and photosynthetic systems, secondary metabolism stimulation, and growth inhibition (Garg and Singla 2011). Stoeva et al. (2004) recorded reduced CO_2 fixation and hampered PSII functional activity as a consequence of As toxicity in young maize plants. In another study, six varieties of *Triticum aestivum* were exposed to increased concentrations of As (III) and As (V). Significant reduction in seedling roots production was recorded along with reduced grain yield in all varieties (Liu et al. 2005).

High concentrations of copper (Cu) in the soil induce stress and cause cytotoxicity and injury in plants. This could lead to leaf chlorosis and growth retardation (Lewis et al. 2001). Interestingly, Copper toxicity has been noticed to increase the activities of all antioxidant enzymes, for example, peroxidase (POD), superoxide dismutase (SOD), and ascorbate peroxidase (APX) in the roots and shoots of *Zea mays* (Tanyolac et al. 2007; Kumar et al. 2008), and *Oryza sativa* (Chen et al. 2000; Thounaojam et al. 2012). In wheat, at mitotic telophase, chromosomal aberrations were attributed to high Cu content (Truta et al. 2013). Atha et al. (2012) reported damaged DNA structure due to excess Cu quantities in *Lolium perenne* and *Raphanus sativus*. However, each crop response varies differently to Cu toxicity. It chiefly depends upon cultivar used and the nature of crop (Adrees et al. 2015). Similarly, in plant exposed to increased quantities of antioxidant enzymes, that is, catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), and superoxide dismutase (SOD) have been reported by researchers (Ahmad et al. 2011;

Arshad et al. 2016). Ali et al. (2014) reported reduced photosynthetic pigments and growth, while ROS, malondialdehyde content, and antioxidant enzymes were increased in *Brassica napus* due to Cd toxicity.

Roots are the first organs of plants that face HMs presence in the soil. Roots absorb water along with nutrients from the soil and transport them to the entire body of the plant under normal conditions. However, their normal function alters when they encounter higher concentrations of toxic metal elements in the soil. In such conditions, roots have to undergo various functional and structural modifications (Noman et al. 2017b). For example, alteration of membrane characteristics and metabolism, hardening of cell wall, certain cellular, molecular, and phenotypic changes and root growth inhibition (Atkinson and Urwin 2012). Some plants are able to accumulate HMs inside vacuoles in root cells rendering their inactivation. Whereas, other plants translocate and concentrate heavy metal ions in shoot system, for example, in hyperaccumulator plants (Noman et al. 2014, 2017b). Reduction in mitotic activity of roots after exposure to toxicity of HMs has been recorded by researchers in many plant species. The consequences abridged mitotic divisions are reduced cell division and elongation and ultimately suppressed root growth (Sundaramoorthy et al. 2010; Thounaojam et al. 2012).

Among all the heavy metals, chromium (Cr) is particularly toxic for the plant roots as its higher concentrations occur in roots as compared to the other plant parts (Kumar and Maiti 2013). The defense strategy of plant roots against Cr toxicity is to immobilize and accumulate Cr ions inside the vacuole and this is the proposed reason for high Cr accumulation in root cells (Nematshahi et al. 2012). Inhibition of root cell elongation and cell division has been attributed to Cr toxicity (Shanker et al. 2005). Likewise, Srivastava and Jain (2011) also recorded extended cell cycle due to the presence of toxic level of Cr inside the root cells. Furthermore, reduced root length in *Helianthus annuus* due to Cr toxicity has also been credited to cell cycle extension (Fozia et al. 2008). Absorption of water and minerals are severely curtailed by restricted root length. Consequently, shoot length and overall plant growth and vigor is also brutally affected. Likewise, the findings of Yuan et al. (2013) and Petö et al. (2011) held copper (Cu)-mediated toxicity responsible for the inhibition of primary root elongation due to induced alterations in the auxin level.

Membranes are the most vulnerable targets of HM attack. Commonly, the degree of stress tolerance is measured by the extent of membrane damage (Gadallah 1999). To protect the cells from injurious effects of HMs and other unwanted substances, certain checkpoints are located on the plasma membranes that are highly controlled. Damaged membranes can cause unbalanced and abrupt water and nutrients uptake. This may lead to decline in stomatal conductance and consequently lowered photosynthetic rate. Reduced transpiration intensity in oat plants due to As-mediated stress was considered to be a consequence of disturbed transport and uptake of water (Stoeva and Bineva 2003). Singh et al. (2006) also reported reduction in membrane stability when they were evaluating the effect of As on membrane conductivity of *Pteris ensiformis* and *Pteris vittata*. Vazquez et al. (2008) also confirmed the membrane damage in mung bean treated with As. Increased amounts of malondialdehyde

along with electrolytes leakage were found in the root cells of mung bean. Similarly, nickel (Ni) has reportedly been involved in inducing cell membrane damage in different plant species. In *Oryza sativa*, Ni toxicity badly affected the H-ATPase activity and lipid composition of plasma membrane (Ros et al. 1992). Excess ROS production due to high iron (Fe) concentrations ultimately cause irreversible damage to cell membrane, cellular structure, proteins, DNA and other vital biomolecules (de Dorlodot et al. 2005).

6.3 Hyperaccumulator Plants and Heavy Metals Up Take

Hyperaccumulator plants take up and accumulate extraordinary quantities of toxic substances in their shoot system during normal growth and reproduction. These plants do not exhibit any phytotoxic effects in spite of concentrating toxic heavy metals (Khalid et al. 2018d). The hyperaccumulator plants share common ability to grow on metal contaminated soils and fall in different families. However, in comparison with non-hyperaccumulators, all of the hyperaccumulator plants proceed with three basic steps in concentrating metal elements inside their bodies, that is, (1) high rate of HMs uptake, (2) faster translocation to the shoots, and (3) detoxification through sequestration in above ground parts. Comparative studies regarding molecular and physiological aspects of hyperaccumulating plants revealed upregulation and overexpression of trans-membrane transporters, for example, HMA, ZIP, YSL, MTP, and MATE families encoding genes in hyperaccumulators (Arbaoui et al. 2014). Hence, the overexpression of these genes is directly proportional to augmented metals uptake, their xylem-based translocation, ability to make phytochelatin-heavy metal complexes, and their final sequestration in the vacuoles (Hossain et al. 2012). Keeping the above facts in view, it has been demonstrated by molecular, genetic, and biotechnological tools that plant's tolerance to HMs can be altered significantly. The three key steps of heavy metal accumulation shown by hyperaccumulators have been given in the following;

6.3.1 Heavy Metal Uptake and Role of Transporters

Many factors regulate the uptake of metal ions from the soil, for example, the metal availability, soil aeration, soil moisture, pH, and temperature. Hence, heavy metal accumulation is not simple increase in concentrations inside the plant against increasing concentrations in the environment. Similarly, microorganisms and root exudates also influence the HMs availability and mobility patterns in the soil (Wenzel et al. 2003). Some metals like Cd are more mobile in the soil as compared to the other; hence, they are taken up swiftly (Spence et al. 2014). Root proliferation system also influences the uptake of heavy metals (Whiting et al. 2000). Nevertheless,

the rate of metal uptake by plants can be estimated by using biological accumulation factor (BAF) under a known set of environmental conditions:

$$BAF = \frac{The\ concentration\ of\ heavy\ metal\ in\ the\ plant}{The\ concentration\ of\ heavy\ metal\ in\ the\ soil}$$

To avoid metal ions uptake, plants secrete various organic substances through the roots into the soil. These root secretions act as first line of defense and prevent metal uptake into the cell by chelating the metal ions (Marschner 1995). For nutrients and metal ions uptake from the soil, plants possess highly specialized transport systems (Fig. 6.2). These transport systems involve different mechanisms to carry metal ions. Most of the HMs move via highly evolved co-transporters and metal pumps on the cell membrane of root cells (Pilon et al. 2009). These transporters are the membrane proteins present at the organ, tissue or at the whole plant level. The intracellular metal ions transport is controlled by a set of these proteins located across the cell membrane, tonoplast, and other subcellular membrane bounded organelles (Krämer et al. 2007; Maestri et al. 2010). On the other hand, some of the HMs like Cd and Pb are very toxic even in traces. Also, these heavy metals do not take part in any of the specialized biological functions in plants. For this reason, it is assumed that these do not have any specific transporters present on cell membrane for their
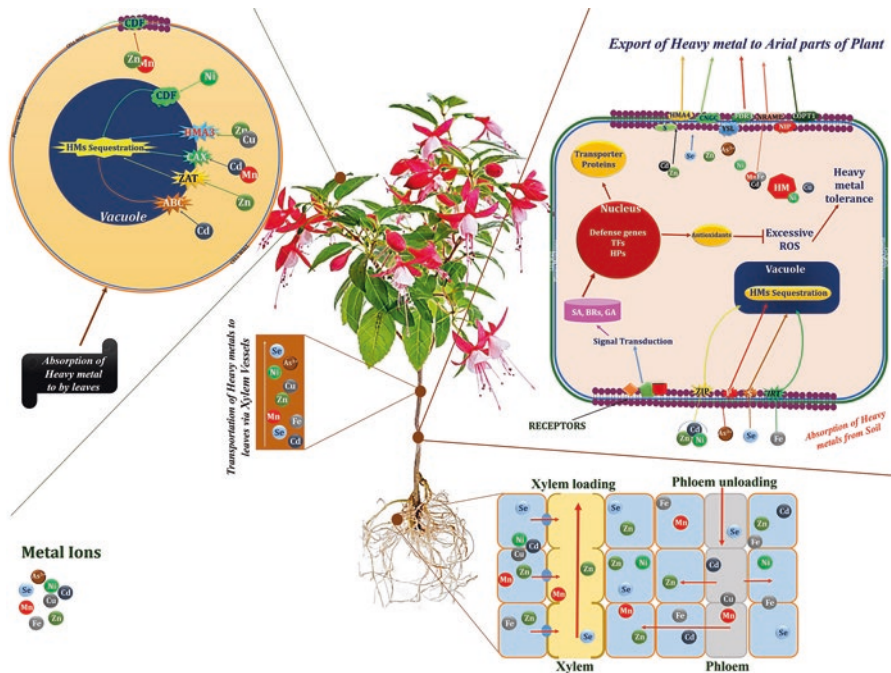

Fig. 6.2 Heavy metal uptake and transport in plants through various metal transporters on plasma membrane

movement. For cellular entry, these toxic metal ions move through wide range of transporters. The most common transporters involved in HM transport belong to ZIP family, NRAMP, CDF, and P1B-ATPases (Guerinot 2000; Williams et al. 2000; Takahashi et al. 2012). Overexpressing genes for these transporters have been noticed in hyperaccumulator plants. Notably, their expression does not have any harmful impact on physiological homeostasis in plants, but instead they confer enhanced heavy metal tolerance (Curie et al. 2000; Verret et al. 2004). In hyperaccumulator plants, expression of zinc–iron permease (ZIP) genes coding for cell membrane cation transporters have been observed irrespective of high Zn availability in the soil (Weber et al. 2004). Contrarily, in non-hyperaccumulator plants, their expression is highly dependent on soil Zn availability. Only detectable fraction of these transporters can be seen under Zn deficiency (Assunção et al. 2010). In Zn hyperaccumulator plant, *Thlaspi caerulescens*, genes coding for Zn transporters express all the times irrespective the Zn status of soil. Therefore, this elucidates the great ability of *T. caerulescens's* to accumulate high amount of Zn (Pence et al. 2000). ZIP expression in plants is directly associated with capacity to accumulate metal ions and they play vital role in enhanced metal accumulation capacity of hyperaccumulator plants (Krämer et al. 2007). In roots of *N. caerulescens,* constitutive, high expression of *NcZNT1* indicates its involvement in root-to-shoot long distance transport by maintaining Zn and Cd influx for xylem loading (Lin et al. 2016).

Overexpression of *IRT1* in the root cells of *Arabidopsis thaliana* against iron (Fe) deficiency in the soil proves the role of *IRT1* in the uptake of Fe from the soil (Vert et al. 2002). Several other heavy metal transporters are present on the cell membrane, for example, in hyperaccumulator plant *Anemone halleri*, *AtHMA4* is involved in hyperaccumulation and root-to-shoot translocation of Cd and Zn (Hanikenne et al. 2008). In *Noccae acaerulescens* ecotypes, the difference in the expression of *NcHMA*3 and *NcHMA*4 is involved in rendering Cd tolerance (Ueno et al. 2011; Craciun et al. 2012).

NRAMP is another class of cell membrane-bound metal transporters involved in the transport of different metal ions (e.g., Ni, Cd, Zn, Mn, and Co). These transporters are localized on the tonoplast and the cell membrane of root and shoot cells (Nevo and Nelson 2006). *OsNRAMP*1 is known to transport Fe through endodermis and pericycle cell membranes in rice. Expression of *OsNRAMP*1 greatly increased the Cd and As tolerance in roots and shoots of *Arabidopsis thaliana* (Tiwari et al. 2014). Arsenic (As) enters the cell through phosphate transporters since it is proved to be analogous to phosphate in chemical structure (Meharg and Hartley-Whitaker 2002; Kanoun-Boulé et al. 2009). Different phosphate/arsenate transporters are present on hyperaccumulator plant *Pteris vittata* as compared to *Pteris tremula,* a non-hyperaccumulator (Caille et al. 2005). Similarly, Galeas et al. (2007) attributed much higher concentrations of Selenium (Se) and Sulfur (S) in Se hyperaccumulator plants *Stanley apinnata* and *Astragalus bisulcatus* as compared to non-hyperaccumulators. This helps us to build opinion that the sulfate transporters in the cell membrane of roots are involved in Se uptake due to structural similarity between the two elements.

6.3.2   Heavy Metals Translocation from Root to Shoot

Non-hyperaccumulator plants retain most of the HMs in their root cells either by storing them in the vacuole or by detoxifying them in the cytoplasm. Conversely, hyperaccumulator plants readily translocate heavy metals to the shoot cells through vascular system (Fig. 6.2). For this process, different root-based chelators have been identified in hyperaccumulators. The chelators translocate heavy metals by binding with them. Organic acids (e.g., citrate, malate) and certain amino acids (e.g., methionine, nicotinamine, histidine) act as chelators and make complexes by binding with the metal ions (Callahan et al. 2006; Anwer et al. 2017). For example, free histidine transports Ni through xylem by making His-Ni complex. The high quantity of histidine in Ni hyperaccumulating plant *Thlaspic aerulescens* have also been reported by Assunção et al. (2003). Various transporter proteins are also involved in the translocation of metals via xylem; for example, P-type ATPases, cation diffusion facilitator proteins (CDF), multidrug and toxin efflux proteins (MATE), natural resistance-associated macrophage proteins (NRAMP), and zinc–iron permease (ZIP) proteins. Among P-type ATPases, P1B-type ATPases proteins are particularly important as they play role in metal tolerance, homeostasis and transport (Axelsen and Palmgren 1998a, b). Genes encoding for mentioned protein types such as *HMA*4 overexpress in *A. halleri* and *T. caerulescens* (Mils et al. 2003; Talke et al. 2006; Hanikenne et al. 2008). Similarly, overexpression of *AtHMA*4 proteins increased translocation of Cd and Zn from root to shoot. Moreover, they also enhance plant tolerance to these metals (Verret et al. 2004). The expression of *AtHMA*4 has also been known to positively influence other genes involved in HMs translocation. However, in another study, Zhang et al. (2016) reported high expression of *HMA*2 in roots of Cd hyperaccumulator plant *Sedum alfredii* as compared to this plant's non-hyperaccumulating populations. Interestingly, they did not find the expression of *HMA4*. Overexpression of *AtHMA*5 has also been correlated to copper (Cu) detoxification and root-shoot transport (Andrés-Colás et al. 2006). Genes encoding for MATE family of protein transporters are *FDR*3. The role of *FDR*3 inside root cells is citrate transport (a ligand for Fe homeostasis) via xylem. The overexpression of these genes was also recorded in the roots of *A. halleri* and *T. caerulescens* (Talke et al. 2006; van de Mortel et al. 2006). The overexpression of *FDR3* suggests its probable role in other heavy metals translocation (Durrett et al. 2007; Krämer et al. 2007). Similarly, in YSL family of proteins, *ZmYS*1 (maize Yellow Stripe 1 protein) transporters are involved in the translocation of Mn, Ni, Zn, Fe, Cu, and Cd (Schaaf et al. 2004).

6.3.3   Heavy Metal Detoxification/Sequestration

After translocation to shoots, cytosolic metal ions concentrations become so high that can cause phytotoxicity. To alleviate the detrimental effects of metal toxicity, plants either compartmentalize metals inside the cell or accumulate them in the vacuole. Hyperaccumulators have great efficiency to detoxify/sequester heavy

metals. This key property enables them to concentrate and tolerate high quantities of toxic metals in their aerial parts without showing phytotoxic effects.

In detoxification and sequestration as well, Various intracellular transporters take part in detoxification and sequestration of HMs, for example ABC, NRAMP, CDF, CaCA, and HMA (Fig. 6.2). Exploration of miRNA targets such as ABC or NRAMP transporters have displayed their direct function in metal tolerance pathways (Noman and Aqeel 2017). Two families - Multidrug Resistance associated Proteins (MRP) and Pleiotropic Drug Resistance proteins (PDR) - of ABC transporters play crucial role in sequestering and transporting of chelated metals and xenobiotics inside the vacuole. Additionally, HMT1 transporters are present in the tonoplast and transport PC–Cd (phytochelatin–cadmium) complexes (Ortiz et al. 1995; Kuriakose and Prasad 2008). In Zn/Ni hyperaccumulator plant leaves, the overexpression of *MTP*1gene has been reported. *MTP1* gene encodes for a protein which is localized to tonoplast and have role in increasing accumulation and tolerance of Zn (Hammond et al. 2006; Dräger et al. 2004; Gustin et al. 2009). After excess Zn transportation to the vacuole, Zn deficiency may occur inside the cytosol leading to the enhanced expression of ZIP transporters in the root cells of hyperaccumulators (Gustin et al. 2009). CDF transporters are found in many eukaryotes. They are known to transport metal cation, for example, Cd, Ni, Fe, Mn, Co, and Zn from cytoplasm to the apoplast, vacuole, and endoplasmic reticulum (Krämer et al. 2007; Montanini et al. 2007; Peiter et al. 2007). *AtHMA*3 overexpression induced tolerance against heavy metals, for example, Zn, Pb, Cd, and Co in hyperaccumulators through storage of these metals in the vacuole (Morel et al. 2009a, b). Furthermore, CaCA and NRAMP transporters are also present on the tonoplast and play role in the transport of metals from cytosol to the vacuole.

6.4   Detoxification of Heavy Metals, ROS Production, and Antioxidant Defense System

Reactive oxygen species (ROS), that is, OH^-, $O_2^{\cdot-}$, H_2O_2, are excited and partially reduced forms of oxygen. These are highly reactive and unstable components (Halliwell and Gutteridge 2007) produced inside the organelles during different cellular metabolic processes (Noman et al. 2014). These are byproduct of aerobic metabolism and mostly produced in plant cell organelles experiencing high electron flow or showing high oxidizing metabolic activities. For example, mitochondria, chloroplast, and peroxisomes are the principle sites for ROS production (Apel and Hirt 2004; Mittler et al. 2004; Mhamdi et al. 2010). However, their production increases many folds after plants exposure to heavy metals. Incremented ROS production is commonly considered as the primary plant response to metal and other abiotic stresses (Mittler et al. 2011; Ali et al. 2016; Ahmad et al. 2017). ROS are known to cause oxidative stress whenever they are in excess amount inside the cell

(Sharma and Dietz 2009). They react with and damage the structure of essential macromolecules, for example, lipids, proteins, DNA, RNA, and many other important biomolecules (Mittler 2002; Valko et al. 2006). The intensity of damage toward different molecules is different depending upon the type of ROS. Besides having toxic effects inside the cellular organelles, ROS also acts as signaling molecules for the regulation of many crucial physiological processes, for example, stomatal movement, cell differentiation, growth, and root hair growth (Kwak et al. 2006; Tsukagoshi et al. 2010). ROS are highly versatile as signaling molecules, and show diverse properties depending upon the sites of production, their reactivity, and potential to cross the cell membrane (Konig et al. 2012; Vaahtera et al. 2014). They regulate stress signaling, systemic responses, redox levels, development, differentiation, and cell death (Mittler et al. 2011; Mignolet-Spruyt et al. 2016). Many complex processes are involved in regulating the fate of ROS whether to act as damaging element or the signaling one.

Besides being important signaling molecules of metal stress, ROS cause lipid peroxidation thus lead to damaged cell membrane. The excessive membrane destruction can culminate in cell death (Foyer and Noctor 2005; Zaheer et al. 2015; Mittler 2017). According to studies, plant tissues generate ROS from 1 to 2% of their total oxygen intake (Bhattachrjee 2005). High levels of superoxide and H_2O_2 inside the chloroplasts during heavy metal stress are the result of reduced CO_2 fixation (Takahashi and Murata 2008). As a result of reduced electron transport, ROS is also produced in mitochondria (Keunen et al. 2011). In chloroplast, reduced PS II efficiency and uncoupling of electron transport can be a result of HMs toxicity after replacement of Mn and Ca in the reaction center of PS II (Faller et al. 2005; Krantev et al. 2008). Similarly, Cd-inhibited electron flow in PS I could be the reason for the ROS generation. In photorespiration, the oxidation of glycolate to glyoxylic acid also produces H_2O_2 (Mittler et al. 2004). It is also produced in leaf peroxisomes due to HM stress (Romero-Puertas et al. 2004). $O_2^{\cdot -}$ and H_2O_2 are also produced by enzymes activities, for example, peroxidases are associated with cell wall and cell membrane-bound NADPH oxidases (Mhamdi et al. 2010). The hydroxyl radicals are formed by the action of free iron due to its role in Fenton reaction (Vaahtera et al. 2014; Mignolet-Spruyt et al. 2016).

Despite the extreme toxic effects of ROS to plants, some plants are found to be tolerant to the high levels of ROS. How plants can survive in high ROS concentrations? From the studies on hyperaccumulator plants, it has been determined that several factors in these plants contribute in resistance to ROS. These factors include several physio-biochemical adaptations, low ROS production, and ROS scavenging. Thus, the production of ROS is balanced by the scavenging mechanism by activating cellular antioxidant defense system (Das and Roychoudhury 2016; Zafar et al. 2016). The two processes are always running parallel in the plant cell, that is, the production of ROS as a byproduct of aerobic metabolism and removal of ROS by antioxidative defense system. Under normal conditions, plants keep an appropriate balance between ROS production and its quenching (Noman et al. 2017c, d).

Exposure to toxic levels of heavy metals can disturb the balance between ROS production and quenching. Thus, intracellular ROS levels rapidly increase (Sharma et al. 2010; Mishra et al. 2011). Plants stimulate the overexpression of antioxidant defense in order to prevent adversaries caused by ROS.

The components of antioxidant defense system may be enzymatic or nonenzymatic (Arshad et al. 2016; Noman and Aqeel 2017). The enzymatic components of antioxidant defense system are ascorbate peroxidase (APX), superoxide dismutase (SOD), and catalase (CAT), monodehydroascorbate reductase (MDHAR), dehydro ascorbate reductase (DHAR), glutathione peroxidase (GPX), glutathione reductase (GR) and glutathione-S-transferase (GST) (Zafar et al. 2016). The nonenzymatic components are glutathione, ascorbate, tocopherols, proline, and carotenoids (Apel and Hirt 2004; Sharma and Dietz 2009; Noman et al. 2014). The level of cellular antioxidants is generally related to increased or decreased stress levels. The ROS concentration at an appropriate level encourages plant growth by developing resistance against stressors. Plants keep the ROS concentration in control by directing the expression of a cascade of signaling pathways and several genes (Neill et al. 2002; Noman et al. 2017e). Considering the role of antioxidants in mitigating the oxidative stress by managing ROS levels, biosynthetic pathway genes for maintaining the appropriate ROS level in organisms/transgenic plants showing increased levels of antioxidants has been given in the Table 6.1.

CAT has high specificity for scavenging H_2O_2. However, its activity for organic peroxides is not very strong. H_2O_2 are mainly produced in peroxisomes during β-oxidation of fatty acids, photorespiratory oxidation, and other systems during stressed conditions (Del Río et al. 2006; Corpas et al. 2008). CAT degrades H_2O_2 in a very energy efficient manner (Mallick and Mohn 2000). From *Brassica juncea*, overexpression of genes for CAT were introduced into tobacco plants rendering them Cd stress tolerant (Guan et al. 2009). The CAT scavenges H_2O_2 very rapidly but its affinity for H_2O_2 is lower than that of APX. In managing metal stress, APX may have a more important role in ROS scavenging. Increased APX expression in plants facing HM toxicity has been reported by many studies (Khan et al. 2007; Malar et al. 2014; Carneiro et al. 2017). Overproduction of APX stimulates the SOD activity, thus improving the tolerance by strengthening the ROS scavenging (Sarowar et al. 2005). In transgenic plants, the overproduction of APX improves the tolerance to heavy metal stress. Similarly, SOD is found in many subcellular compartments and catalyzes the removal of O_2, H_2O_2, and O_2^-. SOD overproduction in plants is related to increased oxidative stress tolerance. Zaefyzadeh et al. (2009) reported that SOD level can be used as a selection standard to screen stress resistant plants. All of the antioxidants are involved in alleviating the heavy metal stress in plants. ROS-based overexpression of antioxidant defense system has been reported by several studies (Ovečka and Takáč 2014; Sytar et al. 2013; Barrameda-Medina et al. 2014; Bashri and Prasad 2015).

Table 6.1 Expression of genes of enzymatic and/or nonenzymatic antioxidants in plants and their role in transgenic plants against heavy metal stress tolerance

Sr. No.	Transgenic organism	Source plant	Gene(s)	Functional response	References
1	Arabidopsis thaliana	Allium sativum/ Saccharomyces	AsPCS1/GSH1	As and Cd tolerance by high production of glutathione and phytochelatin	Guo et al. (2008)
		Nicotiana tabacum	NtPox parB	Provide protection against oxidative stress and Al toxicity	Ezaki et al. (2001)
			NtPox parB AtPox	Provide protection against oxidative stress and Al toxicity	Ezaki et al. (2000)
		Synechocystis PCC 6803	GPX-2	Fe tolerance along with tolerance to H_2O_2, MV, chilling, and drought stresses	Gaber et al. (2006)
2	Brassica juncea	Arabidopsis	APS1	Se tolerance caused by its quick reduction	Pilon-Smits et al. (1999)
		Bacterial	cytGR/cpGR	Cause reduced accumulation of Cd and increase GR activity	Pilon-Smits et al. (2000)
		Escherichia coli	gshII	Enhances Cd tolerance by increasing thiols, phytochelatins, and glutathione concentrations in transgenic plants	Zhu et al. (1999)
3	Brassica napus	Triticum aestivum	MnSOD	Imparts Al tolerance by showing greater activity of SOD	Basu et al. (2001)
4	Escherichia coli	Anabaena sp. PCC7120	PCS	Enhances Cu and Cd tolerance by increasing the production of phytochelatins	Chaurasia et al. (2008)
		Anabaena sp. PCC7120	Alkyl hydroperoxide reductase	Enhances Cu and Cd tolerance by increased H_2O_2 and reactive sulfur species scavenging	Mishra et al. (2009)
		Brassica rapa	GR	Tolerance to Al, Cd, and Zn by enhancing GR activity	Kim et al., (2009)
		Oryza sativa	DHAR	Imparts tolerance toward heavy metals and oxidative stresses	Shin et al. (2008)
		Thlaspi goesingense	Serine acetyltransferase	Tolerance to Co and Ni by overexpressing glutathione	Freeman et al. (2005)
		Nicotiana tabacum	Mn SOD + APX	Enhance tolerance to As, Cd, Cu, and H_2O_2, reduce chlorophyll degradation, ion leakage, and increase APX activity	Lee et al. (2007)

(continued)

Table 6.1 (continued)

Sr. No.	Transgenic organism	Source plant	Gene(s)	Functional response	References
5	*Hebeloma cylindrosporum*	*Paxillus involutus*	*MT1*	Tolerance to Cd and Cu	Bellion et al. (2007)
6	*Nicotiana tabacum*	*Arabidopsis*	*MDHAR/DHAR*	Tolerance to Al by regulating level of ascorbate	Yin et al. (2010)
		Arabidopsis	*PCs*	Increases Cd tolerance and regulates its root-to-shoot transport	Pomponi et al. (2006)
		Brassica juncea	*CAT3*	Increases Cd tolerance, roots and seedling growth	Gichner et al. (2004)
		Brassica juncea	*CAT*	Induces tolerance to Cd and Zn by lowering H_2O_2 level and increasing CAT activity	Guan et al. (2009)
		Escherichia coli	*DHAR/GR/GST*	Enhance heavy metal tolerance by maintaining level of glutathione and ascorbate	Le Martret et al. (2011)
		Mus musculus	*MT1*	Increases Hg tolerance and its accumulation	Ruiz et al. (2011)
		Thlaspi caerulescens	*TcPCS1*	Increase Cd tolerance by enhancing CAT, SOD, and POD activities and by reducing lipid peroxidation	Liu et al. (2011)
		Trichoderma virens	*GST*	Enhance Cd tolerance by reducing its uptake, Increase APX, CAT, SOD, GST, and GPX activities	Dixit et al. (2011)
		Methanothermobacter thermoautotrophicum	*MTH1745*	Increase Hg tolerance by enhancing POD and SOD activity, induce high photosynthesis	Chen et al. (2012)

6.5   Heavy Metal Stress and Plant Signaling

The intracellular responses of plants to HMs are a result of functioning of intricate signaling networks to transmit the stimuli from the outside of the cell. Certain biochemical and molecular mechanisms are regulated by the plants as a response to HMs stress in the environment. A large number of signal transduction units work in this regard by making up a signal transduction network. Several signaling cascades work in this regard; they regulate several defense-related genes by perceiving the signals from the receptors at upstream and transmitting them to the nucleus. These signals perceiving receptors in stress conditions have been studied by researchers, for example, *EF-Tu* receptor (*EFR*), protein kinases (*RLK*s), *ERECTA* (ER), and ethylene resistance1/2 (*ETR1/2*) etc. (Sinha et al. 2011; Jalmi and Sinha 2015). In HM stress conditions, hormones signaling, calcium signaling, and MAPK signaling are the major signaling networks. To counteract the HM stress with a network of signaling pathways, metal binding and transporting proteins are synthesized (Peng and Gong 2014).

There are several calcium-sensing proteins which take part in calcium signaling such as CaM like proteins (CMLs), Ca^{2+}-dependent protein kinases (CDPKs), calmodulins (CaMs), and calcineurin B-like proteins (CBLs) (Steinhorst and Kudla 2014). Transmitting a signal in response to environmental heavy metal stress, CDPKs and MAPK work together (Opdenakker et al. 2012). According to a report, Ca plays a role in plants under high Pb stress causing cell death and during this process, CDPK kinases trigger the activity of MAPKs through CDPK-mediated pathway (Huang and Huang 2008).

Different plant hormones are also involved in HM stress responses via hormone signaling (Chen et al. 2014). These plant hormones, for example, auxin, gibberellins, ethylene, cytokinin, etc. help the plants in tolerating the HM stress by remodeling the root system. Auxin homeostasis directly influences the plant responses under HM stress (Potters et al. 2007). *AUX1* and *PIN2* genes are responsible for basipetal transport of auxin via outer cell layers in roots (Rashotte et al. 2000). Similarly, *PIN1* is involved in the inhibition of primary roots elongation by redistributing auxin under Cu toxicity (Yuan and Huang 2016). Expression of auxin-related genes such as Gretchen Hagen (*GH3*) genes, *CYP79B2*, *CYP79B3*, Phosphoribosyl Anthranilate Transferase 1 (*PAT1*), ABCB family, and PIN family is related to HM stress in plants (Wang et al. 2016). In Cd toxicity, lateral root growth is promoted by an increase in the IAA concentration; the process is controlled by *NITRILASE* (*NIT*) biosynthetic gene thus trying to protect the plant from toxic Cd (Vitti et al. 2013). Cytokinins and ethylene also appear in plants when they want to overcome the adverse effects of HMs. For example, Piotrowska-Niczyporuk et al. (2012) reported that Cd destroyed the chloroplast membranes and inhibited the photosynthetic pigments in green alga *Chlorella vulgaris*; however, application of cytokinin restored the primary metabolite level and increased photosynthetic activity. Ethylene induces Al tolerance in plants by regulating the malate ions efflux using *ET8* (Yu et al. 2016).

6.6   Biotechnological Approaches for Improving Plant System Biology Against Metal Stress

6.6.1   Genomics

6.6.1.1   Plant Gene Expression in Response to Heavy Metals

Understanding the plant responses to HMs stress is imperative for improving crop productivity. Specific structural genes maintain the function of cellular components that lead to metal stress tolerance. Therefore, identification, validation, and characterization of genes associated with HM stress is very important. Most of the plant molecular responses against metal stress can be a result of changes in gene expression. Generally, plant stress-responsive genes can be categorized as early responsive genes and delayed responsive genes. The early responsive genes are quickly activated but for a short time. Contrarily, the delayed responsive genes are induced gradually with long-lasting expression. Transcription factors (TFs) which are involved in downstream delayed responses are encoded by early responsive genes. This utilizes signaling components of the cell and no new metabolites are synthesized in the process. Of note, most of the stress-responsive genes are encoded by delayed responsive genes which are expressed as a result of complex regulation.

Predominantly, different researchers have elaborated the effects of heavy metal stress on plant genome, that is, *Arabidopsis thaliana* (Wintz et al. 2003; Weber et al. 2004; Yamasaki et al. 2007; Jagadeeswaran et al. 2014). Although *A. thaliana* does not have strong tolerance toward the metal stress, however, comparison of its genome with that of heavy metal tolerant or hyperaccumulator plants can be interesting. *Thlaspi caerulescens* is a hyperaccumulator model plant species for genomics, proteomics, transcriptomics, and metabolomics technologies as it is well able to grow and thrive in soils contaminated with Cd, Pb, Ni, and Zn (Assunção et al. 2003). Comparison between the genomes of *A. thaliana* and *T. caerulescens* display remarkable differences in gene expression (van de Mortel et al. 2006). Over 2200 genes in roots were differentially expressed in *T. caerulescens* and *A. thaliana.* Similarly, using Affymetrix arrays, Hammond et al. (2006) reported 5000 differentially expressed genes in the shoots of *T. caerulescens* as compared with non-hyperaccumulating plant species *T. arvense.* In both of these studies, the overexpression of genes in *T. caerulescens* was related to metal transport, compartmentalization and homeostasis. The more interesting part was non-induction/expression of these genes in non-adapted plants.

The heavy metal ATPase (*HMA*) genes family in plants is associated with metal absorption, transport and resistance. *HMAs* can be classified into two major subgroups based upon metal substrate specificity, that is, the Zn/Co/Cd/Pb P_{1B}-ATPase group and Cu/Ag P_{1B}-ATPase group (Axelsen and Palmgren 1998a, b). Eight members of P_{1B}-ATPases have been identified in *A. thaliana* (Williams and Mills 2005). Some members of P_{1B}-type ATPases has also been identified in *Arabidopsis halleri*, *T. caerulescens*, wheat, and barley (Deng et al. 2013). Li et al. (2015a) observed differential expression of various *HMA* genes in different tissues against Ag, Cu,

Cd, Zn, Pb, Co, and Mn toxicity in *Populus trichocarpa*. Tissue-specified actions revealed upregulation of *HMA*1 and *HMA*4 genes in leaves. Whereas, *HMA*5.1 and *HMA*8 had high expression levels in roots under metal stress. In some cases, metal ion accumulation and gene overexpression are in direct correlation. For example, Cd accumulation in roots and shoots increases with overexpression of *HMA*3 (Morel et al. 2009a, b). Similarly, rice *HMA*5 is overexpressed under elevated soil Cu concentrations (Deng et al. 2013). *Aeluropus littoralis* manages its phytoremediation potential for Pb and Hg metals by regulating *H⁺-ATPase* gene on the plasma membrane (Jam et al. 2014). Similarly, treatment with Cd resulted in overexpressed serine acetyltransferase (*SAT*) genes family in *Arabidopsis* (Howarth et al. 2003). Evidence suggest that genes encoding for MAPK pathway have important roles in stress signaling and hormone action. Gene functional analysis at large scale with high-throughput technology can be done with functional genomics, that is, proteomics, transcriptomics, ionomics, metabolomics, and phenomics (Rout and Panigrahi 2015; Soda et al. 2015).

6.6.1.2 Utilizing Functional Approaches to Elucidate Plant Stress Responses

Transcriptomics

Studying plant responses to various stresses including HM stress widely use plant transcriptomics. An extensive range of defense-related pathways have been exposed by the overexpression of genes during metal stress. Cell-specific studies and spatial profiling of plant tissues have become possible with more advanced technology of microarrays (Brady et al. 2007; Spollen et al. 2008). Different technologies are in use for transcriptomic studies such as sequence-based approaches, cap analysis of gene expression (CAGE), serial analysis of gene expression (SAGE), massively parallel signature sequencing (MPSS), next generation sequencing (NGS), and RNA sequencing (Kodzius et al. 2006; Tan et al. 2009; Wang et al. 2009; Paicu et al. 2017; Lowe et al. 2017). Using these technologies, specific transcripts of many plant species have been quantified and identified stress tolerant pathways in plants like rice, maize, soybean, and *Arabidopsis* (Deyholos 2010; Pu and Brady 2010; Rogers et al. 2012).

The genes triggered by stressors are categorized into two types, that is, (1) regulatory genes and (2) functional genes (Tran et al. 2010). Regulatory type of genes constitutes a gene network and encode various TFs. Whereas, functional type genes encode various metabolites, for example, alcohols, sugars, and amines. These metabolites are crucial in rendering plant tolerant to heavy metal stress. The TFs are normally members of multigenic families and control expressions of many genes, thus considered as master regulators. TFs bind at specific binding sites of cis-acting elements in the target gene promoters and thereby regulate the genes expression (Wray et al. 2003). Several TFs families have been recognized so far to regulate the stress responses in plants such as, *EMF1, CCAATDR1, AP2/EREBP, AREB/ABF,*

C2H2, C2C2-Dof, C3H, C2C2-Gata, C2C2-YABBY, C2C2-CO-like, ARID, CCAAT-HAP2, CCAAT-HAP3, CCAAT-HAP5, CPP, SBP, E2F-DP, WRKY, bZIP, HSF, E2F-DP, AtSR, MADS, MYB, MYC, bHLH, HB, ABI3VP1, DREB1/CBF, ARF, NAC, and *TUB* (Singh et al. 2002; Shameer et al. 2009; Noman et al. 2017e).

Ramos et al. (2007) reported induction of zinc finger and basic region leucine zipper (bZIP) TFs in *B. juncea* and *A. thaliana* in response to Cd stress. TFs were also found in *A. halleri* under Cd stress (Weber et al. 2006). Similarly, two other TFs, that is, *ERF*1 and *ERF*5 from *AP2/ERF* super family have been induced in *A. thaliana* facing Cd toxicity (Herbette et al. 2006). In these plants, differential expression of TFs might be due to different levels of Cd stress. Norton et al. (2008) applied sodium arsenate to arsenate tolerant rice plants to perform transcriptomic analysis and found the expression of several TFs, stress protein genes and transporters. They found about 576 upregulated and 622 downregulated genes. Of note, most of the genes were related to glutathione metabolism, synthesis, and transport. Similarly, transcriptomic profiling of Al-sensitive genotype of maize (S1587-17) was performed under high level of Al in the soil. Differential expression of genes was evident with upregulation of genes for ethylene, auxin, and lignin biosynthesis which is positively correlated with inhibition of root growth (Mattiello et al. 2010). Under Fe deficiency, several genes are found to be upregulated in leaves and roots of *A. thaliana* such as *FIT*, *AtbHLH*38, *AtbHLH*39, *AtbHLH*100, and *AtbHLH*101 (Wang et al. 2007; Yuan et al. 2008). Due to upregulation of these genes, TFs are generated for ferrous transporters and ferric chelate reductases that are critical in regulating Fe uptake in plants under Fe deficiency (Varotto et al. 2002; Yuan et al. 2008). Moreover, several other Fe transporters get activated when *FIT* and *AtbHLH*38 or *AtbHLH*39 genes are expressed together, for example. *HMA*3, Iron-Regulated Transporter 2 (*IRT*2), and *MTP*3 and adequate intercellular Fe level (Wu et al. 2012).

Mitogen-Activated Protein Kinase (MAPK) cascade also get activated in response to HM stress. There are three types of kinases, that is, MAPK, MAPK kinase (MAPKK), and MAPK kinase kinase (MAPKKK) which play a major role for hormone synthesis and signal transduction pathway activation under stressed conditions (Jonak et al. 2002). In *Medicago sativa,* these pathways get activated under Cd and/or Cu stress that results in regulation of TFs for biosynthesis of defense, chelating compounds and metal transporters (Jonak et al. 2004).

Micro RNAs (miRNAs) are well modulators of heavy metal stress tolerance by regulating the number of TFs for stress signaling (Noman et al. 2017a; Noman and Aqeel 2017). miRNAs fight with stressors by regulating various cellular and metabolic processes, for example, cell proliferation, transcription regulation, and apoptosis. Their role in regulating abiotic stress tolerance has been identified in a number of plant species, for example, *Arabidopsis, Medicago*, and *Phaseolus* (Trindade et al. 2010; Budak et al. 2015). In *B. vulgaris,* 13 different miRNAs were identified for their involvement in metal stress regulation (Li et al. 2015a, b). miRNAs aid molecular mechanisms by making plants tolerant against heavy metal stress (Fang et al. 2013). Different transcripts, for example, miR156, miR171, and miR396a have reportedly been downregulated in *Brassica napus* exposed to Cd stress (Zhou et al. 2012a, b). In modified miRNAs expression profiles of *Medicago* under various levels of Cd stress, miR166 was reported to be downregulated while miR393,

miR171, miR319, and miR529 were found to be upregulated (Zhou et al. 2008). Similarly, Al stress in plants is also regulated by miRNAs. Comparison between two subspecies of rice, that is, *Oryza indica* and *Oryza japonica* for miRNAs expression levels revealed different responses under Al stress. A complex response was indicated by the appearance of 16 different kinds of miRNAs (Lima et al. 2011). Under Hg stress, miR171, miR319, miR393, and miR529 were found upregulated in *M. truncatula* (Zhou et al. 2008). Similarly, other scientists reported the downregulation of miR398 in response to Hg or Cd toxicity and nutrient deficiency in plants (Kuo and Chiou 2011; Yang and Chen 2013) indicating the miRNA398-mediated ROS regulation under nutrient stress conditions.

In As stress, on account of differential expression, 18 different miRNAs were identified in Chinese rice (Liu and Zhang 2012) and 69 novel miRNAs were reported in *B. juncea* (Srivastava et al. 2012). In another study, exogenously applied JA and IAA changed the expression of miR167, miR319, and miR854 under As stress, which in turn positively influenced the plant growth (Gupta et al. 2014). Similarly, oxidative stress in rice lead to differential expression of seven miRNAs having genes encoding for nutrient transport, transcriptional regulation, programmed cell death, auxin homeostasis, and cell proliferation (Li et al. 2010).

Metabolomics

Physiological state of living cells can be determined with the help of metabolites which carry energy for growth and maintenance. All the metabolites characterization of an organism under a set of environmental conditions is called metabolomics. They denote physiological state as they are key link between phenotype and genetics of an organism. They reflect the genetic information of an individual because they correlate with variety of biosynthetic pathways. The quantity of metabolites is directly proportional to physiological activities of plants depending upon surrounding environmental conditions (Bundy et al. 2005). Physiological activities of heavy metal tolerant plants also expected to be changed in stress conditions. Therefore, under normal and stressed physiological status, acquiring the detailed knowledge of metabolome in the plant can be significant and is called metabolomics. Stress signals stimulate the plant receptors thereby activating the stress-responsive genes which synthesize specific metabolites to help in adapting environmental stress (Nakabayashi and Saito 2015). In this way, typical stress-responsive genes and alterations in physiological pathways can be identified to screen stress tolerant plants. Metabolomics is overwhelmingly used to understand plants responses to abiotic stress tolerance (Jia et al. 2016). In the past few years, high-throughput methodologies were developed to analyze metabolites. A number of analytical technologies such as liquid and gas chromatography, nuclear magnetic resonance spectroscopy (NMR), matrix-assisted laser desorption ionization (MALDI), and inductively coupled mass spectrometry are used to analyze metabolites in plants. Metabolomic studies have been get boosted by the recent advancements in molecular techniques (Morrow 2010). The role of various metabolites in tolerating heavy metal stress is discussed in the next section.

Free Amino Acids

Plants accumulate various compatible solutes in response to different environmental stresses (Serraj and Sinclair 2002; Akula and Ravishankar 2011). These include proline, trehalose, polyols, sucrose, histidine, proline, glycine, betaine, pipecolate betaine, and alanine betaine (Ashraf and Harris 2004; Sharma and Dietz 2006). The plants produce extraordinary high levels of these free amino acids as a nonenzymatic response to combat the negative effects of environmental stresses including HM stress. They provide protection to plants by regulating ROS detoxification, cellular osmotic adjustment, protein/enzymes stabilization, and providing protection to membrane integrity (Bohnert and Jensen 1996; Ashraf and Foolad 2007). The level of proline increases many folds in plants under heavy metal toxicity (Talanova et al. 2000; Sharma and Dietz 2006; Szabados and Savouré 2010). When it comes to opposing stress, proline has been identified to play diverse roles in plants, for example, as signaling molecule, metal chelator, and antioxidant defense molecule (Hayat et al. 2012). It also strengthens the metal-quenching ability of other antioxidant enzymes (Emamverdian et al. 2015). Elevated levels of proline have been seen in lower plants, for example, algae and lichens (Siripornadulsil et al. 2002; Bačkor and Loppi 2009) as well as in higher plants under heavy metal toxicity (Nedjimi and Daoud 2009; Belhaj et al. 2016). Genes encoding proline synthesis can be cloned to confer heavy metal tolerance to transgenic plants.

Another important metabolite, putrescine has been found to play crucial role in plants under abiotic stresses. It is also precursor of spermine and spermidine in plants. Significantly high quantity of putrescine was found in bean and oat plants exposed to Cu and Cd stress (Weinstein et al. 1986). Similarly, Lin and Kao (1999) also reported increased quantity of putrescine in rice under Cu stress. Increased levels of histidine were also reported by Krämer et al. (1996) in xylem sap of Ni hyperccumulating plant *Alyssum lesbiacum* under Ni stress (Singh et al. 2016).

α-Tocopherol

In plants, alpha-Tocopherols are normally synthesized in plastids and are active forms of vitamin E. Their concentration increases under stress conditions. Besides scavenging ROS very efficiently, they are involved in terminating chain reactions in lipid peroxidation (Munne-Bosch 2005; Maeda and DellaPenna 2007). Therefore, these appear to be guard of cell membranes against the damaging effects of stresses. Before being degraded by resonance energy transfer, one tocopherol molecule can deactivate up to 220 molecules of O_2. The tocopherol level inside the plants alters dramatically with the intensity of environmental stresses. The involvement of α-tocopherol in regulating plant activities under heavy metal stress has been addressed by many studies (Collin et al. 2008; Lushchak and Semchuk 2012). Collin et al. (2008) reported the accumulation of α-tocopherol in *A. thaliana* under Cd and Cu stress. Comparison between two mutants of *A. thaliana* clearly proved the link between heavy metal stress alleviation and α-tocopherol production.

Ascorbate

Ascorbate is a low molecular weight and abundantly found antioxidant in plants also known as vitamin C. It is crucial element of defense against ROS activity (Noman et al. 2014). Ascorbate normally exists as reduced form in chloroplast and actively protects the macromolecules from detrimental consequences of oxidative stress. It plays role in many important physiological functions of plants such as growth, metabolism, and differentiation. Because of its great ability to donate electrons to various enzymatic and nonenzymatic pathways, it is known as a powerful antioxidant. D-mannose/L-galactose are main contributing elements of ascorbate through Smirnoff-Wheeler pathway (Wheeler et al. 1998). It is synthesized in mitochondria and transported by facilitated diffusion or through a proton-electrochemical gradient to other cell components. It is abundantly found in photosynthetic tissues; however, it has also been spotted in other cell types, apoplasts, cytoplasm, and organelles in plants (Smirnoff et al. 2004; Shao et al. 2008). Among these, apoplastic ascorbic acid performs the most important role and considered as prime defense line against ROS (Barnes et al. 2002). The level of ascorbate changes in plants in response to heavy metal stresses (Maheshwari and Dubey 2009; Mishra et al. 2011; Srivastava and Dubey 2011; Soares et al. 2016). The level of ascorbate in plants depends upon the intensity of environmental stress (Chaves et al. 2002). Overexpression of biosynthetic enzymes of ascorbate is involved in enhancing plant tolerance to abiotic stress. In tomato plants, overexpression of GME gene family members is involved in biosynthesis and accumulation ascorbate in response to oxidative stress (Zhang et al. 2011). Similarly, in *A. thaliana*, enhanced ascorbate content helped combating oxidative stress (Wang et al. 2010).

Glutathione

Glutathione (γ-glutamylcysteinyl-glycine, GSH) is a nonprotein low molecular weight thiol that acts against the toxic effects of ROS inside the cells. It is found in all cellular organelles such as chloroplasts, vacuoles, endoplasmic reticulum, mitochondria, and cytosol (Foyer and Noctor 2003). GSH has good reducing power and in several ways it acts as an antioxidant. It acts as free radical scavenger by directly reacting with H_2O_2, $\cdot OH$, and $O_2^{\cdot-}$. It is involved in many cellular processes such as signal transduction, cell growth, synthesis of phytochelatins, synthesis of nucleic acids and proteins, enzymatic regulation, conjugation of metabolites, detoxification of xenobiotics, regulation of sulfate transport, and expression of the stress-responsive genes (Foyer et al. 1997). GSH is formed from oxidized glutathione (GSSG) by the enzyme glutathione reductase (GR). Under metal stress, the disulfide bridge of GR breaks off (Lee et al. 1998). Hence, GR enzyme plays crucial role in antioxidant defense by maintaining a high GSH/GSSG ratio. In the presence of ROS, GSH care for macromolecules by acting either as proton donor or by forming adducts with reactive electrophiles and yield GSSG (Asada 1994). GSH is also involved in the regeneration of ascorbate. Singh et al. (2015) reported increments in

GR activity in *Luffa* seedlings with increasing As concentration. Similarly, increased activity of GSH was noted in *Helianthus annuus* in response to multimetal toxicity (Belhaj et al. 2016).

Phenols and Carotenoids

Phenols are abundantly found diverse secondary metabolites in plants such as tannins, lignin, hydroxycinnamate esters, and flavonoids (Grace and Logan 2000). Due to their great ability to donate hydrogen atoms or electrons, they have strong antioxidant properties. They have great ability to chelate metal ions and reduce heavy metal toxicity (Jun et al. 2003). These inhibit lipid peroxidation and directly scavenge ROS. Phenols hinder free radical diffusion through the membranes by modifying lipid packing order and decreasing fluidity of cell membranes (Arora et al. 2000). Phenols are involved in chelating Fe ions and thus reduce ROS production by suppressing Fenton's reaction. Janas et al. (2009) reported the accumulation of phenolic compounds in lentil roots exposed to Cu stress. They were of the opinion that the accumulation of phenols is in response to production of ROS. Tannins chelate Mn ions reducing Mn toxicity in tannin rich tea plants (Lavid et al. 2001). Zayneb et al. (2015) reported high phenolic and flavonoid contents in *Trigonella foenum-graecum* exposed to Cd stress. Increased flavonoid contents were also seen in wheat growing in the presence of heavy metals (Copaciu et al. 2016). In another study, improved antioxidant capacity of transgenic potato plants was due to the accumulation of flavonoids (Lukaszewicz et al. 2004). Carotenoids are lipophilic antioxidants and detoxify various kinds of ROS (Young 1991). Carotenoids absorb light energy for chlorophyll. They scavenge ROS and protect the photosynthetic apparatus by preventing the formation of O_2 in excited chlorophyll molecules. They also act as signaling molecule to environmental stress responses (Li et al. 2008). Their concentrations increase in plants exposed to heavy metal stress (Wang et al. 2014; Piotrowska-Niczyporuk et al. 2015; Soares et al. 2016).

Phenomics

Phenotypes of plants are a result of genotypes or a set of genetic instructions and interactions with the environment. It is generally difficult to predict phenotype from genotype due to the involvement of so many genes and gene products under the influence of complex and instable environmental conditions. Study of full set of phenotypic traits translated by genetic instructions from a gene or the entire genome of an organism is called phenomics. Phenotypes can be described at several levels, from a single molecule to complex cellular, physiological, and developmental dynamic metabolic networks. Additional effects of phenotypic complexity can be created by interactions with competing organisms, pathogens, and symbionts. Moreover, phenotypes are dynamic and change tremendously over the course of time.

For genetic improvement in plants, accurate and precise measurement of traits is very critical. It has broad spectrum importance for yield improvement in food crops

and environmental remediation by plants. Several large-scale approaches are used to study phenomics including forward and reverse phenomics which can help to identify desirable traits which make a genotype the best. Genotype of a species, genus, several representatives of a population or phylogenetic group can be determined with next generation sequencing methods. The high-throughput technologies include near infrared imaging for the measurement of cellular and soil water contents; far infrared imaging for measuring the temperature of leaf/canopy; color imaging for plant structure, leaf health, phenology, and biomass; fluorescence imaging for assessing the physiological efficiency of photosynthetic machinery (Kumar et al. 2015).

Interactions between genotypes and environment pressurize the phenotype. Hence, collecting large data at different developmental stages for multiple organ types across multiple environments would be ideal. For studying a specific problem, cell types, organs, or the whole plants are identified with genome of interest. Then, genome sequencing and/or transcriptomics are used for characterizing mRNAs and gene spaces. The candidate genes are discovered through construction of metabolic networks and large-scale proteomic analysis. A tremendous number of molecular markers can be generated by resequencing of genetic variants. Traditional genetic mapping or genome-wide association and other modern approaches are used to find target genes for specific phenotypes (Rounsley and Last 2010).

6.7 Conclusions and Future Prospects

Heavy metal stress tolerance is difficult to control and engineer due to the involvement of several signaling pathway components and many genes. The original systems for metal uptake, its translocation, sequestration, and detoxification help plants to combat HMs stress. To confer heavy metal stress tolerance in plants, we require extensive knowledge for the identification and validation of novel HM responsive genes, TFs, miRNAs, gene products and linkage of their expression profiles with the consequent targets. Advanced understanding of the dogmatic mechanisms and linked components like TFs or miRNAs would result in the purposeful development of the plant tolerance to metal stress. It is now possible to identify specific changes in biochemical and molecular networks of tissues and cells in response to heavy metal stress by using functional genomics and phenomics technologies. By using techniques like omics approach, genes and their target validation will offer sufficient evidence for approval or disapproval of their role in metal tolerance. These omics approaches have amplified our understanding of gene expression mechanisms. The production of stress tolerant crops is becoming more possible with increased understanding of molecular pathways behind heavy metal stress tolerance. Modifications in the target genes expression level of newly identified miRNAs or other genes would also give new elements of plants HM tolerance mechanism.

With the advancement and advent of new omic technologies for investigation of cellular complexity, we are in a position to have a better look at stress tolerant mechanisms. Thousands of stress-related genes have been identified so far and this

trend is increasing with improvement of sequencing technologies. Surprising results have been obtained by genetic engineering of metabolites, proteins, and heavy metal stress-responsive genes. There is a need to exploit full potential of functional genomics and phenomics. With the employment of multidisciplinary approach using transcriptomics, metabolomics, and phenomics, heavy metal stress tolerant valuable crop plants can be produced in future. Amalgamation of described knowledge about omics, regulatory networks and targets would guide us to incremented regulation of HM stress in different crops.

References

Adrees M, Ali S, Rizwan M, Ibrahim M, Abbas F, Farid M, Zia-ur-Rehman M, Irshad MK, Bharwana SA (2015) The effect of excess copper on growth and physiology of important food crops: a review. Environ Sci Pollut Res 22(11):8148–8162

Ahmad P, Nabi G, Ashraf M (2011) Cadmium-induced oxidative damage in mustard [*Brassica juncea* (L.) Czern. & Coss.] plants can be alleviated by salicylic acid. South Afric J Bot 77(1):36–44

Ahmad R, Ali S, Hannan F, Rizwan M, Iqbal M, Hassan Z, Akram NA, Maqbool S, Abbas F (2017) Promotive role of 5-aminolevulinic acid on chromium-induced morphological, photosynthetic, and oxidative changes in cauliflower (*Brassica oleraceabotrytis* L.). Environ Sci Pollut Res 24:8814–8824

Akula R, Ravishankar GA (2011) Influence of abiotic stress signals on secondary metabolites in plants. Plant Signal Behav 6(11):1720–1731

Ali B, Qian P, Jin R, Ali S, Khan M, Aziz R, Tian T, Zhou W (2014) Physiological and ultra-structural changes in *Brassica napus* seedlings induced by cadmium stress. Biol Plant 58(1):131–138

Ali S, Rizwan M, Ullah N, Bharwana SA, Waseem M, Farooq MA, Abbasi GH, Farid M (2016) Physiological and biochemical mechanisms of silicon-induced copper stress tolerance in cotton (*Gossypium hirsutum* L.). Acta Physiol Plant 38:1–11

Andrés-Colás N, Sancenón V, Rodríguez-Navarro S, Mayo S, Thiele DJ, Ecker JR, Puig S, Peñarrubia L (2006) The Arabidopsis heavy metal P-type ATPase HMA5 interacts with metal-lochaperones and functions in copper detoxification of roots. Plant J 45:225–236

Anwer S, Khan S, Ashraf MY, Noman A, Baloch SU, Zafar S, Fahad S (2017) Impact of chelator-induced phytoextraction of cadmium on yield and ionic uptake of maize. Int J Phytoremediation 19(6):505–513. https://doi.org/10.1080/15226514.2016.1254153

Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55:373–399. https://doi.org/10.1146/annurev.arplant.55.031903.141701

Arbaoui S, Slimane RB, Rezgui S, Bettaieb T (2014) Metal transporters for uptake, sequestration and translocation. In: Gupta DK, Chatterjee S (eds) Heavy metal remediation: transport and accumulation in plants. Nova Science Publishers, New York, pp 29–44

Arora A, Byrem TM, Nair MG, Strasburg GM (2000) Modulation of liposomal membrane fluidity by flavonoids and isoflavonoid. Arch Biochem Biophys 373(1):102–109

Arshad M, Ali S, Noman A, Ali Q, Rizwan M, Farid M, Irshad MK (2016) Phosphorus amendment decreased cadmium (Cd) uptake and ameliorates chlorophyll contents, gas exchange attributes, antioxidants and mineral nutrients in wheat (*Triticum aestivum* L.) under Cd stress. Arch Agron Soil Sci 62(4):533–546. https://doi.org/10.1080/03650340.2015.1064903

Asada K (1994) Production and action of active oxygen species in photosynthetic tissues. In: Foyer CH, Mullineaux PM (eds) Causes of photooxidative stress and amelioration of defense systems in plants. CRC Press, Boca Raton, FL, pp 77–104

Ashraf M, Foolad MR (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ Exp Bot 59:206–216. https://doi.org/10.1016/j.envexpbot.2005.12.006

Ashraf M, Harris PJC (2004) Potential biochemical indicators of salinity tolerance in plants. Plant Sci 166:316. https://doi.org/10.1016/j.plantsci.2003.10.024

Assunção AGL, Schat H, Aarts MGM (2003) *Thlaspi caerulescens*, an attractive model species to study heavy metal hyperaccumulation in plants. New Phytol 159:351–360

Assunção AGL, Herrero E, Lin Y, Huettel B, Talukdar S, Smaczniak C, Immink RGH, Eldik M, Fiers M, Schat H, Aarts MGM (2010) *Arabidopsis thaliana* transcription factors bZIP19 and bZIP23 regulate the adaptation to zinc deficiency. Proc Nat Acad Sci USA 107:10296–10301

Atha DH, Wang H, Petersen EJ, Cleveland D, Holbrook RD, Jaruga P, Nelson BC (2012) Copper oxide nanoparticle mediated DNA damage in terrestrial plant models. Environ Sci Technol 46:1819–1827

Atkinson NJ, Urwin PE (2012) The interaction of plant biotic and abiotic stresses: from genes to the field. J Exp Bot 63:3523–3544. https://doi.org/10.1093/jxb/ers100

ATSDR (Agency for Toxic Substances and Disease Registry) (2015) Toxicological profile for cadmium. http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=48&tid=15. Accessed 21 June 2017

Axelsen KB, Palmgren MG (1998a) Evolution of substrate specificities in the P-type ATPase superfamily. J Mol Evol 46:84–101. https://doi.org/10.1007/PL00006286

Axelsen KB, Palmgren MG (1998b) Inventory of the superfamily of P-Type ion pumps in Arabidopsis. Plant Physiol 126:696–706

Bačkor M, Loppi S (2009) Interactions of lichens with heavy metals. Biol Plant 53(2):214–222

Barnes JD, Zheng Y, Lyons TM (2002) Plant resistance to ozone: the role of ascorbate. In: Omasa K, Saji H, Youssefian S, Kondo N (eds) Air pollution and plant biotechnology. Springer, Tokyo, Japan, pp 235–254

Barrameda-Medina Y, Montesinos-Pereira D, Romero L, Blasco B, Ruiz JM (2014) Role of GSH homeostasis under Zn toxicity in plants with different Zn tolerance. Plant Sci 227:110–121

Bashri G, Prasad SM (2015) Indole acetic acid modulates changes in growth, chlorophyll a fluorescence and antioxidant potential of *Trigonella foenum-graecum* L. grown under cadmium stress. Acta Physiol Plant 37:1745. https://doi.org/10.1007/s11738-014-1745-z

Basu U, Good AG, Taylor GJ (2001) Transgenic *Brassica napus* plants overexpressing aluminium-induced mitochondrial manganese superoxide dismutase cDNA are resistant to aluminium. Plant Cell Environ 24:1278–1269. https://doi.org/10.1046/j.0016-8025.2001.00783.x

Belhaj D, Elloumi N, Jerbi B, Zouari M, Abdallah FB, Ayadi H, Kallel M (2016) Effects of sewage sludge fertilizer on heavy metal accumulation and consequent responses of sunflower (*Helianthus annuus*). Environ Sci Pollut Res 23(20):20168–20177

Bellion M, Courbot M, Jacob C, Guinet F, Blaudez D, Chalot M (2007) Metal induction of a *Paxillus involutus* metallothionein and its heterologous expression in *Hebeloma cylindrosporum*. New Phytol 174:151–158. https://doi.org/10.1111/j.1469-8137.2007.01973.x

Bhattachrjee S (2005) Reactive oxygen species and oxidative burst: roles in stress, senescence and signal transduction in plant. Curr Sci 89:1113–1121

Bohnert HJ, Jensen RG (1996) Strategies for engineering water-stress tolerance in plants. Trends Biotechnol 14:89–97. https://doi.org/10.1016/01677799(96)80929-2

Brady SM, Orlando DA, Lee JY, Wang JY, Koch J, Dinneny JR et al (2007) A high-resolution root spatiotemporal map reveals dominant expression patterns. Science 318:801–806

Budak H, Kantar M, Bulut R, Akpinar BA (2015) Stress responsive miRNAs and isomiRs in cereals. Plant Sci 235:1–13. https://doi.org/10.1016/j.plantsci.2015.02.008

Bundy JG, Willey TL, Castell RS, Ellar DJ, Brindle KM (2005) Discrimination of pathogenic clinical isolates and laboratory strains of *Bacillus cereus* by NMR-based metabolomic profiling. FEMS Microbiol Lett 242:127–136. https://doi.org/10.1016/j.femsle.2004.10.048

Caille N, Zhao FJ, McGrath SP (2005) Comparison of root absorption, translocation and tolerance of arsenic in the hyperaccumulator *Pteris vittata* and the nonhyperaccumulator *Pteris tremula*. New Phytol 165:755–761. https://doi.org/10.1111/j.1469-8137.2004.01239.x

Callahan DL, Baker AJM, Kolev SD, Wedd AG (2006) Metal ion ligands in hyperaccumulating plants. J Biol Inorg Chem 11:2–12

Carneiro JM, Chacón-Madrid K, Galazzi RM, Campos BK, Arruda SC, Azevedo RA, Arruda MA (2017) Evaluation of silicon influence on the mitigation of cadmium-stress in the development of *Arabidopsis thaliana* through total metal content, proteomic and enzymatic approaches. J Trace Elem Med Biol 44:50–58

Chaurasia N, Mishra Y, Rai LC (2008) Cloning expression and analysis of phytochelatin synthase (pcs) gene from *Anabaena* sp. PCC 7120 offering multiple stress tolerance in *Escherichia coli*. Biochem Biophys Res Commun 376:225–230. https://doi.org/10.1016/j.bbrc.2008.08.129

Chaves MM, Pereira JS, Maroco J et al (2002) How plants cope with water stress in the field. Photosynth Growth Ann Botany 89:907–916

Chen LM, Lin CC, Kao CH (2000) Copper toxicity in rice seedlings: changes in antioxidative enzyme activities, H_2O_2 level, and cell wall peroxidase activity in roots. Bot Bull Acad Sinica 41:99–103

Chen Z, Pan Y, Wang S, Ding Y, Yang W, Zhu C (2012) Overexpression of a protein disulfide isomerase-like protein from *Methanothermobacter thermoautotrophicum* enhances mercury tolerance in transgenic rice. Plant Sci 197:10–20. https://doi.org/10.1016/j.plantsci.2012.08.005

Chen YA, Chi WC, Trinh NN, Huang LY, Chen YC, Cheng KT (2014) Transcriptome profiling and physiological studies reveal a major role for aromatic amino acids in mercury stress tolerance in rice seedlings. PLoS One 9:95163. https://doi.org/10.1371/journal.pone.0095163

Collin VC, Eymery F, Genty B, Rey P, Havaux M (2008) Vitamin E is essential for the tolerance of *Arabidopsis thaliana* to metal induced oxidative stress. Plant Cell Environ 31:244–257. https://doi.org/10.1111/j.1365-3040.2007.01755.x

Copaciu F, Opriş O, Niinemets Ü, Copolovici L (2016) Toxic influence of key organic soil pollutants on the total flavonoid content in wheat leaves. Water Air Soil Pollut 227(6):196

Corpas FJ, Palma JM, Sandalio LM, Valderrama R, Barroso JB, del Río LA (2008) Peroxisomal xanthine oxidoreductase: characterization of the enzyme from pea (*Pisum sativum* L.) leaves. J Plant Physiol 165(13):1319–1330

Craciun AR, Meyer C-L, Chen J, Roosens N, Groodt RD, Hilson P et al (2012) Variation in HMA4 gene copy number and expression among *Noccaea caerulescens* populations presenting different levels of Cd tolerance and accumulation. J Exp Bot 63:4179–4189

Curie C, Alonso JM, Le Jean M, Ecker JR, Briat JF (2000) Involvement of NRAMP1 from *Arabidopsis thaliana* in iron transport. Biochem J 347:749–755

DalCorso G, Manara A, Furini A (2013) An overview of heavy metal challenge in plants: from roots to shoots. Metallomics 5:1117–1132. https://doi.org/10.1039/c3mt00038a

Das K, Roychoudhury A (2016) Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. In: Khan NA, Anjum NA, Sofo A, Kizek R, Baier M (eds) Redox homeostasis managers in plants under environmental stresses. Frontiers in Environmental Science, pp 53–65

De Dorlodot S, Lutts S, Bertin P (2005) Effects of ferrous iron toxicity on the growth and mineral composition of an interspecific rice. J Plant Nutr 28:1–20. https://doi.org/10.1081/PLN-200042144

Del Río LA, Sandalio LM, Corpas FJ, Palma JM, Barroso JB (2006) Reactive oxygen species and reactive nitrogen species in peroxisomes. Production, scavenging, and role in cell signaling. Plant Physiol 141(2):330–335

Deng F, Yamaji N, Xia J, Ma JF (2013) A member of the heavy metal P-type ATPase OsHMA5 is involved in xylem loading of copper in rice. Plant Physiol 163:1353–1362. https://doi.org/10.1104/pp.113.226225

Deyholos MK (2010) Making the most of drought and salinity transcriptomics. Plant Cell Environ 33:648–654

Dixit P, Mukherjee PK, Ramachandran V, Eapen S (2011) Glutathione transferase from *Trichoderma virens* enhances cadmium tolerance without enhancing its accumulation in transgenic *Nicotiana tabacum*. PLoS One 6:16360. https://doi.org/10.1371/journal.pone.0016360

Dräger BD, Desbrosses-Fonrouge AG, Krach C, Chardonnens AN, Meyer RC, Saumitou-Laprade P, Krämer U (2004) Two genes encoding *Arabidopsis halleri* MTP1 metal transport proteins co-segregate with zinc tolerance and account for high MTP1 transcript levels. Plant J 39:425–439

Durrett TP, Gassmann W, Rogers EE (2007) The FRD3-mediated efflux of citrate into the root vasculature is necessary for efficient iron translocation. Plant Physiol 144:197–205

Emamverdian A, Ding Y, Mokhberdoran F, Xie Y (2015) Heavy metal stress and some mechanisms of plant defense response. Sci World J 2015:756120. https://doi.org/10.1155/2015/756120

Ezaki B, Gardner RC, Ezaki Y, Matsumoto H (2000) Expression of aluminum induced genes in transgenic *Arabidopsis* plants can ameliorate aluminum stress and/or oxidative stress. Plant Physiol 122:657–666

Ezaki B, Katsuhara M, Kawamura M, Matsumoto H (2001) Different mechanisms of four aluminum (Al)-resistant transgenes for Al toxicity in Arabidopsis. Plant Physiol 127:918–927

Faller P, Kienzler K, Krieger-Liszkay A (2005) Mechanism of Cd2+ toxicity: Cd2+ inhibits photo activation of Photosystem II by competitive binding to the essential Ca2+ site. Biochimica et BiophysicaActa 1706(1–2):158–164

Fang X, Zhao Y, Ma Q, Huang Y, Wang P, Zhang J, Nian H, Yang C (2013) Identification and comparative analysis of cadmium tolerance associated miRNAs and their targets in two soybean genotypes. PLoS One 8:81471

Farias JG, Antes FLG, Nunes PAA, Nunes ST, Schaich G, Rossato LV et al (2013) Effects of excess copper in vineyard soils on the mineral nutrition of potato genotypes. Food Energy Security 2:49–69. https://doi.org/10.1002/fes3.16

Foyer CH, Noctor G (2003) Redox sensing and signaling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. Physiol Plant 119(3):355–364

Foyer CH, Noctor G (2005) Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. Plant Cell 17:1866–1875

Foyer CH, Lopez-Delgado H, Dat JF, Scott JM (1997) Hydrogen peroxide- and glutathione-associated mechanisms of acclimatory stress tolerance and signaling. Physiol Plant 100(2):241–254

Fozia A, Muhammad AZ, Muhammad A, Zafar MK (2008) Effect of chromium on growth attributes in sunflower (*Helianthus annuus* L.). J Environ Sci 20(12):1475–1480

Freeman JL, Garcia D, Kim D, Hopf A, Salt DE (2005) Constitutively elevated salicylic acid signals glutathione-mediated nickel tolerance in *Thlaspi* nickel hyperaccumulators. Plant Physiol 137:1082–1091. https://doi.org/10.1104/pp.104.055293

Gaber A, Yoshimura K, Yamamoto T, Yabuta Y, Takeda T, Miyasaka H, Nakano Y, Shigeoka S (2006) Glutathione peroxidase-like protein of Synechocystis PCC 6803 confers tolerance to oxidative and environmental stresses in transgenic *Arabidopsis*. Physiol Plant 128:251–262

Gadallah MAA (1999) Effects of proline and glycine betaine on *Vicia faba* responses to salt stress. Biol Plant 42(2):249–257

Galeas ML, Zhang LH, Freeman JL, Wegner M, Pilon-Smits EAH (2007) Seasonal fluctuations of selenium and sulphur accumulation in selenium hyperaccumulators and related nonaccumulators. New Phytol 173:517–525

Garg N, Singla P (2011) Arsenic toxicity in crop plants: physiological effects and tolerance mechanisms. Environ Chem Lett 9(3):303–321

Garzón T, Gunsé B, Moreno AR, Tomos AD, Barceló J, Poschenrieder C (2011) Aluminium-induced alteration of ion homeostasis in root tip vacuoles of two maize varieties differing in Al tolerance. Plant Sci 180:709–715. https://doi.org/10.1016/j.plantsci.2011.01.022

Gichner T, Patkova Z, Szakova J, Demnerova K (2004) Cadmium induces DNA damages in tobacco roots, but no DNA damage, somatic mutations or homologous recombinations in tobacco leaves. Mut Res Genet Toxicol Environ Mutagen 559:49–57. https://doi.org/10.1016/j.mrgentox.2003.12.008

Gill SS, Hasanuzzaman M, Nahar K, Macovei A, Tuteja N (2013) Importance of nitric oxide in cadmium stress tolerance in crop plants. Plant Physiol Biochem 63:254–261. https://doi.org/10.1016/j.plaphy.2012.12.001

Grace SG, Logan BA (2000) Energy dissipation and radical scavenging by the plant phenylpropanoid pathway. Philos Trans R Soc B 355(1402):1499–1510

Guan Z, Chai T, Zhang Y, Xu J, Wei W (2009) Enhancement of Cd tolerance in transgenic tobacco plants overexpressing a Cd-induced catalase cDNA. Chemosphere 76(5):623–630

Guerinot ML (2000) The ZIP, family of metal transporters. Biochim Biophys Acta 1465:190–198

Guo J, Dai X, Xu W, Ma M (2008) Overexpressing *GSHI* and *AsPCSI* simultaneously increases the tolerance and accumulation of cadmium and arsenic in *Arabidopsis thaliana*. Chemosphere 72:1020–1026. https://doi.org/10.1016/j.chemosphere.2008.04.018

Gupta O, Sharma P, Gupta R, Sharma I (2014) MicroRNA mediated regulation of metal toxicity in plants: present status and future perspectives. Plant Mol Biol 84:1–18

Gustin JL, Loureiro ME, Kim D, Na G, Tikhonova M, Salt DE (2009) MTP1-dependent Zn sequestration into shoot vacuoles suggests dual roles in Zn tolerance and accumulation in Zn hyperaccumulating plants. Plant J 57:1116–1127

Halliwell B, Gutteridge JMC (2007) Free radicals in biology and medicine, 5th edn. Oxford University Press

Hammond JP, Bowen HC, White PJ, Mills V, Pyke KA, Baker AJ, Whiting SN, May ST, Broadley MR (2006) A comparison of the *Thlaspi caerulescens* and *Thlaspi arvense* shoot transcriptomes. New Phytol 170:239–260

Hanikenne M, Talke IN, Haydon MJ, Lanz C, Nolte A, Motte P, Kroymann J, Weigel D, Krämer U (2008) Evolution of metal hyperaccumulation required cisregulatory changes and triplication of HMA4. Nature 453:391–395

Hayat S, Hayat Q, Alyemeni MN, Wani AS, Pichtel J, Ahmad A (2012) Role of proline under changing environments: a review. Plant Signal Behav 7(11):1456–1466

Herbette S, Taconnat L, Hugouvieux V, Piette L, Magniette ML, Cuine S et al (2006) Genome-wide transcriptome profiling of the early cadmium response of *Arabidopsis* roots and shoots. Biochimie 88:1751–1765. https://doi.org/10.1016/j.biochi.2006.04.018

Hossain Z, Komatsu S (2013) Contribution of proteomic studies towards understanding plant heavy metal stress response. Front Plant Sci 3:310. https://doi.org/10.3389/fpls.2012.00310

Hossain MA, Piyatida P, da Silva JAT, Fujita M (2012) Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. J Bot 2012:872875. https://doi.org/10.1155/2012/87287

Howarth JR, Dominguez-Solis JR, Gutierrez-Alcala G, Wray JL, Romero LC, Gotor C (2003) The serine acetyltransferase gene family in *Arabidopsis thaliana* and the regulation of its expression by cadmium. Plant Mol Biol 51:589–598

Huang TL, Huang HJ (2008) ROS and CDPK-like kinase-mediated activation of MAP kinase in rice roots exposed to lead. Chemosphere 71:1377–1385

Jagadeeswaran G, Li YF, Sunkar R (2014) Redox signaling mediates the expression of a sulfate-deprivation-inducible microRNA395 in Arabidopsis. Plant J 77:85–96. https://doi.org/10.1111/tpj.12364

Jalmi SK, Sinha AK (2015) ROS mediated MAPK signaling in abiotic and biotic stress-striking similarities and differences. Front Plant Sci 6:769. https://doi.org/10.3389/fpls.2015.00769

Jam M, Alemzadeh A, Tale AM, Esmaeili-Tazangi S (2014) Heavy metal regulation of plasma membrane H+-ATPase gene expression in halophyte *Aeluropus littoralis*. Mol Biol Res Commun 3(2):129–139

Janas KM, Amarowicz R, Zielinska-Tomaszewska J, Kosinska A, Posmyk MM (2009) Induction of phenolic compounds in two dark-grown lentil cultivars with different tolerance to copper ions. Acta Physiol Plant 31(3):587–595

Jewell MC, Campbell BC, Godwin ID (2010) Transgenic plants for abiotic stress resistance. In: Kole C, Michler CH, Abbott AG, Hall TC (eds) Transgenic crop plants. Springer, Berlin; Heidelberg, pp 67–31. https://doi.org/10.1007/978-3-642-04812-8_2

Jia X, Sun C, Zuo Y, Li G, Li G, Ren L et al (2016) Integrating transcriptomics and metabolomics to characterise the response of *Astragalus membranaceus* Bge. var. mongolicus (Bge.) to progressive drought stress. BMC Genomics 17:188. https://doi.org/10.1186/s12864-016-2554-0

Jonak C, Okrész L, Bögre L, Hirt H (2002) Complexity, cross talk and integration of plant MAP kinase signalling. Curr Opin Plant Biol 5:415–424. https://doi.org/10.1016/S1369-5266(02)00285-6

Jonak C, Nakagami H, Hirt H (2004) Heavy metal stress. Activation of distinct mitogen-activated protein kinase pathways by copper and cadmium. Plant Physiol 136:3276–3283. https://doi.org/10.1104/pp.104.045724

Jun M, Fu HY, Hong J, Wan X, Yang CS, Ho CT (2003) Comparison of antioxidant activities of isoflavones from kudzu root (PuerarialobataOhwi). J Food Sci 68:2117–2122. https://doi.org/10.1111/j.1365-2621.2003.tb07029.x

Kanoun-Boulé M, Vicente JA, Nabais C, Prasad MNV, Freitas H (2009) Ecophysiological tolerance of duckweeds exposed to copper. Aquat Toxicol 9(1):1–9. https://doi.org/10.1016/j.aquatox.2008.09.009

Keunen E, Remans T, Bohler S, Vangronsveld J, Cuypers A (2011) Metal induced oxidative stress and plant mitochondria. Int J Mol Sci 12:6894–6918. https://doi.org/10.3390/ijms12106894

Khalid N, Hussain M, Hameed M, Ahmad R (2017) Physiological, biochemical and defense system responses of *Parthenium hysterophorus* to vehicular exhaust pollution. Pak J Bot 49(1):67–75

Khalid N, Hussain M, Young HS, Ashraf M, Hameed M, Ahmad R (2018a) Lead concentrations in soils and some wild plant species along two busy roads in Pakistan. Bull Environ Contam Toxicol 100(2):250–258

Khalid N, Hussain M, Ashraf M, Masood A, Akhtar Y (2018b) Spatio-Temporal variation in cadmium released by automobiles along two roads in Pakistan. Pak J Bot 50(2):529–536

Khalid N, Noman A, Sanaullah T, Akram MA, Aqeel A (2018c) Vehicle pollution toxicity induced changes in physiology, defence system and biochemical characteristics of *Calotropis procera* L. Chem Ecol 34(6):565–581

Khalid N, Noman A, Aqeel M, Masood A, Tufail A (2018d) Phytoremediation potential of *Xanthium strumarium* for heavy metals contaminated soils at roadsides. Int J Environ Sci Technol 16(4):2091–2100. https://doi.org/10.1007/s13762-018-1825-5

Khalid N, Young HS, Hussain M, Boyce B, Aqeel M, Noman A (2018e) Effects of road proximity on heavy metal concentrations in soils and some common wild plants in Southern California. Environ Sci Pollut Res 25(35):35257–35265

Khan NA, Samiullah SS, Nazar R (2007) Activities of antioxidative enzymes, sulphur assimilation, photosynthetic activity and growth of wheat (*Triticum aestivum*) cultivars differing in yield potential under cadmium stress. J Agron Crop Sci 193:435–444

Kim IS, Shin SY, Kim YS, Kim HY, Yoon HS (2009) Expression of a glutathione reductase from *Brassica rapa subsp. pekinensis* enhanced cellular redox homeostasis by modulating antioxidant proteins in *Escherichia coli*. Mol Cells 28:479–487. https://doi.org/10.1007/s10059-009-0168-y

Kodzius R, Kojima M, Nishiyori H, Nakamura M, Fukuda S, Taqami M et al (2006) CAGE: cap analysis of gene expression. Nat Methods 3:211–222

Konig J, Muthuramalingam M, Dietz KJ (2012) Mechanisms and dynamics in the thiol/disulfide redox regulatory network: transmitters, sensors and targets. Curr Opin Plant Biol 15:261–268

Krämer U, Cotter-Howells JD, Charnock JM, Baker AJM, Smith JAC (1996) Free histidine as a metal chelator in plants that accumulate nickel. Nature 379:635–638. https://doi.org/10.1038/379635a0

Krämer U, Talke IN, Hanikenne M (2007) Transition metal transport. FEBS Lett 581:2263–2272

Krantev A, Yordanova R, Janda T, Szalai G, Popova L (2008) Treatment with salicylic acid decreases the effect of cadmium on photosynthesis in maize plants. J Plant Physiol 165(9):920–931

Kumar A, Maiti SK (2013) Availability of chromium, nickel and other associated heavy. metals of ultramafc and serpentine soil/rock and in plants. Int J Emerg Technol Adv Eng 3(2):256–268

Kumar P, Tewari RK, Sharma PN (2008) Modulation of copper toxicity induced oxidative damage by excess supply of iron in maize plants. Plant Cell Rep 27:399–409

Kumar J, Pratab A, Kumar S (2015) Plant phenomics: an overview. In: Kumar J, Pratab A, Kumar S (eds) Phenomics in crop plants: trends, options and limitations. Springer, New Delhi, India, pp 1–10

Kuo HF, Chiou TJ (2011) The role of microRNAs in phosphorus deficiency signaling. Plant Physiol 156:1016–1024

Kuriakose SV, Prasad MNV (2008) Cadmium stress affects seed germination and seedling growth in *Sorghum bicolor* (L.) Moench by changing the activities of hydrolyzing enzymes. Plant Growth Regul 54:143–156. https://doi.org/10.1007/s10725-007-9237-4

Kwak JM, Nguyen V, Schroeder JI (2006) The role of reactive oxygen species in hormonal responses. Plant Physiol 141:323–329. https://doi.org/10.1104/pp.106.079004

Lavid N, Schwartz A, Yar Den O, Tel-Or E (2001) The involvement of polyphenols and peroxidase activities in heavy metal accumulation by epidermal glands of water lily (*Nymphaeceaea*). Planta 212:323. https://doi.org/10.1007/s004250000400

Le Martret B, Poage M, Shiel K, Nugent GD, Dix PJ (2011) Tobacco chloroplast transformants expressing genes encoding dehydroascorbate reductase, glutathione reductase, and glutathione-S-transferase, exhibit altered anti-oxidant metabolism and improved abiotic stress tolerance. Plant Biotechnol J 9:661–673. https://doi.org/10.1111/j.1467-7652.2011.00611.x

Lee H, Jo J, Son D (1998) Molecular cloning and characterization of the gene encoding gluta-thione reductase in *Brassica campestris*. Biochim Biophys Acta 1395:309–314. https://doi.org/10.1016/S0167-4781(97)00198-X

Lee SH, Ahsan N, Lee KW, Kim DH, Lee DG, Kwak SS et al (2007) Simultaneous overexpression of both CuZn superoxide dismutase and ascorbate peroxidase in transgenic tall fescue plants confers increased tolerance to a wide range of abiotic stresses. J Plant Physiol 164:1626–1638. https://doi.org/10.1016/j.jplph.2007.01.003

Lewis S, Donkin ME, Depledge MH (2001) Hsp 70 expression in *Enteromorpha intestinalis* (Chlorophyta) exposed to environmental stressors. Aqua Toxicol 51:277–291

Li F, Vallabhaneni R, Yu J, Rocheford T, Wurtzel ET (2008) The maize phytoene synthase gene family: overlapping roles for carotenogenesis in endosperm, photomorphogenesis, and thermal stress tolerance. Plant Physiol 147(3):1334–1346

Li YF, Zheng Y, Addo-Quaye C, Zhang L, Saini A, Jagadeeswaran G et al (2010) Transcriptome-wide identification of microRNA targets in rice. Plant J62:742–759. https://doi.org/10.1111/j.1365-313X.2010.04187.x

Li D, Xu X, Hu X, Liu Q, Wang Z, Zhang H, Wang H, Wei M, Wang H, Liu H, Li C (2015a) Genome-wide analysis and heavy metal-induced expression profiling of the HMA gene family in *Populus trichocarpa*. Front Plant Sci 6:1149. https://doi.org/10.3389/fpls.2015.01149

Li JL, Cui J, Cheng DY (2015b) Computational identification and characterization of conserved miRNAs and their target genes in beet (*Beta vulgaris*). Genet Mol Res 14:9103–9108. https://doi.org/10.4238/2015

Lima JC, Arenhart RA, Margis-Pinheiro M, Margis R (2011) Aluminum triggers broad changes in microRNA expression in rice roots. Genet Mol Res 10:2817–2832. https://doi.org/10.4238/2011.November.10.4

Lin CC, Kao CH (1999) Excess copper induces an accumulation of putrescine in rice leaves. Bot Bull Acad Sinica 40:213–218

Lin YF, Hassan Z, Talukdar S, Schat H, Aarts MG (2016) Expression of the ZNT1 zinc transporter from the metal hyperaccumulator *Noccaea caerulescens* confers enhanced zinc and cadmium tolerance and accumulation to *Arabidopsis thaliana*. PLoS One 11(3). https://doi.org/10.1371/journal.pone.0149750

Liu Q, Zhang H (2012) Molecular identification and analysis of arsenite stress responsive miRNAs in rice. J Agric Food Chem 60:6524–6536

Liu X, Zhang S, Shan X, Zhu YG (2005) Toxicity of arsenate and arsenite on germination seedling growth and amylolytic activity of wheat. Chemosphere 61:293–301

Liu GY, Zhang YX, Chai TY (2011) Phytochelatin synthase of *Thlaspi caerulescens* enhanced tolerance and accumulation of heavy metal when expressed in yeast and tobacco. Plant Cell Rep 30:1067–1076. https://doi.org/10.1007/s00299-011-1013-2

Lowe R, Shirley N, Bleackley M, Dolan S, Shafee T (2017) Transcriptomics technologies. PLoS Comput Biol 13(5):e1005457. https://doi.org/10.1371/journal.pcbi.1005457

Luckett BG, Su LJ, Rood JC, Fontham ETH (2012) Cadmium exposure and pancreatic cancer in South Louisiana. J Environ Public Health 2012:180186. https://doi.org/10.1155/2012/180186

Lukaszewicz M, Matysiak-Kata I, Skala J, Fecka I, Cisowski W, Szopa J (2004) Antioxidant capacity manipulation in transgenic potato tuber by changes in phenolic compounds content. J Agric Food Chem 52(6):1526–1533

Lushchak VI, Semchuk NM (2012) Tocopherol biosynthesis: chemistry, regulation and effects of environmental factors. Acta Physiol Plant 34:1607–1628. https://doi.org/10.1007/s11738-012-0988-9

Maeda H, DellaPenna D (2007) Tocopherol functions in photosynthetic organisms. Curr Opin Plant Biol 10:260–265

Maestri E, Marmiroli M, Visioli G, Marmiroli N (2010) Metal tolerance and hyperaccumulation: costs and trade-offs between traits and environment. Environ Exp Bot 68:1–13

Maheshwari R, Dubey RS (2009) Nickel-induced oxidative stress and the role of antioxidant defence in rice seedlings. Plant Growth Regul 59(1):37–49

Malar S, Vikram SS, Favas PJ, Perumal V (2014) Lead heavy metal toxicity induced changes on growth and antioxidative enzymes level in water hyacinths [*Eichhornia crassipes* (Mart.)]. Bot Stud 55(1):54. https://doi.org/10.1186/s40529-014-0054-6

Malar S, Vikram SS, Favas PJ, Perumal V (2016) Lead heavy metal toxicity induced changes on growth and antioxidative enzymes level in water hyacinths [*Eichhornia crassipes* (Mart.)]. Bot Stud 55(1):54. https://doi.org/10.1186/s40529-014-0054-6

Mallick N, Mohn FH (2000) Reactive oxygen species: response of algal cells. J Plant Physiol 157(2):183–193

Manara A (2012) Plant responses to heavy metal toxicity. In: Furini A (ed) Plants and heavy metals. Springer Briefs in Molecular Science, pp 27–53

Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic Press, Toronto

Mattiello L, Kirst M, Da Silva FR, Jorge RA, Menossi M (2010) Transcriptional profile of maize roots under acid soil growth. BMC Plant Biol 10:196

Meharg AA, Hartley-Whitaker J (2002) Arsenic uptake and metabolism in arsenic resistant and non-resistant plant species. New Phytol 154:29–42

Mhamdi A, Queval G, Chaouch S, Vanderauwera S, Van Breusegem F, Noctor G (2010) Catalase function in plants: a focus on *Arabidopsis* mutants as stress-mimic models. J Exp Bot 61(15):4197–4220

Mignolet-Spruyt L, Idänheimo N, Hoeberichts FA, Mühlenbock P, Brosché M, Van Breusegem F, Kangasjärvi J (2016) Spreading the news: subcellular and organellar reactive oxygen species production and signalling. J Exp Bot 67:3831–3844

Mils RF, Krjiger GC, Baccarini PJ, Hall JL, Williams LE (2003) Functional expression of AtHMA4, a P-1B-type ATPase of the Zn/Co/Cd/Pb subclass. Plant J 35:164–176

Mishra Y, Chaurasia N, Rai LC (2009) AhpC (alkyl hydroperoxide reductase) from *Anabaena* sp. PCC 7120 protects *Escherichia coli* from multiple abiotic stresses. Biochem Biophys Res Commun 381:606–611. https://doi.org/10.1016/j.bbrc.2009.02.100

Mishra S, Jha AB, Dubey RS (2011) Arsenite treatment induces oxidative stress, upregulates antioxidant system, and causes phytochelatin synthesis in rice seedlings. Protoplasma 248(3):565–577

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7:405–410. https://doi.org/10.1016/S1360-1385(02)02312-9

Mittler R (2017) ROS are good. Trends Plant Sci 22(1):11–19. https://doi.org/10.1016/j.tplants.2016.08.002

Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) Reactive oxygen gene network of plants. Trends Plant Sci 9:490–498

Mittler R, Vanderauwera S, Suzuki N, Miller G, Tognetti VB, Vandepoele K et al (2011) ROS signaling: the new wave? Trends Plant Sci 16:300–309

Mohamed HI (2011) Molecular and biochemical studies on the effect of gamma rays on lead toxicity in cowpea (*Vigna sinensis*) plants. Biol Trace Elem Res 144:1205–1218

Montanini B, Blaudez D, Jeandroz S, Sanders D, Chalot M (2007) Phylogenetic and functional analysis of the cation diffusion facilitator (CDF) family: improved signature and prediction of substrate specificity. BMC Genomics 8:107. https://doi.org/10.1186/1471-2164-8-107

Morel M, Crouzet J, Gravot A, Auroy P, Leonhardt N, Vavasseur A, Richaud P (2009a) AtHMA3, a P1B-ATPase allowing Cd/Zn/Co/Pb vacuolar storage in Arabidopsis. Plant Physiol 149:894–904

Morel M, Crouzet J, Gravot A, Auroy P, Leonhardt N, Vavasseur A et al (2009b) AtHMA3, a P$_{1B}$-ATPase allowing Cd/Zn/Co/Pb vacuolar storage in *Arabidopsis*. Plant Physiol 149:894–904. https://doi.org/10.1104/pp.108.130294v

Morrow KJJ (2010) Mass spec central to metabolomics. Gen Eng Biotechnol News 30:1–3

Munne-Bosch S (2005) The role of a9-tocopherol in plant stress tolerance. J Plant Physiol 162:743–748. https://doi.org/10.1016/j.jplph.2005.04.022

Nakabayashi R, Saito K (2015) Integrated metabolomics for abiotic stress responses in plants. Curr Opin Plant Biol 24:10–16

Nedjimi B, Daoud Y (2009) Cadmium accumulation in *Atriplex halimus* subsp. schweinfurthii and its influence on growth, proline, root hydraulic conductivity and nutrient uptake. Flora Morphol Distrib Funct Ecol Plants 204(4):316–324

Neill S, Desikan R, Hancock J (2002) Hydrogen peroxide signaling. Curr Opin Plant Biol 5:388–395. https://doi.org/10.1016/S1369-5266(02)00282-0

Nematshahi N, Lahouti M, Ganjeali A (2012) Accumulation of chromium and its effect on growth of (*Allium cepa* cv. Hybrid). Eur J Exp Biol 2(4):969–974

Nevo Y, Nelson N (2006) The NRAMP family of metal-ion transporters. Biochim Biophys Acta 1763:609–620

Noman A, Aqeel M (2017) miRNA-based heavy metal homeostasis and plant growth. Environ Sci Pollut Res 24:10068–10082

Noman A, Ali Q, Hameed M, Mehmood T, Iftikhar T (2014) Comparison of leaf anatomical characteristics of *Hibiscus rosa-sinensis* grown in Faisalabad region. Pak J Bot 46(1):199–206

Noman A, Fahad S, Aqeel M, Ali U, Ullah A, Anwer S, Khan S, Zainab M (2017a) miRNAs: major modulators for crop growth and development under abiotic stresses. Biotechnol Lett 39(5):685–700. https://doi.org/10.1007/s10529-017-2302-9

Noman A, Aqeel M, Javed MT, Zafar S, Ali Q, Islam W, Irshad MK, Buriro M, Kanwal H, Khalid N, Khan S (2017b) Histological changes in *Hibiscus rosa-sinensis* endorse acclimation and phytoremediation of industrially polluted sites. J Anim Plant Sci 27(5):1637–1648

Noman A, Aqeel M, Deng J, Khalid N, Sanaullah T, Shuilin H (2017c) Biotechnological advancements for improving floral attributes in ornamental plants. Front Plant Sci 8:530

Noman A, Kanwal H, Khalid N, Sanaullah T, Tufail A, Masood A, Sabir S, Aqeel M, He S (2017d) Perspective research progress in cold responses of *Capsella bursa-pastoris*. Front Plant Sci 8:1388. https://doi.org/10.3389/fpls.2017.01388

Noman A, Liu ZQ, Aqeel M, Zaynab M, Khan MI, Hussain A, Ashraf MF, Li X, Weng Y, He SL (2017e) Basic leucine zipper domain transcription factors: the vanguards in plant immunity. Biotechnol Lett 39(12):1779–1791. https://doi.org/10.1007/s10529-017-2431-1

Noman A, Ali Q, Maqsood J, Iqbal N, Javed MT, Rasool N, Naseem J (2018a) Deciphering physio-biochemical, yield, and nutritional quality attributes of water-stressed radish (*Raphanus sativus* L.) plants grown from Zn-Lys primed seeds. Chemosphere 195:175–189

Noman A, Ali Q, Nasim J, Javed MT, Kanwal H, Islam W, Aqeel M, Khalid N, Zafar S, Tayyeb M, Iqbal N, Buriro M, Maqsood J, Shahid S (2018b) Sugar beet extract acts as a natural biostimulant for physio-biochemical attributes in water stressed wheat (*Triticum aestivum* L.). Acta Physiol Plant 40:110

Norton GJ, Lou-Hing DE, Meharg AA, Price AH (2008) Rice-arsenate interactions in hydrophonics: whole genome transcriptional analysis. J Exp Bot 59:2267–2276

Opdenakker K, Remans T, Vangronsveld J, Cuypers A (2012) Mitogen-activated protein (MAP) kinases in plant metal stress: regulation and responses in comparison to other biotic and abiotic stresses. Int J Mol Sci 13:7828–7853

Ortiz DF, Ruscitti T, McCue KF, Ow DW (1995) Transport of metal-binding peptides by HMT1, a fission Yeast ABC-type vacuolar membrane protein. J Biol Chem 270:4721–4728

Ovečka M, Takáč T (2014) Managing heavy metal toxicity stress in plants: biological and biotechnological tools. Biotechnol Adv 32(1):73–86

Paicu C, Mohorianu I, Stocks M, Xu P, Coince A, Billmeier M, Dalmay T, Moulton V, Moxon S (2017) miRCat2: accurate prediction of plant and animal microRNAs from next-generation sequencing datasets. Bioinformatics 33(16):2446–2454

Peiter E, Montanini B, Gobert A, Pedas P, Husted S, Maathuis FJM, Blaudez D, Chalot M, Sanders D (2007) A secretory pathway-localized cation diffusion facilitator confers plant manganese tolerance. Proc Natl Acad Sci U S A 104:8532–8537

Pence NS, Larsen PB, Ebbs SD, Letham DL, Lasat MM, Garvin DF, Eide D, Kochian LV (2000) The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumulator *Thlaspi caerulescens*. Proc Natl Acad Sci 97(9):4956–4960

Peng JS, Gong JM (2014) Vacuolar sequestration capacity and long-distance metal transport in plants. Front Plant Sci 5:19. https://doi.org/10.3389/fpls.2014.00019

Petö A, Lehotai N, Lozano-Juste J, León J, Tari I, Erdei L et al (2011) Involvement of nitric oxide and auxin in signal transduction of copper-induced morphological responses in *Arabidopsis* seedlings. Ann Bot 108:449–457. https://doi.org/10.1093/aob/mcr176

Pilon M, Cohu CM, Ravet K, Abdel-Ghany SE, Gaymard F (2009) Essential transition metal homeostasis in plants. Curr Opin Plant Biol 12:347–357

Pilon-Smits EAH, Hwang S, Lytle CM, Zhu Y, Tai JC, Bravo RC et al (1999) Overexpression of ATP sulfurylase in indian mustard leads to increased selenate uptake, reduction, and tolerance. Plant Physiol 119:1123–1132. https://doi.org/10.1104/pp.119.1.123

Pilon-Smits EAH, Zhu YL, Sears T, Terry N (2000) Overexpression of glutathione reductase in *Brassica juncea*: effects on cadmium accumulation and tolerance. Physiol Plant 110:455–460. https://doi.org/10.1111/j.1399-3054.2000.1100405.x

Piotrowska-Niczyporuk A, Bajguz A, Zambrzycka E, Godlewska-Zyłkiewicz B (2012) Phytohormones as regulators of heavy metal biosorption and toxicity in green alga *Chlorella vulgaris* (*Chlorophyceae*). Plant Physiol Biochem 52:52–65

Piotrowska-Niczyporuk A, Bajguz A, Talarek M, Bralska M, Zambrzycka E (2015) The effect of lead on the growth, content of primary metabolites, and antioxidant response of green alga *Acutodesmus obliquus* (Chlorophyceae). Environ Sci Pollut Res 22(23):19112–19123

Pomponi M, Censi V, Di Girolamo V, De Paolis A, di Toppi LS, Aromolo R et al (2006) Overexpression of *Arabidopsis* phytochelatin synthase in tobacco plants enhances Cd (2+) tolerance and accumulation but not translocation to the shoot. Planta 223:180–190. https://doi.org/10.1007/s00425-005-0073-3

Potters G, Pasternak TP, Guisez Y, Palme KJ, Jansen MA (2007) Stress-induced morphogenic responses: growing out of trouble? Trends Plant Sci 12:98–105

Pu L, Brady S (2010) Systems biology update: cell type-specific transcriptional regulatory networks. Plant Physiol 152:411–419

Ramos J, Clemente MR, Naya L, Loscos J, Perez-Rontome C, Sato S et al (2007) Phytochelatin synthases of the model legume *Lotus japonicus*. A small multigene family with different responses to cadmium and alternatively spiced variants. Plant Physiol 143:110–118. https://doi.org/10.1104/pp.106.090894

Rashotte AM, Brady SR, Reed RC, Ante SJ, Muday GK (2000) Basipetal auxin transport is required for gravitropism in roots of Arabidopsis. Plant Physiol 122:481–490

Rogers ED, Jackson T, Moussaieff A, Aharoni A, Benfey PN (2012) Cell type-specific transcriptional profiling: implications for metabolite profiling. Plant J 70:5–17

Romero-Puertas MC, Rodr'ıguez-Serrano M, Corpas FJ, Gomez M, Del LA, Sandalio LM (2004) Cadmium induced subcellular accumulation of O_2.- and H_2O_2 in pea leaves. Plant Cell Environ 27(9):1122–1134

Ros R, Morales A, Segura J, Picazo I (1992) In vivo and in vitro effects of nickel and cadmium on the plasmalemma ATPase from rice (*Oryza sativa* L.) shoots and roots. Plant Sci 83:1–6

Rounsley SD, Last RL (2010) Shotguns and SNPs: how fast and cheap sequencing is revolutionizing plant biology. Plant J 61:922–927

Rout GR, Panigrahi J (2015) Analysis of signaling pathways during heavy metal toxicity: a functional genomics perspective. In: Pandey GK (ed) Elucidation of abiotic stress signaling in plants. Springer, New York, pp 295–322

Ruiz ON, Alvarez D, Torres C, Roman L, Daniell H (2011) Metallothionein expression in chloroplasts enhances mercury accumulation and phytoremediation capability. Plant Biotechnol J 9:609–617. https://doi.org/10.1111/j.1467-7652.2011.00616.x

Sarowar S, Kim EN, Kim YJ, Ok SH, Kim KD, Hwang BK, Shin JS (2005) Overexpression of a pepper ascorbate peroxidase-like 1 gene in tobacco plants enhances tolerance to oxidative stress and pathogens. Plant Sci 169:55–63

Schaaf G, Ludewig U, Erenoglu BE, Mori S, Kitahara T, von Wirén N (2004) ZmYS1 functions as a proton-coupled symporter for phytosiderophore- and nicotianamine-chelated metals. J Biol Chem 279:9091–9096

Serraj R, Sinclair TR (2002) Osmolyte accumulation: can it really help increase crop yield under drought conditions? Plant Cell Environ 25:333–341. https://doi.org/10.1046/j.13653040.2002.00754.x

Shahid M, Pinelli E, Dumat C (2012) Review of Pb availability and toxicity to plants in relation with metal speciation; role of synthetic and natural organic ligands. J Hazard Mater 219–220:1–12. https://doi.org/10.1016/j.jhazmat.2012.01.060

Shameer K, Ambika S, Varghese SM, Karaba N, Udayakumar M, Sowdhamini R (2009) STIFDB–*Arabidopsis* stress-responsive transcription factor DataBase. Int J Plant Genomics 2009:583429. https://doi.org/10.1155/2009/583429

Shanker AK, Cervantes C, Loza-Tavera H, Avudainayagam S (2005) Chromium toxicity in plants. Environ Int 31(5):739–753

Shao HB, Chu LY, Lu ZH, Kang CM (2008) Primary antioxidant free radical scavenging and redox signaling pathways in higher plant cells. Int J Biol Sci 4(1):8–14

Sharma RK, Agrawal M (2005) Biological effects of heavy metals: an overview. J Environ Biol 26:301–313

Sharma SS, Dietz KJ (2006) The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. J Exp Bot 57:711–726. https://doi.org/10.1093/jxb/erj073

Sharma SS, Dietz KJ (2009) The relationship between metal toxicity and cellular redox imbalance. Trends Plant Sci 14:43–50. https://doi.org/10.1016/j.tplants.2008.10.007

Sharma P, Jha AB, Dubey RS (2010) Oxidative stress and antioxidative defense system in plants growing under abiotic Stresses. In: Pessarakli M (ed) Handbook of plant and crop stress, 3rd edn. CRC Press, Taylor and Francis Publishing Company, Boca Raton, FL, pp 89–138

Shin SY, Kim IS, Kim YH, Park HM, Lee JY, Kang HG et al (2008) Scavenging reactive oxygen species by rice dehydroascorbate reductase alleviates oxidative stresses in *Escherichia coli*. Mol Cells 26:616–620

Singh K, Foley RC, Oñate-Sánchez L (2002) Transcription factors in plant defense and stress responses. Curr Opin Plant Biol 5:430–436. https://doi.org/10.1016/S1369-5266(02)00289-3

Singh N, Ma LQ, Srivastava M, Rathinasabapathi B (2006) Metabolic adaptations to arsenic induced oxidative stress in *Pteris vittata* L. and *Pteris ensiformis* L. Plant Sci 170:274–282

Singh HP, Kaur G, Batish DR, Kohli RK (2011a) Lead (Pb)-inhibited radicle emergence in *Brassica campestris* involves alterations in starch-metabolizing enzymes. Biol Trace Elem Res 144:1295–1301

Singh R, Gautam N, Mishra A, Gupta R (2011b) Heavy metals and living systems: an overview. Indian J Pharm 43(3):246–253. https://doi.org/10.4103/0253-7613.81505

Singh VP, Srivastava PK, Prasad SM (2012) Differential effect of UV-B radiation on growth, oxidative stress and ascorbate-glutathione cycle in two cyanobacteria under copper toxicity. Plant Physiol Biochem 61:61–70. https://doi.org/10.1016/j.plaphy.2012.09.005

Singh VP, Singh S, Kumar J, Prasad SM (2015) Investigating the roles of ascorbate-glutathione cycle and thiol metabolism in arsenate tolerance in ridged Luffa seedlings. Protoplasma 252:1217–1229. https://doi.org/10.1007/s00709-014-0753-6

Singh S, Parihar P, Singh R, Singh VP, Prasad SM (2016) Heavy metal tolerance in plants: role of transcriptomics, proteomics, metabolomics, and ionomics. Front Plant Sci 6:1143. https://doi.org/10.3389/fpls.2015.01143

Sinha AK, Jaggi M, Raghuram B, Tuteja N (2011) Mitogen-activated protein kinase signaling in plants under abiotic stress. Plant Signal Behav 6:196–203

Siripornadulsil S, Traina S, Verma DPS, Sayre RT (2002) Molecular mechanisms of proline-mediated tolerance to toxic heavy metal in transgenic microalgae. Plant Cell 14:2837–2847. https://doi.org/10.1105/tpc.004853

Smirnoff N, Running JA, Gatzek S (2004) Ascorbate biosynthesis: a diversity of pathways. In: Asard H, May JM, Smirnoff N (eds) Vitamin C: its functions and biochemistry in animals and plants. BIOS Scientific, New York, NY, pp 7–29

Soares C, de Sousa A, Pinto A, Azenha M, Teixeira J, Azevedo RA, Fidalgo F (2016) Effect of 24-epibrassinolide on ROS content, antioxidant system, lipid peroxidation and Ni uptake in *Solanum nigrum* L. under Ni stress. Environ Exp Bot 122:115–125

Soda N, Wallace S, Karan R (2015) Omics study for abiotic stress responses in plants. Adv Plant Agric Res 2(1):00037

Spence A, Hanson RE, Grant CN, Fung LH, Rattray R (2014) Assessment of the bioavailability of cadmium in Jamaican soils. Environ Monit Assess 186(7):4591–4603

Spollen WG, Tao W, Valliyodan B, Chen K, Hejlek LG, Kim JJ et al (2008) Spatial distribution of transcript changes in the maize primary root elongation zone at low water potential. BMC Plant Biol 8:32. https://doi.org/10.1186/1471-2229-8-32

Srivastava S, Dubey RS (2011) Manganese-excess induces oxidative stress, lowers the pool of anti-oxidants and elevates activities of key antioxidative enzymes in rice seedlings. Plant Growth Regul 64:1–16

Srivastava S, Jain R (2011) In-situ monitoring of chromium cytotoxicity in sugarcane. J Environ Biol 32(6):759–763

Srivastava S, Suprasanna PS, D'Souza SF (2012) Mechanisms of arsenic tolerance and detoxi-fication in plants and their application in transgenic technology: a critical appraisal. Int J Phytoremediation 14:506–517

Steinhorst L, Kudla J (2014) Signaling in cells and organisms—calcium holds the line. Curr Opin Plant Biol 22:14–21

Stoeva N, Bineva T (2003) Oxidative changes and photosynthesis in Oat plants grown in As-contaminated soil. Bulg J Plant Physiol 29(1–2):87–95

Stoeva N, Berova M, Zlatez Z (2004) Physiological response of maize to arsenic contamination. Biol Plantarum 47(3):449–452

Sundaramoorthy P, Chidambaram A, Ganesh KS, Unnikannan P, Baskaran L (2010) Chromium stress in paddy: (i) nutrient status of paddy under chromium stress; (ii) phytoremediation of chromium by aquatic and terrestrial weeds. C R Biol 333:597–607. https://doi.org/10.1016/j.crvi.2010.03.002

Sytar O, Kumar A, Latowski D, Kuczynska P, Strzałka K, Prasad MN (2013) Heavy metal-induced oxidative damage, defense reactions, and detoxification mechanisms in plants. Acta Physiol Plant 35(4):985–999

Szabados L, Savouré A (2010) Proline: a multifunctional amino acid. Trends Plant Sci 15(2):89–97

Takahashi S, Murata N (2008) How do environmental stresses accelerate photoinhibition? Trends Plant Sci 13(4):178–182

Takahashi R, Bashir K, Ishimaru Y, Nishizawa NK, Nakanishi H (2012) The role of heavy-metal ATPases, HMAs, in zinc and cadmium transport in rice. Plant Signal Behav 7:1799–1801

Talanova VV, Titov AF, Boeva NP (2000) Effect of increasing concentrations of lead and cadmium on cucumber seedlings. Biol Plant 43:441–444. https://doi.org/10.1023/A:1026735603890

Talke IN, Hanikenne M, Krämer U (2006) Zinc-dependent global transcriptional control, tran-scriptional deregulation, and higher gene copy number for genes in metal homeostasis of the hyperaccumulator *Arabidopsis halleri*. Plant Physiol 142:148–167

Tan KC, Ipcho SVS, Trengove RD, Oliver RP, Solomon PS (2009) Assessing the impact of transcriptomics, proteomics and metabolomics on fungal phytopathology. Mol Plant Pathol 10:703–715

Tanyolac D, Ekmekçi Y, Ünalan Ş (2007) Changes in photochemical and antioxidant enzyme activities in maize (*Zea mays* L.) leaves exposed to excess copper. Chemosphere 67:89–98

Thounaojam TC, Panda P, Mazumdar P, Kumar D, Sharma G, Sahoo L, Panda S (2012) Excess copper induced oxidative stress and response of antioxidants in rice. Plant Physiol Biochem 53:33–39

Tiwari M, Sharma D, Dwivedi S, Singh M, Tripathi RD, Trivedi PK (2014) Expression in Arabidopsis and cellular localization reveal involvement of rice NRAMP, OsNRAMP1, in arsenic transport and tolerance. Plant Cell Environ 37:140–152. https://doi.org/10.1111/pce.12138

Tran LSP, Nishiyama R, Yamaguchi-Shinozaki K, Shinozaki K (2010) Potential utilization of NAC transcription factors to enhance abiotic stress tolerance in plants by biotechnological approach. GM Crops 1:32–39. https://doi.org/10.4161/gmcr.1.1.10569

Trindade I, Capitao C, Dalmay T, Fevereiro MP, Santos DM (2010) miR398 and miR408 are up-regulated in response to water deficit in *Medicago truncatula*. Planta 231:705–716. https://doi.org/10.1007/s00425-009-1078-0

Truta E, Vochita G, Zamfirache MM, Olteanu Z, Rosu CM (2013) Copper-induced genotoxic effects in root meristems of *Triticum aestivum* L. cv. beti. Carpath J Earth Environ Sci 8:83–92

Tsukagoshi H, Busch W, Benfey PN (2010) Transcriptional regulation of ROS controls transition from proliferation to differentiation in the root. Cell 143:606–616. https://doi.org/10.1016/j.cell.2010.10.020

Ueno D, Milner MJ, Yamaji N, Yokosho K, Koyama E, Zambrano CM et al (2011) Elevated expression of TcHMA3 plays a key role in the extreme Cd tolerance in a Cd-hyperaccumulating eco-type of *Thlaspi caerulescens*. Plant J 66:852–862

Vaahtera L, Brosché M, Wrzaczek M, Kangasjärvi J (2014) Specificity in ROS signaling and tran-script signatures. Antioxid Redox Signal 21:1422–1441

Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M (2006) Free radicals, metals and antioxi-dants in oxidative stress-induced cancer. Chem Biol Interact 160:1–40

Van de Mortel JE, Villanueva LA, Schat H, Kwekkeboom J, Coughlan S, Moerland PD, Themaat EVL, Koornneef M, Aarts MGM (2006) Large expression differences in genes for iron and zinc homeostasis, stress response and lignin biosynthesis distinguish roots of *Arabidopsis thaliana* and the related metal hyperaccumulator *Thlaspi caerulescens*. Plant Physiol 142:1127–1147

Varotto C, Maiwald D, Pesaresi P, Jahns P, Salamini F, Leister D (2002) The metal ion transporter IRT1 is necessary for iron homeostasis and efficient photosynthesis in *Arabidopsis thaliana*. Plant J 31:589–599. https://doi.org/10.1046/j.1365-313X.2002.01381.x

Vassilev A, Perez-Sanz A, Semane B, Carteer R, Vangronsveld J (2005) Cadmium accumulation and tolerance of two salix genotypes hydro-ponically grown in presence of cadmium. J Plant Nutr 28:2159–2177

Vazquez S, Esteban E, Carpena RO (2008) Evolution of arsenate toxicity in nodulated White Lupine in a long-term culture. J Agric Food Chem 56(18):8580–8587

Verret F, Gravot A, Auroy P, Leonhardt N, David P, Nussaume L, Vavasseur A, Richaud P (2004) Overexpression of AtHMA4 enhances root-to-shoot translocation of zinc and cadmium and plant metal tolerance. FEBS Lett 576:306–312

Vert G, Grotz N, Dédaldéchamp F, Gaymard F, Guerinot ML, Briat JF et al (2002) IRT1, an *Arabidopsis* transporter essential for iron uptake from the soil and for plant growth. Plant Cell 14:1223–1233. https://doi.org/10.1105/tpc.001388

Vitti A, Nuzzaci M, Scopa A, Tataranni G, Remans T, Vangronsveld J et al (2013) Auxin and cytokinin metabolism and root morphological modifications in *Arabidopsis thaliana* seed-lings infected with *Cucumber mosaic virus* (CMV) or exposed to cadmium. Int J Mol Sci 14:6889–6902

Wang HY, Klatte M, Jakoby M, Bäumlein H, Weisshaar B, Bauer P (2007) Iron deficiency-mediated stress regulation of four subgroup Ib BHLH genes in *Arabidopsis thaliana*. Planta 226:897–908. https://doi.org/10.1007/s00425-007-0535-x

Wang Z, Gerstein M, Snyder M (2009) RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet 10:57–63

Wang Z, Xiao Y, Chen W, Tang K, Zhang L (2010) Increased vitamin C content accompanied by an enhanced recycling pathway confers oxidative stress tolerance in *Arabidopsis*. J Integr Plant Biol 52(4):400–409

Wang CL, Liu YG, Zeng GM, Hu XJ, Ying YC, Xi HU, Lu ZH, Wang YQ, Li HY (2014) Mechanism of exogenous selenium alleviates cadmium induced toxicity in *Bechmeria nivea* (L.) Gaud (Ramie). Trans Nonferrous Metals Soc China 24(12):3964–3970

Wang S, Ren X, Huang B, Wang G, Zhou P, An Y (2016) Aluminium-induced reduction of plant growth in alfalfa (*Medicago sativa*) is mediated by interrupting auxin transport and accumula-tion in roots. Sci Rep 6:30079. https://doi.org/10.1038/srep30079

Weber M, Harada E, Vess C, Roepenack-Lahaye E, Clemens S (2004) Comparative microarray analysis of *Arabidopsis thaliana* and *Arabidopsis halleri* roots identifies nicotianamine synthase, a ZIP transporter and other genes as potential metal hyperaccumulation factors. Plant J 37:269–281

Weber M, Trampczynska A, Clemens S (2006) Comparative transcriptome analysis of toxic metal responses in *Arabidopsis thaliana* and the Cd2+− hypertolerant facultative metallophyte *Arabidopsis halleri*. Plant Cell Environ 29:950–963. https://doi.org/10.1111/j.1365-3040.2005.01479.x

Weinstein LH, Kaur-Sawhney R, Venkat Rajam M, Wettlaufer SH, Galston AW (1986) Cadmium-induced accumulation of putrescine in oat and bean leaves. Plant Physiol 82:641–645. https://doi.org/10.1104/pp.82.3.641

Wenzel WW, Bunkowski M, Puschenreiter M, Horak O (2003) Rhizosphere characteristics of indigenously growing nickel hyperaccumulator and excluder plants on serpentine soil. Environ Pollut 123:131–138

Wheeler GL, Jones MA, Smirnoff N (1998) The biosynthetic pathway of vitamin C in higher plants. Nature 393(6683):365–369

Whiting SN, Leake JR, McGrath SP, Baker AJM (2000) Positive responses to zinc and cadmium by roots of the hyperaccumulator *Thlaspi caerulescens*. New Phytol 145:199–210. https://doi.org/10.1046/j.1469-8137.2000.00570.x

Williams LE, Mills RF (2005) P_{1B}-ATPases-an ancient family of transition metal pumps with diverse functions in plants. Trends Plant Sci 10:491–502. https://doi.org/10.1016/j.tplants.2005.08.008

Williams LE, Pittman JK, Hall JL (2000) Emerging mechanisms for heavy metal transport in plants. Biochim Biophys Acta 1465:104–126

Wintz H, Fox T, Wu YY, Feng V, Chen W, Chang HS, Zhu T, Vulpe C (2003) Expression profiles of *Arabidopsis thaliana* in mineral deficiencies reveal novel transporters involved in metal homeostasis. J Biol Chem 278:47644–47653

Wray GA, Hahn MW, Abouheif E, Balhoff JP, Pizer M, Rockman MV et al (2003) The evolution of transcriptional regulation in eukaryotes. Mol Biol Evol 20:1377–1419. https://doi.org/10.1093/molbev/msg140

Wu H, Chen C, Du J, Liu H, Cui Y, Zhang Y et al (2012) Cooverexpression FIT with AtbHLH38 or AtbHLH39 in *Arabidopsis*-enhanced cadmium tolerance via increased cadmium sequestration in roots and improved iron homeostasis of shoots. Plant Physiol 158:790–800. https://doi.org/10.1104/pp.111.190983

Yamasaki H, AbdelGhany SE, Cohu CM, Kobayashi Y, Shikanai T, Pilon M (2007) Regulation of copper homeostasis by microRNA in Arabidopsis. J Biol Chem 282:16369–16378

Yang ZM, Chen J (2013) A potential role of microRNAs in plant response to metal toxicity. Metallomics 5:1184–1190

Yang X, Feng Y, He Z, Stoffell PJ (2005) Molecular mechanisms of heavy metal hyperaccumulation and phytoremediation. J Trace Elem Med Biol 18:339–353. https://doi.org/10.1016/j.jtemb.2005.02.007

Yin L, Wang S, Eltayeb AE, Uddin MI, Yamamoto Y, Tsuji W et al (2010) Overexpression of dehydroascorbate reductase, but not monodehydroascorbate reductase, confers tolerance to aluminum stress in transgenic tobacco. Planta 231:609–621. https://doi.org/10.1007/s00425-009-1075-3

Young J (1991) The photoprotective role of carotenoids in higher plants. Physiol Plant 83(4):702–708

Yu Y, Jin C, Sun C, Wang J, Ye Y, Zhou W et al (2016) Inhibition of ethylene production by putrescine alleviates aluminium-induced root inhibition in wheat plants. Sci Rep 6:8888. https://doi.org/10.1038/srep18888

Yuan HM, Huang X (2016) Inhibition of root meristem growth by cadmium involves nitric oxide-mediated repression of auxin accumulation and signaling in Arabidopsis. Plant Cell Environ 39:120–135

Yuan Y, Wu H, Wang N, Li J, Zhao W, Du J et al (2008) FIT interacts with AtbHLH38 and AtbHLH39 in regulating iron uptake gene expression for iron homeostasis in Arabidopsis. Cell Res 18:385–397. https://doi.org/10.1038/cr.2008.26

Yuan HM, Xu HH, Liu WC, Lu YT (2013) Copper regulates primary root elongation through PIN1-mediated auxin redistribution. Plant Cell Physiol 54:766–778. https://doi.org/10.1093/pcp/pct030

Zaefyzadeh M, Quliyev RA, Babayeva SM, Abbasov MA (2009) The effect of the interaction between genotypes and drought stress on the superoxide dismutase and chlorophyll content in durum wheat landraces. Turk J Biol 33(1):1–7

Zafar S, Ashraf MY, Ali Q, Ashraf A, Anwer S, Iqbal N, Kausar A, Noman A, Ali M, Zafar MA, Feroz K (2016) Antioxidant activity and secondary metabolites in selected vegetables irrigated with sewage water. Appl Ecol Environ Res 14(5):35–48. https://doi.org/10.15666/aeer/1405_035048

Zaheer IE, Ali S, Rizwan M, Farid M, Shakoor MB, Gill RA, Najeeb U, Iqbal N, Ahmad R (2015) Citric acid assisted phytoremediation of copper by *Brassica napus* L. Ecotoxicol Environ Saf 120:310–317

Zayneb C, Bassem K, Zeineb K, Grubb CD, Noureddine D, Hafedh M, Amine E (2015) Physiological responses of fenugreek seedlings and plants treated with cadmium. Environ Sci Pollut Res 22(14):10679–10689

Zhang C, Liu J, Zhang Y et al (2011) Overexpression of SlGMEs leads to ascorbate accumulation with enhanced oxidative stress, cold, and salt tolerance in tomato. Plant Cell Rep 30(3):389–398

Zhang Z, Yu Q, Du H, Ai W, Yao X, Mendoza-Cózatl DG, Qiu B (2016) Enhanced cadmium efflux and root-to-shoot translocation are conserved in the hyperaccumulator *Sedum alfredii* (Crassulaceae family). FEBS Lett 590(12):1757–1764

Zhou J, Jiao F, Wu Z, Li Y, Wang X, He X, Zhong W, Wu P (2008) OsPHR2 is involved in phosphate-starvation signaling and excessive phosphate accumulation in shoots of plants. Plant Physiol 146:1673–1686. https://doi.org/10.1104/pp.107.111443

Zhou H, Liu Q, Li J, Jiang D, Zhou L, Wu P, Lu S, Li F, Zhu L, Liu Z (2012a) Photoperiod and thermosensitive genic male sterility in rice are caused by a point mutation in a novel noncoding RNA that produces a small RNA. Cell Res 22:649–660

Zhou ZS, Song JB, Yang ZM (2012b) Genome wide identification of *Brassica napus* microRNAs and their targets in response to cadmium. J Exp Bot 63:4597–4613

Zhu LY, Pilon-Smits EAH, Jouanin L, Terry N (1999) Overexpression of glutathione synthetase in Indian mustard enhances cadmium accumulation and tolerance. Plant Physiol 119:173–180