

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Towards Understanding Plant Response to Heavy Metal Stress

Zhao Yang and Chengcai Chu

*State Key Laboratory of Plant Genomics, National Centre for Plant Gene Research
Institute of Genetics and Developmental Biology, Chinese Academy of Sciences
China*

1. Introduction

Metals like zinc, iron and copper are essential micronutrients required for a wide range of physiological processes in all plant organs for the activities of various metal-dependent enzymes and proteins. However, they can also be toxic at elevated levels. Metals like arsenic, mercury, cadmium and lead are nonessential and potentially highly toxic. Once the cytosolic metal concentration in plant turns out of control, phytotoxicity of heavy metal inhibits transpiration and photosynthesis, disturbs carbohydrate metabolism, and drives the secondary stresses like nutrition stress and oxidative stress, which collectively affect the plant development and growth (Krämer & Clemens, 2005).

Plants have developed a complex network of highly effective homeostatic mechanisms that serve to control the uptake, accumulation, trafficking, and detoxification of metals. Components of this network have been identified continuously, including metal transporters in charge of metal uptake and vacuolar transport; chelators involved in metal detoxification via buffering the cytosolic metal concentrations; and chaperones helping delivery and trafficking of metal ions (Clemens, 2001).

This chapter summarizes heavy metal stress and detoxification in plant. Special focus is given to metallothionein, yet vacuolar metal transporters, phytochelatins as well as certain organic acids, amino acids, and chaperones are also addressed with recent advances. Besides, heavy metal-induced oxidative stress and tolerance as an example of abiotic stress cross-talk will be discussed.

1.1 The vacuolar compartmentation mediated by transporter families CDF and Nramp

A balanced cytosolic metal concentration has to be maintained all the time via strict compartmentation and chelation. The plant vacuole is a main storage compartment site for heavy metals present in excess (Ernst et al., 1992). Nickel-hyperaccumulator plant *Alyssum serpyllifolium* keeps its 72% of the cellular Ni in the vacuole (Brooks et al., 1980). Analysis with leaves from barley grown at heavy metal-polluted environment showed that cadmium, molybdenum, and zinc are mainly subjected to vacuolar compartmentation (Brune et al., 1995). Study on *Phragmites australis* under zinc pollution revealed that most Zn was immobilized in the apoplast or sequestered into the vacuolar lumen (Jiang & Wang, 2008).

The CDF (cation diffusion facilitator) transporters, once named as MTP for metal tolerance protein, are involved in mediating the cytoplasmic efflux of transition metal cations such as

Zn^{2+} , Cd^{2+} , Co^{2+} , Ni^{2+} or Mn^{2+} . In *S.cerevisiae*, two proteins COT1 and ZRC1 confer overexpression lines cobalt and zinc/cadmium tolerance respectively (Conklin et al., 1992; Kamizono et al., 1989), and both are localized to the vacuolar membrane, indicating a role in metal sequestration (Li & Kaplan, 1998). The identification of *Arabidopsis* ZAT/MTP1, a member of CDF family, provides the first information for a possible vacuolar zinc transporter in plant. AtMTP1 is localized to vacuolar membranes, and overexpression of the complete protein-coding domain of ZAT results in enhanced Zn resistance and strongly increased Zn content in the roots under high Zn exposure (Kobae et al., 2004; van der Zaal et al., 1999). The ectopic expression of poplar *PtdMTP1* in yeast was able to complement the hypersensitivity of mutant strains to Zn, and transgenic *Arabidopsis* exhibited enhanced zinc tolerance (Blaudez et al., 2003). The vacuolar membrane-localized TgMTP1 of hyperaccumulator *Thlaspi goesingense* confers tolerance to a broad spectrum of heavy metals including Ni, Cd, Zn, and Co, and complements the metal sensitivity of the yeast COT1/ZRC1 mutant strains (Persans et al., 2001), and could particularly increase zinc tolerance by initiating a systemic Zn deficiency response including up-regulation of Zn transporter genes (*ZIP3*, *ZIP4*, *ZIP5* and *ZIP9*) (Gustin et al., 2009). The *Stylosanthes hamata* ShMTP8 conferred manganese tolerance when heterologously overexpressed in yeast and *Arabidopsis*. Further analysis demonstrated that ShMTP8 is localized to the tonoplast, and the Mn tolerance in yeast was managed by internal sequestration rather than by efflux of Mn^{2+} (Delhaize et al., 2003).

It's interesting to note the other side, releasing metal ions from the vacuole into the cytosol if required by metabolism. Which transporter takes the challenge? The NRAMPs might be a possible candidate. The plant NRAMP (natural resistance associated macrophage protein) family transport divalent metal cations into the cytoplasm. *Arabidopsis* AtNRAMP3 and AtNRAMP4 can be induced by iron starvation, complement Fe-uptake yeast mutant, and mediate the remobilization of Fe from vacuolar stores, which is crucial for seed germination during early Fe deficiency period (Lanquar et al., 2005; Thomine et al., 2000). AtNRAMP3 protein, localized to the vacuolar membrane, affects metal accumulation and gene expression of Fe uptake transporter *IRT1* and a root ferric chelate reductase *FRO2* by mobilizing vacuolar metal pools to the cytosol (Thomine et al., 2003). In the metal hyperaccumulator *Thlaspi caerulescens*, TcNRAMP3 and TcNRAMP4, the closest homologues to AtNRAMP3 and AtNRAMP4, have been characterized as highly expressed, vacuolar membrane-localized, and transporting Fe, Mn, Cd and Zn with respective preferences (Oomen et al., 2009; Wei et al., 2009).

Progressive reports implicated that *Arabidopsis* NRAMP proteins have an important role in manganese homeostasis and cadmium toxicity. The *nramp3nramp4* double mutant displayed lower Mn release from mesophyll vacuoles, and it's suggested that AtNRAMP3 and AtNRAMP4 export Mn from vacuoles to maintain mitochondrial MnSOD activity and optimal photosynthesis under Mn deficiency (Lanquar et al., 2010). The transgenic plants with disruption of AtNRAMP6 exhibits enhanced cadmium tolerance whereas the overexpression causes Cd^{2+} hypersensitivity (Cailliatte et al., 2009). AtNRAMP3 showed the similar result (Thomine et al., 2000), implying these two metal transporters might affect remobilization and distribution of cadmium within the cell.

1.2 Chelation of cadmium ions by phytochelatin

Chelation of metals in the cytosol is a very important mechanism of heavy metal detoxification and tolerance (Fig 1). The principal classes of known metal chelators in plant

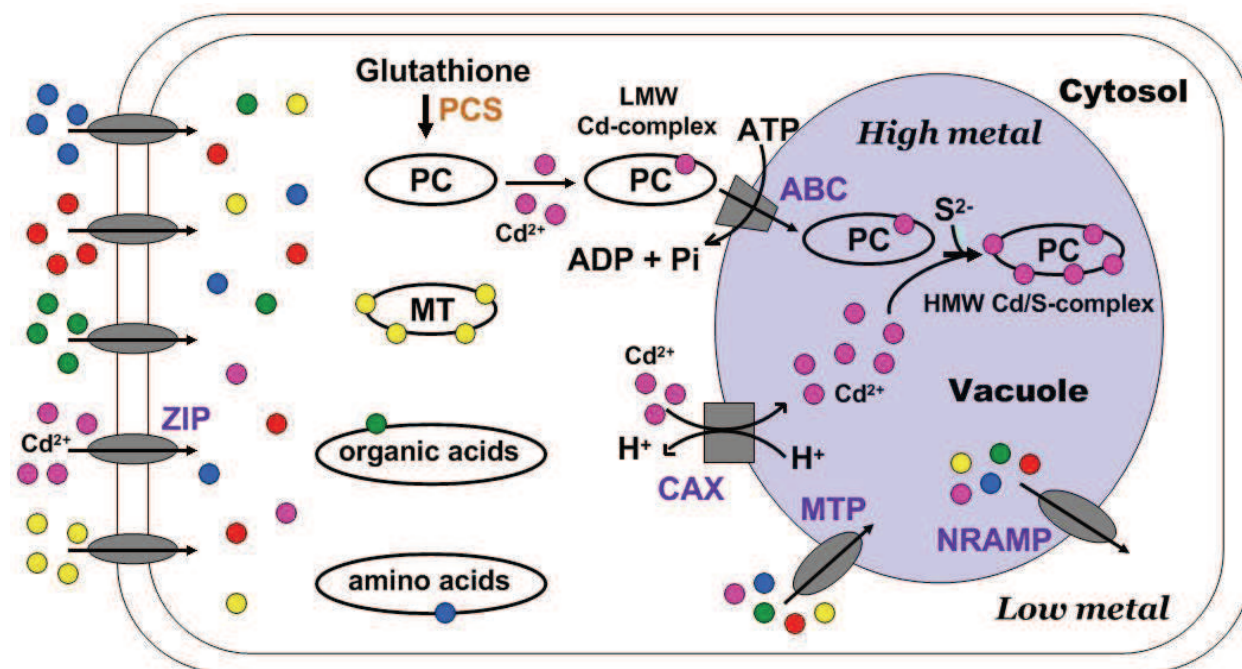


Fig. 1. Vacuolar sequestration of heavy metals in plant cell. Following the uptake through transporters such as ZIP (zinc/iron-regulated transporters) family members, heavy metal ions like Cd^{2+} enters the cytosol and it stimulates the glutathione-derived synthesis of phytochelatins (PCs) by PC synthases (PCS). PCs bind cytosolic Cd^{2+} to form the low-molecular-weight (LMW) complex first, which is transported into the vacuole via a tonoplast-localized ATP-binding-cassette (ABC) transporter. In the vacuole, LMW Cd-complex then accumulate into high-molecular-weight (HMW) complex with more Cd^{2+} , which may enter the vacuole via a direct exchange with protons by tonoplast-localized cation/proton exchanger (CAX) transporters. Transporters MTPs (metal tolerance protein) and NRAMPs (natural resistance associated macrophage protein) residing in the tonoplast mediate passage of metal ions for compartmentation or remobilization. Other chelators including metallothioneins (MTs), organic acids and amino acids help buffering the cytosolic metal concentrations to the safe low metal state.

are metallothioneins (MTs), phytochelatins (PCs), organic acids and amino acids (Clemens, 2001). Metallothionein and phytochelatin are proteins or peptides with low molecular weight, high cysteine content, and unique metal-binding capacity. In early reports lack of detail amino acid sequence data, metal-binding proteins in plants were generally assumed to be MTs, which in fact covered at least part of PCs. In an old classification system of three-class MT proteins, phytochelatins are somewhat confusingly described as enzymatically-synthesized class III whereas other two are gene-encoded class I and II (Cherian & Chan, 1993). Later the classification system has been improved and now it's clear plants express both PCs and MTs, which play relatively independent roles in metal detoxification and/or metabolism (Cobbett & Goldsbrough, 2002).

Phytochelatins have been identified in many plants and photosynthetic organisms, ranging from algae, gymnosperms to monocots and dicots. Phytochelatins (PCs) are synthesized from glutathione (GSH) (in some cases, related compounds) by PC synthases (PCS), and play a role in the distribution and accumulation of Cd and some other highly toxic metals

like Ag, Hg, As (Cobbett, 2000; Rauser, 1999). Modern techniques including X-ray absorption spectroscopy (XAS), high performance liquid chromatography-mass spectrometry (HPLC-MS), inductively coupled plasma optical emission spectrometer (ICP-OES) help to reveal that cadmium ions are generally bound to phytochelatins in plant. The percentage of Cd bound to PCs in Indian mustard seedlings increased from 34% after 6 hours of Cd exposure to 60% after 72 hours (Salt et al., 1997). In a Cd-hyperaccumulator desert plant tumbleweed (*Salsola kali*), cadmium was attached to oxygen and sulfur groups in stems and leaves, implying a great possibility of phytochelatins production in the stems, which later coordinates the absorbed cadmium for transport and storage in cell structures (de la Rosa et al., 2004). The mushroom *Boletus edulis* presented PC-Cd complex under Cd exposure and the more PC complexes were correlated with reduced level of GSH (Collin-Hansen et al., 2007). In wheat, phytochelatins bound 82% of the Cd in roots, 19% in young leaves and 12% in old leaves, suggesting the speciality of PC-based Cd sequestration varies with tissues even in the same plant (Marentes & Rauser, 2007). And it's demonstrated that the chemical structure of thiol and carboxyl groups is essential for the metal-binding ability and formation of a Cd-PCs complex (Satofuka et al., 2001).

There are two types of Cd-PC complexes produced during Cd sequestration: low-molecular-weight (LMW) and high-molecular-weight (HMW). The LMW complex serves as the transient form for transporting Cd^{2+} from cytosol to vacuole where more Cd and sulfide are incorporated to produce the HMW complex, which turns the main storage form of Cd^{2+} (Rauser, 1995). The first molecular insight into transporting the PC-Cd complex comes from the *S.pombe hmt1* mutant, which is unable to form the HMW complexes. SpHMT1 is a half size ATP-binding cassette (ABC) transporter protein, located in the vacuolar membrane, and mediates the ATP-required transport of LMW PC-Cd complexes into vacuolar membrane vesicles (Ortiz et al., 1992; Ortiz et al., 1995). An ATP-dependent, similar-to-SpHMT1 activity has been identified capable of transporting both PCs and PC-Cd complexes in oat roots (Salt & Rauser, 1995). Using a cDNA-microarray approach, some ATP-binding cassette (ABC) transporters in *Arabidopsis* genome were found to be differentially regulated under cadmium treatments, implying their role in Cd sequestration and redistribution (Bovet et al., 2005). A subfamily of ABC transporters, MRPs (multidrug resistance-associated protein) have been implicated mediating PC-Cd complex transport across the tonoplast in plants (Rea, 2007). Expression of *Chlamydomonas reinhardtii* CrMRP2 not only complements the yeast mutant, but also helps accumulating and sequestering more Cd in the stable HMW PC-Cd complex (Wang & Wu, 2006). Song et al. report the identification of the long-sought and major vacuolar PC transporters recently (Song et al., 2010). Two *Arabidopsis* ABCC-type transporters, AtABCC1 and AtABCC2 mediating transport of As(III)-PC though, may as well offer us a good perspective of identifying more specific PC vacuolar transporters for other heavy metals in addition to Cd-PC complex.

Besides the phytochelatin- Cd^{2+} complex transported by ABC transporters, cadmium ions can also reach the vacuole via a direct exchange with protons by tonoplast-localized cation/proton exchanger (CAX) transporters. In oat roots, the pH-dependent Cd^{2+} accumulation in vesicles was accompanied by efflux of protons, which offers the first clue of $\text{Cd}^{2+}/\text{H}^{+}$ antiport in plant (Salt & Wagner, 1993). Then several *Arabidopsis* CAX genes have been cloned and characterized. Expression of AtCAX2 in tobacco increased Cd^{2+} and Mn^{2+} transport in isolated root tonoplast vesicles (Hirschi et al., 2000). The *cax4* loss-of-function mutant and CAX4 RNAi lines displayed altered root growth in response to Cd^{2+} , Mn^{2+} and auxin treatment (Mei et al., 2009). The transgenic tobacco overexpressing AtCAX4 and

AtCAX2 exhibited high Cd^{2+} transport and certain selectivity in tonoplast vesicles, indicating some CAX transporters are more selective for particular divalent cations (Korenkov et al., 2007). Comparative analysis of CAX2 transporters between different plant species including *Arabidopsis*, tomato and barley proposed that there are diverse regulatory mechanisms with regard to CAX antiporter diverse functions (Edmond et al., 2009).

1.3 Metallothionein: metal-binding protein and more

Metallothioneins (MTs) are ubiquitous low-molecular-weight, cysteine-rich proteins that can bind metals via mercaptide bonds. Since the first MT was characterized from horse kidneys as cadmium-binding proteins in 1957 (Margoshes & Vallee, 1957), plenty of MT genes have been identified in a wide variety of organisms including bacteria, fungi, and all eukaryotic animal and plant species (Robinson et al., 1993).

The spatial structures of MTs have been uncovered as a dumbbell-like shape with two separate domains, α and β , containing in their core clusters built up of several tetrahedral Metal-Cys units (Fig 2). The different metal reactivity and metal affinity of two domains prompt different functional roles of the two metal clusters, that is, N-terminal β domain is involved in the homeostasis of essential metal ions (Kagi & Schaffer, 1988; Willner et al., 1987), and C-terminal α domain, the tight binding sequestration of excess and/or toxic metal ions (Cherian et al., 1994; Wright et al., 1987). As for the spacer region linking the α and β domains, it may contribute to stability or subcellular localization of MT proteins (Domenech et al., 2005), and is necessary for MT metal detoxification function (Domenech et al., 2007; Zhou & Goldsbrough, 1994).

MT proteins are generally classified into mammalian Class I and plant Class II, and plant MTs can be further subdivided into four types based on the number and arrangement of cysteine residues and the length of spacer region (Cobbett & Goldsbrough, 2002). These four-type plant MTs exhibited certain tissue-preferential expression patterns. Type 1 MTs

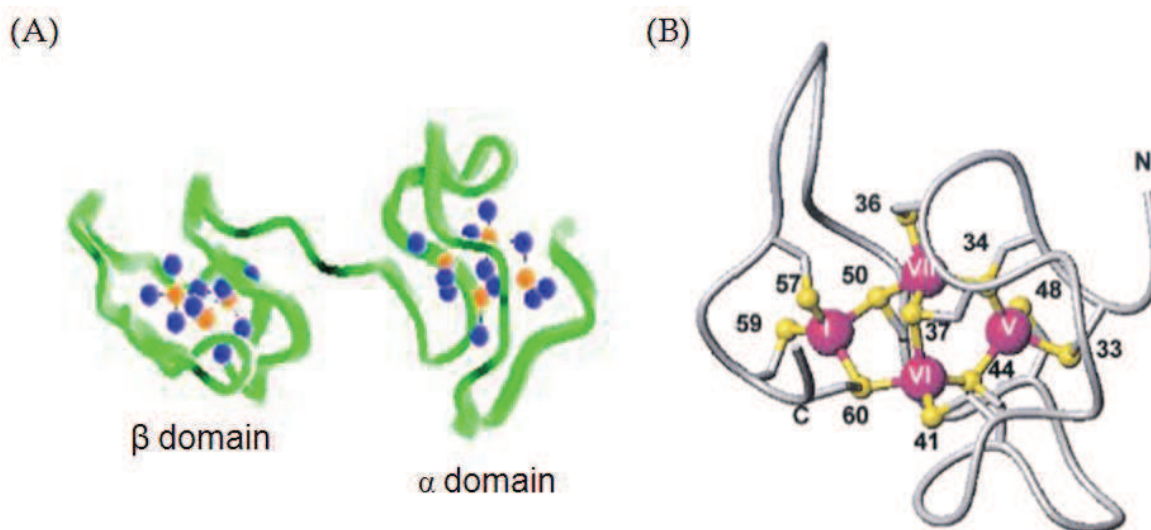


Fig. 2. The spatial structure of metallothionein. (A) general structure of MTs: a dumbbell-like shape with two separate globular domains α and β ; (B) structure of the $[\text{Cd}_4]$ α -domain of rat MT-2 showing the example of a tetrahedral Me(II)-Cys units formed by MTs (adapted from Blindauer et al., 2001)

are expressed much higher in roots than in shoots (Hudspeth et al., 1996), whereas Type 2 MTs are found mainly in leaves (Hsieh et al., 1995; Zhou & Goldsbrough, 1994). Type 3 MTs are expressed abundantly in the ripe fruits (Clendennen & May, 1997; Ledger & Gardner, 1994; Reid & Ross, 1997), and expression of Type 4 MTs, also known as the Ec type, was only found in developing seeds so far (Chyan et al., 2005; Lane et al., 1987).

A vast number of stimuli have been demonstrated capable of inducing MT genes expression in plants, including natural senescence (Bhalerao et al., 2003), hormones like ABA (Reynolds & Crawford, 1996), ethylene (Coupe et al., 1995), wounding and virus infection (Choi et al., 1996), heat shock (Hsieh et al., 1995), sucrose starvation (Hsieh et al., 1996), UV-light (Foley & Singh, 1994), cold and salt stress (Reid & Ross, 1997), etc. Apparently, different types of MTs respond to different factors, which is especially true when treated with heavy metal stress under different concentrations. Copper increased *AtMT1a* expression more than 10-fold in 5-to-8-day *Arabidopsis* seedlings, while the expression of *AtMT2a* varied insignificantly during the same stage under the same treatment (Garcia-Hernandez et al., 1998). 1 mM Cu²⁺, 100 μM Cd²⁺ and 1 mM Fe²⁺ were found to be efficient to decrease the bean MT1 expression, when other concentrations had no pronounced effect (Foley et al., 1997).

Ever since the first identification of MTs, its striking metal-binding property has been brought into sharp focus, which suggests MTs play the principal role in metal homeostasis and detoxification. In animals, MTs are well-known metal-binding proteins protecting against cadmium toxicity (Klaassen et al., 1999), while in plant PCs mainly take the charge of Cd detoxification (Zenk, 1996). MTs seem to have a broader spectrum of metal affinity than PCs, which points to more complicated functions. It's proposed that MTs participate in maintaining the homeostasis of essential copper (Cu) or zinc (Zn) at micronutrient levels, and also in the detoxification of non-essential toxic metals such as cadmium (Cd) and arsenic (As) (Lee et al., 2004; Merrifield et al., 2004; Roosens et al., 2004).

Though modulation of metal concentrations has great impact on cellular redox balance (Bell & Vallee, 2009), MTs may just scavenge reactive oxygen species (ROS) directly. With a large quantity of nucleophilic sulphhydryl groups in the structure, MTs provide a fine nucleophilic "sink" to trap electrophiles and free radicals, that is, the multiple cysteine residues can react with superoxide ($\cdot\text{O}_2^-$) and hydroxyl radicals ($\cdot\text{OH}$) leading to their degradation (Klaassen & Cagen, 1981; Sato & Bremner, 1993). Moreover, MTs can be recycled via thiolate exchange with GST (Vasak et al., 1985). Now accumulating evidences support hypothesis that MTs function as an antioxidant in plants. In wild watermelon, drought-induced CLMT2 showed an extraordinarily high activity for detoxifying hydroxyl radicals *in vitro* (Akashi et al., 2004). Three recombinant metallothionein proteins, the rice OsMT2b (Wong et al., 2004), cotton GhMT3a (Xue et al., 2009) and rubber tree (*Hevea brasiliensis*) HbMT2 (Zhu et al., 2010), possessed hydroxyl radical-scavenging activities, even higher than the positive control GSH in the hydroxyl radical inhibition assays.

Thanks to dynamic instability of metal ions in clusters, MTs can exchange metal ions with other metalloproteins universally necessary for a life cycle. There's zinc transfer between metallothionein and zinc transporter ZnT1 (Palmiter, 2004), chelator EDTA (Leszczyszyn & Blindauer, 2010), SOD (Koh & Kim, 2001; Suzuki & Kuroda, 1995), and other zinc proteins (Jacob et al., 1998). The metal-transfer mechanism should be a cornerstone for MTs' dual role abstracting the toxic metals arsenic (Ngu et al., 2010) or cadmium (Roesijadi, 2000), as well as donating the essential metals like zinc or copper (Liu et al., 2000). In this sense, metal-binding protein MTs are involved not merely in the

coordination of metal concentrations, but contribute more to diverse physiological processes like development or senescence. The wheat type 4 MT Ec gene was specially expressed during pollen embryogenesis, and its accumulation correlates well with increase of the plant hormone abscisic acid (ABA). It's suggested that induced by the ABA signal, this zinc-containing Ec may regulate certain gene expression via zinc trafficking with zinc-dependent DNA/RNA polymerase or zinc-finger proteins (Reynolds & Crawford, 1996). MTs have been implicated during senescence in many plants (Bhalerao et al., 2003; Breeze et al., 2004; Buchanan-Wollaston & Morris, 2000), and hypotheses for MT's role in senescence primarily reckon on either MTs' chelating and detoxifying abilities which alleviate the senescence-induced metal ion disturbance and oxidative burst, or the release of necessary metal ions to required places for nutrient recycling.

The positive correlation between MT expression in diverse organisms and the environmental metal concentration suggests that MTs can be effective biomarkers of heavy metal pollution. Such monitoring programs have already gained great potential comprehensively in aquatic and terraneous invertebrates (Chu et al., 2006; Dallinger et al., 2004; Navarro et al., 2009). In plants, MTs are favorable candidates for phytoremediation of heavy metal contaminants, a low-cost, effective, and sustainable plant-based approach for environment governance (Eapen & D'Souza, 2005; Memon & Schroder, 2009). On the other side, biofortification of mineral micronutrients in food crops for the benefit of human health, is another application and extension for metal research in plants, and MTs could also be contributive. The *rgMT*-overexpressing rice had the cysteine content in seed protein increased about seven-fold, which promises further enhancement of iron bioavailability (Lucca et al., 2002). Overexpression of *OsMT1a* in transgenic rice yielded significant increase of the zinc content in grain by 40-50% compared to wild type, making first step of possibility to fight zinc deficiency with zinc-rich rice (Yang et al., 2009).

1.4 Organic acids, amino acids and chaperones

The reactive interactions between metal ions and S, N, and O made organic acids and amino acids potential ligands for metal chelation. Citrate has been proposed the major ligand for Cd^{2+} at low Cd concentration within cell (Wagner, 1993), and can form Nickel-citrate complex in Ni-hyperaccumulating plant *Sebertia acuminata* (Sagner et al., 1998). The efflux of organic acids including citric acid has been elucidated for aluminium (Al) tolerance mechanisms in plant (Delhaize & Ryan, 1995). Malate and oxalate are also implicated in metal tolerance, metal transport through xylem sap and vacuolar metal sequestration (Rauser, 1999).

The coordination of nickel with histidine has been confirmed with analyses of Ni-hyperaccumulating and non-accumulating species. Upon Ni exposure, a large and proportional increase of free histidine was detected in xylem sap in Ni-hyperaccumulating *A. lesbiacum*. When supplying histidine to a non-accumulating species *A. montanum*, transgenic plant exhibited great increase of both nickel tolerance and capacity for nickel transport to the shoot (Krämer et al., 1996). And such histidine-dependent root-to-shoot translocation of Ni was also observed in *Brassica juncea* (Kerkeb & Kramer, 2003). Nicotianamine (NA), synthesized from three molecules of S-adenosyl-L L-methionine by nicotianamine synthase (NAS), has been primarily linked with Fe and Cu homeostasis (Hell & Stephan, 2003; Herbik et al., 1996; Pich et al., 2001). Through studies on NAS, NA has also been implicated in Zn homeostasis and tolerance (Weber et al., 2004). Other amino acid chelators including proline, glutathione, polyamines, etc, appear to play roles in metal

binding, metal hyperaccumulation, metal stress defence as well as signalling and antioxidation (Sharma & Dietz, 2006).

Copper chaperones are a novel class of proteins involved in intracellular trafficking and delivery of copper to copper-containing proteins such as copper-ATPases or copper/zinc superoxide dismutase. *Arabidopsis* AtCCS is necessary for activation of all three types of Cu/ZnSOD activity (Chu et al., 2005). AtATX1 interacts *in vivo* with two Cu-transporting P-type ATPases HMA5 (Andres-Colas et al., 2006) and RAN1 (Puig et al., 2007) by yeast two-hybrid. The intracellular metal trafficking pathway model composed of Cu transporter, Cu pump and Cu chaperone has been proposed (O'Halloran & Culotta, 2000), and based on such cooperative work, chaperones make a great contribution to the metal transport, detoxification and remobilization (Himmelblau & Amasino, 2000; Robinson & Winge, 2010).

2. Heavy metal-induced oxidative stress and stress tolerance: Cross-talk

Seen from a systemic view, different abiotic stresses may bring general effects on plant growth and development. For example, drought, salt, and cold stresses can all interrupt the cellular water balance leading to osmotic stress, and generate a phytohormone abscisic acid (ABA) for osmotic adjustment (Wang et al., 2003). ABA acts as a key endogenous messenger in stress response, and hence the ABA signalling pathway is more or less involved during plant cross-adaptive processes (Tuteja, 2007). In addition, all abiotic stresses can accumulate excess ROS (reactive oxygen species) at certain stage of stress exposure leading to oxidative stress. However, ROS are not only toxic compounds, but sometimes play as important regulators for many biological processes in plants such as cell cycle, programmed cell death, hormone signaling, biotic and abiotic cell responses (Laloi et al., 2004). As common consequences of abiotic stresses, osmotic stress and the ubiquitous oxidative stress have been extensively studied and offer more and more evidences for cross-talk at various steps or levels in the complicated network of abiotic stress signalling pathways.

Reactive oxygen species (ROS) such as $\cdot\text{O}_2^-$, H_2O_2 and $\cdot\text{OH}$ are unavoidable by-products of aerobic metabolism, and also commonly generated under various stress conditions. The unwelcome result of ROS overproduction is the oxidative stress, which can cause extensive cellular damages (Miller et al., 2008). Therefore, a delicate antioxidant system is indispensably required to supervise the cytotoxic effects of ROS tightly. The plant antioxidant system consists of ROS-scavenging enzymes, such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), as well as low-molecular-weight antioxidants like glutathione, ascorbate, carotenoids, metallothionein, etc (Table 1). Analysis with transgenic plants overexpressing these antioxidant genes revealed that maintenance of a high antioxidant capacity in cells is linked to increased tolerance against various adverse conditions (Guo et al., 2009; Jayaraj & Punja, 2008; Tseng et al., 2007; Wang et al., 2010).

Heavy metal stresses can shift the cellular balance of free radical homeostasis into terrible accumulation of H_2O_2 . For those redox-active transition metals like copper or iron, autooxidation in Fenton reaction and Haber-Weiss reaction will convert H_2O_2 to the highly reactive $\cdot\text{OH}$ molecule in a metal-catalyzed way. Non-redox-active metals like cadmium or mercury can also result in H_2O_2 accumulation and an oxidative burst via depletion of the antioxidant glutathione (GSH) pool and inhibition of antioxidative enzymes, especially glutathione reductase (GR) (Mithofer et al., 2004; Schutzendubel & Polle, 2002). To cope with heavy metal stress and associated oxidative stress, metallothionein, a well-known metal chelator and also antioxidant would possibly be a good way out.

Low molecular weight antioxidants	
Compounds	Target
Ascorbate	$O_2(^1\Delta_g)$, $\cdot OH$, $O_2\cdot$, $HO_2\cdot$
β -Carotene	$O_2(^1\Delta_g)$, $RO_2\cdot$
α -Tocopherol	$RO_2\cdot$
Glutathione	Nonspecific
Urate	$O_2(^1\Delta_g)$, metal
Metallothionein	$\cdot OH$, metal
Flavonoid	$\cdot OH$ and $HOCl$
Phytochelatin	Metal
Enzyme antioxidants	
Enzyme	Reaction catalyzed
Superoxide dismutase	$2O_2\cdot^- + 2H^+ \rightarrow H_2O_2 + O_2$
Catalase	$2H_2O_2 \rightarrow 2H_2O + O_2$
Glutathione peroxidase	H_2O_2 or $ROOH + 2GSH \rightarrow 2H_2O$ or $ROH + GSSG$
Ascorbate peroxidase	$H_2O_2 + \text{Ascorbate} \rightarrow H_2O + \text{Monodehydroascorbate}$
Thioredoxin	$\text{Prot-S}_2 + \text{Prot}'(SH)_2 \rightarrow \text{Prot}(SH)_2 + \text{Prot}'\text{-S}_2$
Peroxiredoxin	$ROOH + R'(SH)_2 \rightarrow ROH + R'S_2 + H_2O$
Glutathione reductase	$GSSG + NAD(P)H + H^+ \rightarrow 2GSH + NAD(P)^+$

Table 1. Cellular antioxidants including low molecular weight antioxidants and enzymes of the ROS-scavenging system. (Adapted from Pinto et al., 2003)

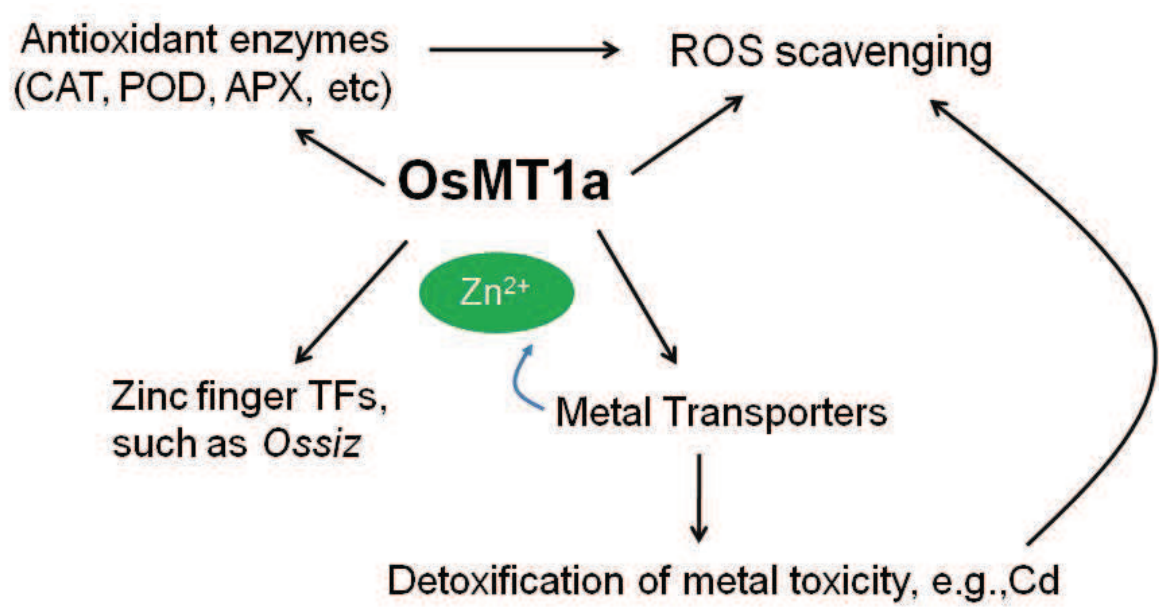


Fig. 3. The proposed model for OsMT1a’s role in stress tolerance and metal detoxification.

Take OsMT1a for example. Yang et al. reported functional characterization of a rice type 1 metallothionein, OsMT1a. A model has been proposed to elucidate how OsMT1a plays a role in drought tolerance in plant (Yang et al., 2009). On the one hand, OsMT1a can directly scavenge ROS via increasing activities of antioxidant enzymes CAT, POD and APX. On the

other hand, OsMT1a lies upstream of some zinc finger transcription factors like Osz1, and may tune up downstream defense genes in virtue of these transcription factors through Zn²⁺ trafficking. Additional data reveal that some zinc/cadmium transporter genes including forecasted vacuolar-membrane-localized ABC transporters *ABC1*, *MRP4* were up-regulated in *OsMT1a* transgenic plants, which probably accounts for uptake enhancement of Zn, as well as detoxification of toxic Cd via compartmentation into vacuole (Yang et al., unpublished data). Whether stomatal closure or ABA signalling is involved in OsMT1a-mediated drought tolerance in rice will be further examined. Altogether, researches on this metal-binding protein metallothionein provide a convincing insight into plant cross-talk combined with zinc homeostasis, cadmium detoxification, ROS scavenging and stress tolerance (Fig 3).

Despite their toxicity, ROS have been reevaluated in recent years as key signal molecules for regulating cell function and development (Rhee, 2006). In plants, the elaborate and efficient network of scavenging mechanisms allowed overcoming ROS toxicity and using some of these toxic molecules, mainly the hydrogen peroxide (H₂O₂) produced by cytosolic membrane-bound NADPH oxidases, as a signal in a wide range of abiotic stress responses (Bailey-Serres & Mittler, 2006; Mittler et al., 2004; Neill et al., 2002). For instance, in response to drought stress, ABA-induced H₂O₂ regulates the stomatal closing of *Arabidopsis* guard cells via activation of calcium-permeable channels in the plasma membrane (Pei et al., 2000), and such ABA-induced ROS production may also be involved in the phosphatidylinositol 3-phosphate (PI3P)-mediated stomatal closure (Park et al., 2003). A vast network of genes have been activated by ROS accumulation, many of which are also central regulators of stress responses, including zinc finger protein Zat family, heat shock and WRKY transcription factors, multiprotein bridging factor 1c, and Rboh genes (Miller et al., 2008). It's implicated that ROS could be an essential intermediate integrating different signals during cross-talk between abiotic stress signalling pathways.

3. Outlook and challenges

As the global population and food demand keep increasing fast, and yet the environment has been endangered worse and worse by water deficit and soil salinization, abiotic stress becomes one of the most harmful factors that limit the growth and productivity of crops worldwide. Although we keep moving forward with the understanding of heavy metal stress and detoxification in plant, there are many components of the complex network yet to be identified. Especially much remains unknown about the signalling molecules of the metal-induced signal transduction, including sensing of the cellular metal change and subsequent transcription regulation of metal-responsive genes (DalCorso et al., 2008). In recent years, next-generation sequencing techniques emerge and develop fast, and the microarray-based analyses become available and efficient for transcriptome or proteome high-throughput screenings, which help to identify regulatory factors for the metal homeostasis and still more metal transporters, low-molecular-weight chelators, chaperones as well. In addition, some heavy metal responsive transcription factors can also be induced by other abiotic stresses such as cold, dehydration, Salicylic Acid (SA) and H₂O₂, suggesting cross-talk exists between heavy metal response and other abiotic stress defense signalling (Fusco et al., 2005; Singh et al., 2002; Suzuki et al., 2001; Weber et al., 2006). Nevertheless, determining the underlying regulatory and cross-talk mechanisms remain a future challenge.

Heavy metal hyperaccumulators are unique plants capable of accumulating high amounts of various toxic elements (Reeves & Baker, 2000), and the active hyperaccumulation is based on mechanisms of internal hypertolerance to cytotoxic metals and a powerful scavenging system compatible for efficient uptake of the pollutants (Salt, 2006). Therefore, comparative studies on hyperaccumulator and non-hyperaccumulator plants will provide us a good view of naturally selected metal hypertolerance and hyperaccumulation. The first core set of candidate genes with high expression in hyperaccumulators has been identified and will be analyzed at biochemical and genetic level (Krämer et al., 2007). Dissecting these genes opens up a wide avenue for understanding the plant metal homeostasis network, and also agricultural genetic engineering for crop tolerance and biofortification, as well as phytoremediation of environmental metal pollution.

4. Acknowledgment

This work was supported by grants from Chinese Academy of Sciences (grant numbers: KSCX2-YW-N-010 & KSCX2-YW-N-056), and National Science Foundation of China (grant numbers: 30825029 & 30621001).

5. References

- Akashi, K.; Nishimura, N.; Ishida, Y. & Yokota, A. (2004). Potent hydroxyl radical-scavenging activity of drought-induced type-2 metallothionein in wild watermelon. *Biochem Biophys Res Commun*, 323, pp. 72-78, ISSN 0006-291X
- Andres-Colas, N.; Sancenon, V.; Rodriguez-Navarro, S.; Mayo, S.; Thiele, D.J.; Ecker, J.R.; Puig, S. & Penarrubia, L. (2006). The Arabidopsis heavy metal P-type ATPase *HMA5* interacts with metallochaperones and functions in copper detoxification of roots. *Plant J*, 45, pp. 225-236, ISSN 0960-7412
- Bailey-Serres, J. & Mittler, R. (2006). The roles of reactive oxygen species in plant cells. *Plant Physiol*, 141, p. 311, ISSN 0032-0889
- Bell, S.G. & Vallee, B.L. (2009). The metallothionein/thionein system: an oxidoreductive metabolic zinc link. *Chembiochem*, 10, pp. 55-62, ISSN 1439-7633
- Bhalerao, R.; Keskitalo, J.; Sterky, F.; Erlandsson, R.; Bjorkbacka, H.; Birve, S.J.; Karlsson, J.; Gardestrom, P.; Gustafsson, P.; Lundeberg, J. & Jansson, S. (2003). Gene expression in autumn leaves. *Plant Physiol*, 131, pp. 430-442, ISSN 0032-0889
- Blaudez, D.; Kohler, A.; Martin, F.; Sanders, D. & Chalot, M. (2003). Poplar metal tolerance protein 1 confers zinc tolerance and is an oligomeric vacuolar zinc transporter with an essential leucine zipper motif. *Plant Cell*, 15, pp. 2911-2928, ISSN 1040-4651
- Blindauer, C.A.; Harrison, M.D.; Parkinson, J.A.; Robinson, A.K.; Cavet, J.S.; Robinson, N.J. & Sadler, P.J. (2001). A metallothionein containing a zinc finger within a four-metal cluster protects a bacterium from zinc toxicity. *Proc Natl Acad Sci USA*, 98, pp. 9593-9598, ISSN 0027-8424
- Bovet, L.; Feller, U. & Martinoia, E. (2005). Possible involvement of plant ABC transporters in cadmium detoxification: a cDNA sub-microarray approach. *Environment International*, 31, pp. 263-267, ISSN 0160-4120
- Breeze, E.; Wagstaff, C.; Harrison, E.; Bramke, I.; Rogers, H.; Stead, A.; Thomas, B. & Buchanan-Wollaston, V. (2004). Gene expression patterns to define stages of post-

- harvest senescence in *Alstroemeria* petals. *Plant Biotechnol J*, 2, pp. 155-168, ISSN 1467-7652
- Brooks, R.R.; Reeves, R.D.; Morrison, R.S. & Malaisse, F. (1980). Hyperaccumulation of Copper and Cobalt - a Review. *Bull Soc Roy Bot Belgique*, 113, pp. 166-172, ISSN 0037-9557
- Brune, A.; Urbach, W. & Dietz, K.J. (1995). Differential toxicity of heavy metals is partly related to a loss of preferential extraplasmic compartmentation - a comparison of Cd-stress, Mo-stress, Ni-stress and Zn-stress. *New Phytol*, 129, pp. 403-409, ISSN 0028-646X
- Buchanan-Wollaston, V. & Morris, K. (2000). Senescence and cell death in *Brassica napus* and *Arabidopsis*. *Symp Soc Exp Biol*, 52, pp. 163-174, ISSN 0081-1386
- Cailliatte, R.; Lapeyre, B.; Briat, J.F.; Mari, S. & Curie, C. (2009). The NRAMP6 metal transporter contributes to cadmium toxicity. *Biochem J*, 422, pp. 217-228, ISSN 1470-8728
- Cherian, G.M. & Chan, H.M. (1993). Biological functions of metallothioneins: a review. In: *Metallothionein III Biological roles and medical implications (Advances in Life Sciences)*, K.T. Suzuki, Imura, N., Kimura, M., (Ed.), pp. 87-109, Birkhäuser Verlag, ISBN 9783764327699, Basel.
- Cherian, M.G.; Howell, S.B.; Imura, N.; Klaassen, C.D.; Koropatnick, J.; Lazo, J.S. & Waalkes, M.P. (1994). Role of metallothionein in carcinogenesis. *Toxicol Appl Pharmacol*, 126, pp. 1-5, ISSN 0041-008X
- Choi, D.; Kim, H.M.; Yun, H.K.; Park, J.A.; Kim, W.T. & Bok, S.H. (1996). Molecular cloning of a metallothionein-like gene from *Nicotiana glutinosa* L. and its induction by wounding and tobacco mosaic virus infection. *Plant Physiol*, 112, pp. 353-359, ISSN 0032-0889
- Chu, C.C.; Lee, W.C.; Guo, W.Y.; Pan, S.M.; Chen, L.J.; Li, H.M. & Jinn, T.L. (2005). A copper chaperone for superoxide dismutase that confers three types of copper/zinc superoxide dismutase activity in *Arabidopsis*. *Plant Physiol*, 139, pp. 425-436, ISSN 0032-0889
- Chu, M.M.; Guo, Z.Q.; Muto, N.; Itoh, N.; Tanaka, K. & Ren, H.W. (2006). Development of ELISA for metallothionein-II allows determination of heavy metal pollution of fresh water. *Front Biosci*, 11, pp. 2113-2122, ISSN 1093-4715
- Chyan, C.L.; Lee, T.T.; Liu, C.P.; Yang, Y.C.; Tzen, J.T. & Chou, W.M. (2005). Cloning and expression of a seed-specific metallothionein-like protein from sesame. *Biosci Biotechnol Biochem*, 69, pp. 2319-2325, ISSN 0916-8451
- Clemens, S. (2001). Molecular mechanisms of plant metal tolerance and homeostasis. *Planta*, 212, pp. 475-486, ISSN 0032-0935
- Clendennen, S.K. & May, G.D. (1997). Differential gene expression in ripening banana fruit. *Plant Physiol*, 115, pp. 463-469, ISSN 0032-0889
- Cobbett, C. (2000). Phytochelatins and their roles in heavy metal detoxification. *Plant Physiol*, 123, pp. 825-832, ISSN 0032-0889
- Cobbett, C. & Goldsbrough, P. (2002). Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. *Annu Rev Plant Biol*, 53, pp. 159-182, ISSN 1543-5008

- Collin-Hansen, C.; Pedersen, S.A.; Andersen, R.A. & Steinnes, E. (2007). First report of phytochelatins in a mushroom: induction of phytochelatins by metal exposure in *Boletus edulis*. *Mycologia*, 99, pp. 161-174, ISSN 0027-5514
- Conklin, D.S.; McMaster, J.A.; Culbertson, M.R. & Kung, C. (1992). COT1, a gene involved in cobalt accumulation in *Saccharomyces cerevisiae*. *Mol Cell Biol*, 12, pp. 3678-3688, ISSN 0270-7306
- Coupe, S.A.; Taylor, J.E. & Roberts, J.A. (1995). Characterisation of an mRNA encoding a metallothionein-like protein that accumulates during ethylene-promoted abscission of *Sambucus nigra* L. leaflets. *Planta*, 197, pp. 442-447, ISSN 0032-0943
- DalCorso, G.; Farinati, S.; Maistri, S. & Furini, A. (2008). How plants cope with cadmium: Staking all on metabolism and gene expression. *J Integr Plant Biol*, 50, pp. 1268-1280, ISSN 1672-9072
- Dallinger, R.; Lagg, B.; Egg, M.; Schipflinger, R. & Chabicosky, M. (2004). Cd accumulation and Cd-metallothionein as a biomarker in *Cepaea hortensis* (Helicidae, Pulmonata) from laboratory exposure and metal-polluted habitats. *Ecotoxicology*, 13, pp. 757-772, ISSN 0963-9292
- de la Rosa, G., Peralta-Videa, J.R., Montes, M., Parsons, J.G., Cano-Aguilera, I., & Gardea-Torresdey, J.L. (2004). Cadmium uptake and translocation in tumbleweed (*Salsola kali*), a potential Cd-hyperaccumulator desert plant species: ICP/OES and XAS studies, *Chemosphere*, 55, 1159-1168, ISSN 0045-6535
- Delhaize, E. & Ryan, P.R. (1995). Aluminum Toxicity and Tolerance in Plants. *Plant Physiol*, 107, pp. 315-321, ISSN 0032-0889
- Delhaize, E.; Kataoka, T.; Hebb, D.M.; White, R.G. & Ryan, P.R. (2003). Genes encoding proteins of the cation diffusion facilitator family that confer manganese tolerance. *Plant Cell*, 15, pp. 1131-1142, ISSN 1040-4651
- Domenech, J.; Mir, G.; Huguet, G.; Capdevila, M.; Molinas, M. & Atrian, S. (2005). Plant metallothionein domains: functional insight into physiological metal binding and protein folding. *Biochimie*, 88, pp. 583-593, ISSN 0300-9084
- Domenech, J.; Orihuela, R.; Mir, G.; Molinas, M.; Atrian, S. & Capdevila, M. (2007). The Cd(II)-binding abilities of recombinant *Quercus suber* metallothionein: bridging the gap between phytochelatins and metallothioneins. *J Biol Inorg Chem*, 12, pp. 867-882, ISSN 0949-8257
- Eapen, S. & D'Souza, S.F. (2005). Prospects of genetic engineering of plants for phytoremediation of toxic metals. *Biotechnol Adv*, 23, pp. 97-114, ISSN 0734-9750
- Edmond, C.; Shigaki, T.; Ewert, S.; Nelson, M.D.; Connorton, J.M.; Chalova, V.; Noordally, Z. & Pittman, J.K. (2009). Comparative analysis of CAX2-like cation transporters indicates functional and regulatory diversity. *Biochem J*, 418, pp. 145-154, ISSN 1470-8728
- Ernst, W.H.O.; Verkleij, J.A.C. & Schat, H. (1992). Metal tolerance in plants. *Acta Bot Neerl*, 41, pp. 229-248, ISSN 0044-5983
- Foley, R.C. & Singh, K.B. (1994). Isolation of a *Vicia faba* metallothionein-like gene: expression in foliar trichomes. *Plant Mol Biol*, 26, pp. 435-444, ISSN 0167-4412
- Foley, R.C.; Liang, Z.M. & Singh, K.B. (1997). Analysis of type 1 metallothionein cDNAs in *Vicia faba*. *Plant Mol Biol*, 33, pp. 583-591, ISSN 0167-4412

- Fusco, N.; Micheletto, L.; Dal Corso, G.; Borgato, L. & Furini, A. (2005). Identification of cadmium-regulated genes by cDNA-AFLP in the heavy metal accumulator *Brassica juncea* L. *J Exp Bot*, 56, pp. 3017-3027, ISSN 0022-0957
- Garcia-Hernandez, M.; Murphy, A. & Taiz, L. (1998). Metallothioneins 1 and 2 have distinct but overlapping expression patterns in Arabidopsis. *Plant Physiol*, 118, pp. 387-397, ISSN 0032-0889
- Guo, X.; Wu, Y.; Wang, Y.; Chen, Y. & Chu, C. (2009). OsMSRA4.1 and OsMSRB1.1, two rice plastidial methionine sulfoxide reductases, are involved in abiotic stress responses. *Planta*, 230, pp. 227-238, ISSN 0032-0935
- Gustin, J.L.; Loureiro, M.E.; Kim, D.; Na, G.; Tikhonova, M. & Salt, D.E. (2009). MTP1-dependent Zn sequestration into shoot vacuoles suggests dual roles in Zn tolerance and accumulation in Zn-hyperaccumulating plants. *Plant J*, 57, pp. 1116-1127, ISSN 1365-313X
- Hell, R. & Stephan, U.W. (2003). Iron uptake, trafficking and homeostasis in plants. *Planta*, 216, pp. 541-551, ISSN 0032-0935
- Herbik, A.; Giritch, A.; Horstmann, C.; Becker, R.; Balzer, H.J.; Baumlein, H. & Stephan, U.W. (1996). Iron and copper nutrition-dependent changes in protein expression in a tomato wild type and the nicotianamine-free mutant chloronerva. *Plant Physiol*, 111, pp. 533-540, ISSN 0032-0889
- Himelblau, E. & Amasino, R.M. (2000). Delivering copper within plant cells. *Curr Opin Plant Biol*, 3, pp. 205-210, ISSN 1369-5266
- Hirschi, K.D.; Korenkov, V.D.; Wilganowski, N.L. & Wagner, G.J. (2000). Expression of arabidopsis CAX2 in tobacco. Altered metal accumulation and increased manganese tolerance. *Plant Physiol*, 124, pp. 125-133, ISSN 0032-0889
- Hsieh, H.M.; Liu, W.K. & Huang, P.C. (1995). A novel stress-inducible metallothionein-like gene from rice. *Plant Mol Biol*, 28, pp. 381-389, ISSN 0167-4412
- Hsieh, H.M.; Liu, W.K.; Chang, A. & Huang, P.C. (1996). RNA expression patterns of a type 2 metallothionein-like gene from rice. *Plant Mol Biol* 32, pp. 525-529, ISSN 0167-4412
- Hudspeth, R.L.; Hobbs, S.L.; Anderson, D.M.; Rajasekaran, K. & Grula, J.W. (1996). Characterization and expression of metallothionein-like genes in cotton. *Plant Mol Biol*, 31, pp. 701-705, ISSN 0167-4412
- Jacob, C.; Maret, W. & Vallee, B.L. (1998). Control of zinc transfer between thionein, metallothionein, and zinc proteins. *Proc Natl Acad Sci USA*, 95, pp. 3489-3494, ISSN 0027-8424
- Jayaraj, J. & Punja, Z.K. (2008). Transgenic carrot plants accumulating ketocarotenoids show tolerance to UV and oxidative stresses. *Plant Physiol Biochem*, 46, pp. 875-883, ISSN 0981-9428
- Jiang, X. & Wang, C. (2008). Zinc distribution and zinc-binding forms in *Phragmites australis* under zinc pollution. *J Plant Physiol*, 165, pp. 697-704, ISSN 1618-1328
- Kagi, J.H. & Schaffer, A. (1988). Biochemistry of metallothionein. *Biochemistry*, 27, pp. 8509-8515, ISSN 0006-2960
- Kamizono, A.; Nishizawa, M.; Teranishi, Y.; Murata, K. & Kimura, A. (1989). Identification of a gene conferring resistance to zinc and cadmium ions in the yeast *Saccharomyces cerevisiae*. *Mol Gen Genet*, 219, pp. 161-167, ISSN 0026-8925

- Kerkeb, L. & Kramer, U. (2003). The role of free histidine in xylem loading of nickel in *Alyssum lesbiacum* and *Brassica juncea*. *Plant Physiol*, 131, pp. 716-724, ISSN 0032-0889
- Klaassen, C.D. & Cagen, S.Z. (1981). Metallothionein as a trap for reactive organic intermediates. *Adv Exp Med Biol*, 136 Pt A, pp. 633-646, ISSN 0065-2598
- Klaassen, C.D.; Liu, J. & Choudhuri, S. (1999). Metallothionein: An intracellular protein to protect against cadmium toxicity. *Annu Rev Pharmacol Toxicol*, 39, pp. 267-294, ISSN 0362-1642
- Kobae, Y.; Uemura, T.; Sato, M.H.; Ohnishi, M.; Mimura, T.; Nakagawa, T. & Maeshima, M. (2004). Zinc transporter of *Arabidopsis thaliana* AtMTP1 is localized to vacuolar membranes and implicated in zinc homeostasis. *Plant Cell Physiol*, 45, pp. 1749-1758, ISSN 0032-0781
- Koh, M. & Kim, H.J. (2001). The effect of metallothionein on the activity of enzymes involved in removal of reactive oxygen species. *Bull Korean Chem Soc*, 22, pp. 362-366, ISSN 0253-2964
- Korenkov, V.; Park, S.; Cheng, N.H.; Sreevidya, C.; Lachmansingh, J.; Morris, J.; Hirschi, K. & Wagner, G.J. (2007). Enhanced Cd²⁺-selective root-tonoplast-transport in tobaccos expressing Arabidopsis cation exchangers. *Planta*, 225, pp. 403-411, ISSN 0032-0935
- Krämer, U.; CotterHowells, J.D.; Charnock, J.M.; Baker, A.J.M. & Smith, J.A.C. (1996). Free histidine as a metal chelator in plants that accumulate nickel. *Nature*, 379, pp. 635-638, ISSN 0028-0836
- Krämer, U. & Clemens, S. (2005). Molecular Biology of Metal Homeostasis and Detoxification. In: *Topics in Current Genetics*, M. Tamás, Martinoia, E., (Ed.), pp. 216-271, Springer Verlag, ISBN 978-3-642-06062-5, New York.
- Krämer, U.; Talke, I.N. & Hanikenne, M. (2007). Transition metal transport. *FEBS Lett*, 581, pp. 2263-2272, ISSN 0014-5793
- Laloi, C.; Apel, K. & Danon, A. (2004). Reactive oxygen signalling: the latest news. *Curr Opin Plant Biol*, 7, pp. 323-328, ISSN 1369-5266
- Lane, B.G.; Kajioka, R. & Kennedy, T.D. (1987). The wheat germ Ec protein is a zinc-containing metallothionein. *Biochemistry and Cellular Biology*, 65, pp. 1001-1005, ISSN 0829-8211
- Lanquar, V.; Lelievre, F.; Bolte, S.; Hames, C.; Alcon, C.; Neumann, D.; Vansuyt, G.; Curie, C.; Schroder, A.; Kramer, U.; Barbier-Brygoo, H. & Thomine, S. (2005). Mobilization of vacuolar iron by AtNRAMP3 and AtNRAMP4 is essential for seed germination on low iron. *Embo J*, 24, pp. 4041-4051, ISSN 0261-4189
- Lanquar, V.; Ramos, M.S.; Lelievre, F.; Barbier-Brygoo, H.; Krieger-Liszkay, A.; Kramer, U. & Thomine, S. (2010). Export of vacuolar manganese by AtNRAMP3 and AtNRAMP4 is required for optimal photosynthesis and growth under manganese deficiency. *Plant Physiol*, 152, pp. 1986-1999, ISSN 1532-2548
- Ledger, S.E. & Gardner, R.C. (1994). Cloning and characterization of five cDNAs for genes differentially expressed during fruit development of kiwifruit. *Plant Mol Biol*, 25, pp. 877-886, ISSN 0167-4412
- Lee, J.; Shim, D.; Song, W.Y.; Hwang, I. & Lee, Y. (2004). Arabidopsis metallothioneins 2a and 3 enhance resistance to cadmium when expressed in *Vicia faba* guard cells. *Plant Mol Biol* 54, pp. 805-815, ISSN 0167-4412

- Leszczyszyn, O.I. & Blindauer, C.A. (2010). Zinc transfer from the embryo-specific metallothionein Ec from wheat: a case study. *Phys Chem Chem Phys*, 12, pp. 13408-13418, ISSN 1463-9084
- Li, L. & Kaplan, J. (1998). Defects in the yeast high affinity iron transport system result in increased metal sensitivity because of the increased expression of transporters with a broad transition metal specificity. *J Biol Chem*, 273, pp. 22181-22187, ISSN 0021-9258
- Liu, S.X.; Fabisiak, J.P.; Tyurin, V.A.; Borisenko, G.G.; Pitt, B.R.; Lazo, J.S. & Kagan, V.E. (2000). Reconstitution of apo-superoxide dismutase by nitric oxide-induced copper transfer from metallothioneins. *Chem Res Toxicol*, 13, pp. 922-931, ISSN 0893-228X
- Lucca, P.; Hurrell, R. & Potrykus, I. (2002). Fighting iron deficiency anemia with iron-rich rice. *J Am Coll Nutr*, 21, pp. 184-190, ISSN 0731-5724
- Marentes, E. & Rauser, W.E. (2007). Different proportions of cadmium occur as Cd-binding phytochelatin complexes in plants. *Physiol Plant*, 131, pp. 291-301, ISSN 1399-3054
- Margoshes, M. & Vallee, B.L. (1957). A cadmium protein from equine kidney cortex. *J Chem Soc*, 79 pp. 4813-4819, ISSN 0022-4936
- Mei, H.; Cheng, N.H.; Zhao, J.; Park, S.; Escareno, R.A.; Pittman, J.K. & Hirschi, K.D. (2009). Root development under metal stress in *Arabidopsis thaliana* requires the H⁺/cation antiporter CAX4. *New Phytol*, 183, pp. 95-105, ISSN 1469-8137
- Memon, A.R. & Schroder, P. (2009). Implications of metal accumulation mechanisms to phytoremediation. *Environ Sci Pollut Res Int*, 16, pp. 162-175, ISSN 0944-1344
- Merrifield, M.E.; Ngu, T. & Stillman, M.J. (2004). Arsenic binding to *Fucus vesiculosus* metallothionein. *Biochem Biophys Res Commun*, 324, pp. 127-132, ISSN 0006-291X
- Miller, G.; Shulaev, V. & Mittler, R. (2008). Reactive oxygen signaling and abiotic stress. *Physiol Plant*, 133, pp. 481-489, ISSN 1399-3054
- Mittler, R., Vanderauwera, S., Gollery, M., & Van Breusegem, F. (2004). Reactive oxygen gene network of plants, *Trends Plant Sci*, 9, pp. 490-498, ISSN 1360-1385
- Mithofer, A.; Schulze, B. & Boland, W. (2004). Biotic and heavy metal stress response in plants: evidence for common signals. *FEBS Lett*, 566, pp. 1-5, ISSN 0014-5793
- Navarro, A.; Quiros, L.; Casado, M.; Faria, M.; Carrasco, L.; Benejam, L.; Benito, J.; Diez, S.; Raldua, D.; Barata, C.; Bayona, J.M. & Pina, B. (2009). Physiological responses to mercury in feral carp populations inhabiting the low Ebro River (NE Spain), a historically contaminated site. *Aquat Toxicol*, 93, pp. 150-157, ISSN 1879-1514
- Neill, S.J.; Desikan, R.; Clarke, A.; Hurst, R.D. & Hancock, J.T. (2002). Hydrogen peroxide and nitric oxide as signalling molecules in plants. *J Exp Bot*, 53, pp. 1237-1247, ISSN 0022-0957
- Ngu, T.T.; Dryden, M.D. & Stillman, M.J. (2010). Arsenic transfer between metallothionein proteins at physiological pH. *Biochem Biophys Res Commun*, 401, pp. 69-74, ISSN 0006-291X
- O'Halloran, T.V. & Culotta, V.C. (2000). Metallochaperones, an intracellular shuttle service for metal ions. *J Biol Chem*, 275, pp. 25057-25060, ISSN 0021-9258
- Oomen, R.J.; Wu, J.; Lelievre, F.; Blanchet, S.; Richaud, P.; Barbier-Brygoo, H.; Aarts, M.G. & Thomine, S. (2009). Functional characterization of NRAMP3 and NRAMP4 from the metal hyperaccumulator *Thlaspi caerulescens*. *New Phytol*, 181, pp. 637-650, ISSN 1469-8137

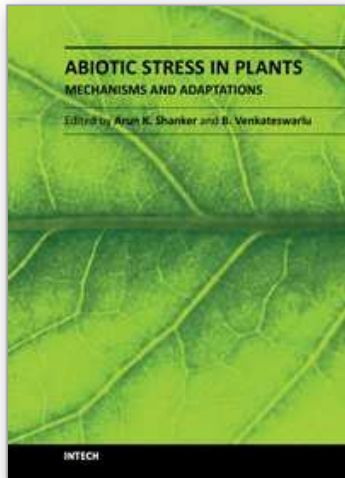
- Ortiz, D.F., Kreppel, L., Speiser, D.M., Scheel, G., McDonald, G., & Ow, D.W. (1992). Heavy metal tolerance in the fission yeast requires an ATP-binding cassette-type vacuolar membrane transporter, *Embo J*, 11, pp. 3491-3499, ISSN 0261-4189
- Ortiz, D.F., Ruscitti, T., McCue, K.F., & Ow, D.W. (1995). Transport of metal-binding peptides by HMT1, a fission yeast ABC-type vacuolar membrane protein, *J Biol Chem*, 270, pp. 4721-4728, ISSN 0021-9258
- Palmiter, R.D. (2004). Protection against zinc toxicity by metallothionein and zinc transporter 1. *Proc Natl Acad Sci USA*, 101, pp. 4918-4923, ISSN 0027-8424
- Park, K.Y.; Jung, J.Y.; Park, J.; Hwang, J.U.; Kim, Y.W.; Hwang, I. & Lee, Y. (2003). A role for phosphatidylinositol 3-phosphate in abscisic acid-induced reactive oxygen species generation in guard cells. *Plant Physiol*, 132, pp. 92-98, ISSN 0032-0889
- Pei, Z.M.; Murata, Y.; Benning, G.; Thomine, S.; Klusener, B.; Allen, G.J.; Grill, E. & Schroeder, J.I. (2000). Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. *Nature*, 406, pp. 731-734, ISSN 0028-0836
- Persans, M.W.; Nieman, K. & Salt, D.E. (2001). Functional activity and role of cation-efflux family members in Ni hyperaccumulation in *Thlaspi goesingense*. *Proc Natl Acad Sci USA*, 98, pp. 9995-10000, ISSN 0027-8424
- Pich, A.; Manteuffel, R.; Hillmer, S.; Scholz, G. & Schmidt, W. (2001). Fe homeostasis in plant cells: does nicotianamine play multiple roles in the regulation of cytoplasmic Fe concentration? *Planta*, 213, pp. 967-976, ISSN 0032-0935
- Pinto, E.; Sigaud-Kutner, T.C.S.; Leitao, M.A.S.; Okamoto, O.K.; Morse, D. & Colepicolo, P. (2003). Heavy metal-induced oxidative stress in algae. *J Phycol*, 39, pp. 1008-1018, ISSN 0022-3646
- Puig, S.; Mira, H.; Dorcey, E.; Sancenon, V.; Andres-Colas, N.; Garcia-Molina, A.; Burkhead, J.L.; Gogolin, K.A.; Abdel-Ghany, S.E.; Thiele, D.J.; Ecker, J.R.; Pilon, M. & Penarrubia, L. (2007). Higher plants possess two different types of ATX1-like copper chaperones. *Biochem Biophys Res Commun*, 354, pp. 385-390, ISSN 0006-291X
- Rausser, W.E. (1999). Structure and function of metal chelators produced by plants: the case for organic acids, amino acids, phytin, and metallothioneins. *Cell Biochem Biophys*, 31, pp. 19-48, ISSN 1085-9195
- Rea, P.A. (2007). Plant ATP-Binding cassette transporters. *Annu Rev Plant Biol*, 58, pp. 347-375, ISSN 1040-2519
- Reeves, R.D. & Baker, A.J.M. (2000). Metal-accumulating plants. In: *Phytoremediation of Toxic Metals: Using Plants to Clean Up the Environment*, I. Raskin, Ensley, B.D., (Ed.), pp. 193-229, John Wiley & Sons Inc, ISBN 978-0-471-19254-1, New York.
- Reid, S.J. & Ross, G.S. (1997). Up-regulation of two cDNA clones encoding metallothionein-like proteins in apple fruit during cool storage. *Physiol Plantarum*, 100, pp. 183 -189, ISSN 1399-3054
- Reynolds, T.L. & Crawford, R.L. (1996). Changes in abundance of an abscisic acid-responsive, early cysteine-labeled metallothionein transcript during pollen embryogenesis in bread wheat (*Triticum aestivum*). *Plant Mol Biol*, 32, pp. 823-829, ISSN 0167-4412
- Rhee, S.G. (2006). H₂O₂, a necessary evil for cell signaling. *Science*, 312, pp. 1882-1883, ISSN 1095-9203
- Robinson, N.J.; Tommey, A.M.; Kuske, C. & Jackson, P.J. (1993). Plant metallothioneins. *Biochem J*, 295, pp. 1-10, ISSN 0264-6021

- Robinson, N.J. & Winge, D.R. (2010). Copper metallochaperones. *Annu Rev Biochem*, 79, pp. 537-562, ISSN 0066-4154
- Roesijadi, G. (2000). Metal transfer as a mechanism for metallothionein-mediated metal detoxification. *Cell Mol Biol (Noisy-le-grand)*, 46, pp. 393-405, ISSN 0145-5680
- Roosens, N.H.; Bernard, C.; Leplae, R. & Verbruggen, N. (2004). Evidence for copper homeostasis function of metallothionein (MT3) in the hyperaccumulator *Thlaspi caerulescens*. *FEBS Lett*, 577, pp. 9-16, ISSN 0014-5793
- Sagner, S.; Kneer, R.; Wanner, G.; Cosson, J.P.; Deus-Neumann, B. & Zenk, M.H. (1998). Hyperaccumulation, complexation and distribution of nickel in *Sebertia acuminata*. *Phytochemistry*, 47, pp. 339-347, ISSN 0031-9422
- Salt, D.E. & Wagner, G.J. (1993). Cadmium transport across tonoplast of vesicles from oat roots. Evidence for a $\text{Cd}^{2+}/\text{H}^{+}$ antiport activity. *J Biol Chem*, 268, pp. 12297-12302, ISSN 0021-9258
- Salt, D.E., & Rauser, W.E. (1995). MgATP-Dependent Transport of Phytochelatins Across the Tonoplast of Oat Roots, *Plant Physiol*, 107, pp. 1293-1301, ISSN 1532-2548
- Salt, D.E.; Pickering, I.J.; Prince, R.C.; Gleba, D.; Dushenkov, S.; Smith, R.D. & Raskin, I. (1997). Metal accumulation by aquacultured seedlings of Indian mustard. *Environ Sci Technol*, 31, pp. 1636-1644, ISSN 0013-936X
- Salt, D.E. (2006). An extreme plant lifestyle: metal hyperaccumulation. In: *Companion to Plant Physiology*, L. Taiz, Zeiger, E., (Ed.), Sinauer Associates, Inc., ISBN 9780878935116, Sunderland, MA.
- Sato, M. & Bremner, I. (1993). Oxygen free radicals and metallothionein. *Free Radic Biol Med*, 14, pp. 325-337, ISSN 0891-5849
- Satofuka, H.; Fukui, T.; Takagi, M.; Atomi, H. & Imanaka, T. (2001). Metal-binding properties of phytochelatin-related peptides. *J Inorg Biochem*, 86, pp. 595-602, ISSN 0162-0134
- Schutzendubel, A. & Polle, A. (2002). Plant responses to abiotic stress: heavy metal induced oxidative stress and protection by mycorrhization. *J Exp Bot*, 53, pp. 1351-1365, ISSN 0022-0957
- Sharma, S.S. & Dietz, K.J. (2006). The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. *J Exp Bot*, 57, pp. 711-726, ISSN 0022-0957
- Singh, K.; Foley, R.C. & Onate-Sanchez, L. (2002). Transcription factors in plant defense and stress responses. *Curr Opin Plant Biol*, 5, pp. 430-436, ISSN 1369- 5266
- Song, W.Y.; Park, J.; Mendoza-Cozatl, D.G.; Suter-Grotemeyer, M.; Shim, D.; Hortensteiner, S.; Geisler, M.; Weder, B.; Rea, P.A.; Rentsch, D.; Schroeder, J.I.; Lee, Y. & Martinoia, E. (2010). Arsenic tolerance in *Arabidopsis* is mediated by two ABCC-type phytochelatin transporters. *Proc Natl Acad Sci USA*, 107, pp. 21187-21192, ISSN 1091-6490
- Suzuki, K.T. & Kuroda, T. (1995). Transfer of copper and zinc from ionic and metallothionein-bound forms to Cu, Zn--superoxide dismutase. *Res Commun Mol Pathol Pharmacol*, 87, pp. 287-296, ISSN 1078-0297
- Suzuki, N.; Koizumi, N. & Sano, H. (2001). Screening of cadmium-responsive genes in *Arabidopsis thaliana*. *Plant Cell Environ*, 24, pp. 1177-1188, ISSN 0140-7791
- Thomine, S.; Wang, R.; Ward, J.M.; Crawford, N.M. & Schroeder, J.I. (2000). Cadmium and iron transport by members of a plant metal transporter family in *Arabidopsis* with

- homology to Nramp genes. *Proc Natl Acad Sci USA*, 97, pp. 4991-4996, ISSN 0027-8424
- Thomine, S.; Lelievre, F.; Debarbieux, E.; Schroeder, J.I. & Barbier-Brygoo, H. (2003). AtNRAMP3, a multispecific vacuolar metal transporter involved in plant responses to iron deficiency. *Plant J*, 34, pp. 685-695, ISSN 0960-7412
- Tseng, M.J.; Liu, C.W. & Yiu, J.C. (2007). Enhanced tolerance to sulfur dioxide and salt stress of transgenic Chinese cabbage plants expressing both superoxide dismutase and catalase in chloroplasts. *Plant Physiol Bioch*, 45, pp. 822-833, ISSN 0981-9428
- Tuteja, N. (2007). Absciscic Acid and abiotic stress signaling. *Plant Signal Behav*, 2, pp. 135-138, ISSN 1559-2316
- van der Zaal, B.J.; Neuteboom, L.W.; Pinas, J.E.; Chardonens, A.N.; Schat, H.; Verkleij, J.A. & Hooykaas, P.J. (1999). Overexpression of a novel *Arabidopsis* gene related to putative zinc-transporter genes from animals can lead to enhanced zinc resistance and accumulation. *Plant Physiol*, 119, pp. 1047-1055, ISSN 0032-0889
- Vasak, M.; Hawkes, G.E.; Nicholson, J.K. & Sadler, P.J. (1985). 113Cd NMR studies of reconstituted seven-cadmium metallothionein: evidence for structural flexibility. *Biochemistry*, 24, pp. 740-747, ISSN 0006-2960
- Wagner, G.J. (1993). Accumulation of Cadmium in Crop Plants And Its Consequences to Human Health *Advances in Agronomy*, 51, pp. 173-212, ISSN 0065-2113
- Wang, T. & Wu, M. (2006). An ATP-binding cassette transporter related to yeast vacuolar ScYCF1 is important for Cd sequestration in *Chlamydomonas reinhardtii*. *Plant Cell Environ*, 29, pp. 1901-1912, ISSN 0140-7791
- Wang, W.; Vinocur, B. & Altman, A. (2003). Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta*, 218, pp. 1-14, ISSN 0032-0935
- Wang, Y.C.; Qu, G.Z.; Li, H.Y.; Wu, Y.J.; Wang, C.; Liu, G.F. & Yang, C.P. (2010). Enhanced salt tolerance of transgenic poplar plants expressing a manganese superoxide dismutase from *Tamarix androssowii*. *Mol Biol Rep*, 37, pp. 1119-1124, ISSN 0301-4851
- 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, pp. 269-281, ISSN 0960-7412
- Weber, M.; Trampczynska, A. & Clemens, S. (2006). Comparative transcriptome analysis of toxic metal responses in *Arabidopsis thaliana* and the Cd(2+)-hypertolerant facultative metallophyte *Arabidopsis halleri*. *Plant Cell Environ*, 29, pp. 950-963, ISSN 0140-7791
- Wei, W.; Chai, T.; Zhang, Y.; Han, L.; Xu, J. & Guan, Z. (2009). The *Thlaspi caerulescens* NRAMP homologue TcNRAMP3 is capable of divalent cation transport. *Mol Biotechnol*, 41, pp. 15-21, ISSN 1073-6085
- Willner, H.; Vasak, M. & Kagi, J.H. (1987). Cadmium-thiolate clusters in metallothionein: spectrophotometric and spectropolarimetric features. *Biochemistry*, 26, pp. 6287-6292, ISSN 0006-2960
- Wong, H.L.; Sakamoto, T.; Kawasaki, T.; Umemura, K. & Shimamoto, K. (2004). Down-regulation of metallothionein, a reactive oxygen scavenger, by the small GTPase OsRac1 in rice. *Plant Physiol*, 135, pp. 1447-1456, ISSN 0032-0889

- Wright, C.F.; Mckenney, K.; Hamer, D.H.; Byrd, J. & Winge, D.R. (1987). Structural and Functional-Studies of the Amino Terminus of Yeast Metallothionein. *J Biol Chem*, 262, pp. 12912-12919, ISSN 0021-9258
- Xue, T.T.; Li, X.Z.; Zhu, W.; Wu, C.G.; Yang, G.G. & Zheng, C.C. (2009). Cotton metallothionein GhMT3a, a reactive oxygen species scavenger, increased tolerance against abiotic stress in transgenic tobacco and yeast. *J Exp Bot*, 60, pp. 339-349, ISSN 0022-0957
- Yang, Z.; Wu, Y.R.; Li, Y.; Ling, H.Q. & Chu, C.C. (2009). OsMT1a, a type 1 metallothionein, plays the pivotal role in zinc homeostasis and drought tolerance in rice. *Plant Mol Biol*, 70, pp. 219-229, ISSN 0167-4412
- Zenk, M.H. (1996). Heavy metal detoxification in higher plants:a review. *Gene*, 179, pp. 21-30, ISSN 0378-1119
- Zhou, J. & Goldsbrough, P.B. (1994). Functional homologs of fungal metallothionein genes from Arabidopsis. *Plant Cell*, 6, pp. 875-884, ISSN 1040-4651
- Zhu, J.; Zhang, Q.; Wu, R. & Zhang, Z. (2010). HbMT2, an ethephon-induced metallothionein gene from *Hevea brasiliensis* responds to H₂O₂ stress. *Plant Physiol Biochem*, 48, pp. 710-715, ISSN 1873-2690

IntechOpen



Abiotic Stress in Plants - Mechanisms and Adaptations

Edited by Prof. Arun Shanker

ISBN 978-953-307-394-1

Hard cover, 428 pages

Publisher InTech

Published online 22, September, 2011

Published in print edition September, 2011

World population is growing at an alarming rate and is anticipated to reach about six billion by the end of year 2050. On the other hand, agricultural productivity is not increasing at a required rate to keep up with the food demand. The reasons for this are water shortages, depleting soil fertility and mainly various abiotic stresses. The fast pace at which developments and novel findings that are recently taking place in the cutting edge areas of molecular biology and basic genetics, have reinforced and augmented the efficiency of science outputs in dealing with plant abiotic stresses. In depth understanding of the stresses and their effects on plants is of paramount importance to evolve effective strategies to counter them. This book is broadly divided into sections on the stresses, their mechanisms and tolerance, genetics and adaptation, and focuses on the mechanic aspects in addition to touching some adaptation features. The chief objective of the book hence is to deliver state of the art information for comprehending the nature of abiotic stress in plants. We attempted here to present a judicious mixture of outlooks in order to interest workers in all areas of plant sciences.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Zhao Yang and Chengcai Chu (2011). Towards Understanding Plant Response to Heavy Metal Stress, Abiotic Stress in Plants - Mechanisms and Adaptations, Prof. Arun Shanker (Ed.), ISBN: 978-953-307-394-1, InTech, Available from: <http://www.intechopen.com/books/abiotic-stress-in-plants-mechanisms-and-adaptations/towards-understanding-plant-response-to-heavy-metal-stress>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the [Creative Commons Attribution-NonCommercial-ShareAlike-3.0 License](https://creativecommons.org/licenses/by-nc-sa/3.0/), which permits use, distribution and reproduction for non-commercial purposes, provided the original is properly cited and derivative works building on this content are distributed under the same license.

IntechOpen

IntechOpen