Exploring the Plant Response to Cadmium Exposure by Transcriptomic, Proteomic and Metabolomic Approaches: Potentiality of High-Throughput Methods, Promises of Integrative Biology

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Abstract With the evolution of high-throughput techniques, performing organism-wide analyses of gene expression, protein content and metabolite profile is becoming easier. This new era of biology opens the way for incredibly promising advances but also extremely challenging difficulties in the aim of integrating these datasets into biologically relevant models. To address questions and unravel networks occurring in cadmium stressed plants, researchers have already taken advantage of such tools. We provide here an overview of the discoveries that have been made regarding plant−cadmium interaction using "omics" methods, and show how their use is relevant to compare species and stresses at the whole plant level. At the end, we propose future breakthroughs to be addressed using these techniques, and discuss the up-coming challenges of data mining and model drawing.

Abbreviations

ACO	Aconitase
ALD	Fructose-bisphosphate aldolase
APS	sulfurylase
AtATX1	Homolog of Anti-oxidant 1
AtCCH	Copper chaperone
CAX	Cation exchanger
Cd	Cadmium
CS	Citrate synthase
Cu	Copper
ENO	Enolase (2-phosphoglycerate hydrolase)
GABA	γ-Aminobutyrate
GAPC	Cytosolic glyceraldehyde 3-phosphate dehydrogenase
GLN1	Glutamine synthetase
GLT	Glutamate synthetase
GSH	Glutathione
GSH1	γ-glutamylcysteine synthase
GSH2	Glutathione synthetase
GST	Glutathione S-transferase
HK	Hexose kinase
HMA3	Heavy metal ATP ASE 3
HSPs	Heat shock proteins
ISO	NADP-specific isocitrate dehydrogenase
MDH	Malate dehydrogenase
MetSO	Methionine sulfoxide
NRAMP	Natural resistance-associated macrophage protein
OAS	*O*-acetylserine (thiol)lyase
PC	Phytochelatin
PDH E1	E2 & E3, pyruvate dehydrogenase complex components
PGM	Phosphoglycerate mutase
PK	Pyruvate kinase
Pr	Pathogenesis-related

Prx	Peroxiredoxin
PSI, II	Photosystem I, II
ROS	Reactive oxygen species
RuBisCO	Ribulose bisphosphate carboxylase oxygenase
SBP1	Selenium binding protein 1
SHMT	Serine hydroxymethyltransferase
SOD	Superoxide dismutase
SUC	Succinate dehydrogenase
TCA	Tricarboxylic acid
TPI	Triose phosphate isomerase
Ub	Ubiquitin

1 Introduction

Cadmium (Cd) is a major pollutant of several industrialized or developing countries and its effects on human health are particularly deleterious (Ishihara et al. 2001). Industrial activities and use of sewage sludge account form the principal sources of Cd, and lead to a continuous increase in its concentration in crop fields and water sources. Cd is considered as a non-essential metal, even though it has been reported to act as a cofactor of carbonic anhydrase in *Thalassiosira weissflogii* and other marine diatoms (Lane et al. 2005). Like other non-essential metals, it is assimilated by plant roots via non-specific transport activity (Clemens 2006). Once in the xylem, it spreads over the entire organism where it affects numerous biological functions probably by replacing metal cofactors, by interacting with protein thiols and by inducing the production of reactive oxygen species. In fact, the mechanistic basis of its toxicity is still poorly understood. In order to cope with Cd, plants have evolved various mechanisms leading to its chelation, exclusion and/or stabilization (Clemens 2006; Cobbett and Goldsbrough 2002; Verbruggen et al. 2009). One of the main well-known protection mechanism in plants involves its chelation by phytochelatins, which are cysteine-rich peptides of general formula $(\gamma\text{Glu-Cys})_n$-X [with $n = 2$–11, and $X =$ Gly (PC) or Ser, Ala or Glu (isoPC)], followed by their transport into the vacuoles (Cobbett 2000; Rauser 1990; Zenk 1996). Understanding the molecular activities underlying these processes could allow one to take advantage of them for phytoremediation of contaminated soils or biofortification for instance to avoid Cd in the edible parts of plants (Pilon-Smits 2005; Palmgren 2008).

Transcriptome-, proteome- and metabolome-wide studies have become increasingly used, and a wide variety of techniques allows for quantitative and/or qualitative analyses of samples at the plant, tissue or organelle level (Agrawal et al. 2011; Baginsky et al. 2010; Fukushima et al. 2009). They provide accurate snapshots of their molecular content at a given time and growth conditions, and comparisons of these datasets are able to give an invaluable dimension of dynamic to this profiling. A number of transcriptomic analysis using microarrays have been performed in the last few years to study the impact of Cd on plants (Herbette et al. 2006; Kovalchuk et al. 2005; van de Mortel et al. 2008; Weber et al. 2006; Yamaguchi et al. 2010; Zhao et al. 2009, 2010). Highly informative in terms of gene expression regulation

over a time and/or concentration course, they allow the identification of early processes to be switched on and off by the plant during exposure to the metal. Recently, the monitoring of transcript levels of *Solanum torvum* plants subjected to low Cd stress was achieved by high-throughput RNA sequencing technologies (RNA seq) (Yamaguchi et al. 2010). This technique, yet still more expensive than microarray-based analyses, allows one to qualitatively and quantitatively explore transcriptome of species for which no microarray chip is commercially available, or species which their genome has not been annotated or even sequenced.

Proteomic analyses usually involves a pre-fractionation step of the sample, followed by a trypsic digestion of the proteins (Agrawal et al. 2011). Peptides are then analyzed by peptide mass fingerprinting (PMF) or tandem MS (MS/MS), often after decomplexification by chromatography (nanoLC-MS). Protein fractionation prior to MS analyses is, in most cases, based on 1D or, more often, 2D-polyacrylamide gel electrophoresis (PAGE), and this applies to most of the studies analyzing the plant response to Cd at the leaf, root and cell culture levels (Aina et al. 2007; Aloui et al. 2009; Alvarez et al. 2009; Durand et al. 2010; Gianazza et al. 2007; Hajduch et al. 2001; Hradilova et al. 2010; Kieffer et al. 2008, 2009a, b; Lee et al. 2010; Rodriguez-Celma et al. 2010; Roth et al. 2006; Sarry et al. 2006; Semane et al. 2010). These comparative 2D-PAGE studies rely on the densitometry intensities of the protein spots on the gel, measured using dedicated analysis software after classical (colloidal Coomassie blue or silver staining) or fluorescent (DIGE) staining. Protein identification of the different spots is then performed by MS. In addition to these techniques, procedures allowing direct quantification in the mass spectrometer are being developed, and here again several methods have been successfully used. They consist in the simultaneous analysis, during a single MS run, of "heavy" and "light" samples, the "heavy" term referring to as stable isotope (^{15}N or ^{13}C for instance) labelling of the sample prior to protein extraction, during cell culture or plant growth. Other methods, such as iTRAQ, require post-extraction labeling, but allow for multiplexing of up to 8 samples. Whichever method being used, peptides from the different samples conserve identical properties, allowing them to enter the mass spectrometer at the same time when a nanoLC-based separation is performed. Then, simultaneous MS or MS/MS analysis of these peptides permits the discrimination of their origin, and thereby relative quantification. Both quantification methods, e.g. ^{15}N- (Lanquar et al. 2007) and iTRAQ- (Alvarez et al. 2009; Schneider et al. 2009) labeling have been used to evaluate Cd-induced changes in plants, as opposed to label-free methods such as spectral counting or, Accurate Mass and Time (AMT) tag approaches, the latter being particularly promising for quantitative proteomic works.

While transcriptomic and proteomic methodologies have been, to some extent, particularly used to study effects of Cd on plants, metabolomic-based work is much less well represented in the literature. A range of analytical platforms can be used to analyze the maximum number of metabolites in a given sample. These include Gas Chromatography–MS (GC–MS), comprehensive GC × GC–MS, Liquid Chromatography–MS (LC–MS) and variants including Ultra Performance Liquid Chromatography (UPLC), Capillary Electrophoresis–MS (CE–MS), Direct Infusion Mass Spectrometry (DIMS), Fourier Transform InfraRed Spectroscopy (FT–IR) and NMR spectroscopy (Fiehn et al. 2000; Goodacre et al. 2003; Hirai et al. 2004; Sato et al. 2004; Tolstikov et al. 2003; Ward et al. 2003; Wolfender et al.

2003). Both global (without any a priori) analyses and more targeted studies have been undertaken. For example, metabolite analyses performed using NMR revealed modifications of organic and amino acids contents after a Cd exposure (Bailey et al. 2003; Hediji et al. 2010) while a global approach performed by LC-MS/MS pointed out that the main variation at the metabolite level came from the presence of several types of chelating peptides (phytochelatins, PC) in Cd-stressed *Arabidopsis thaliana* cells (Sarry et al. 2006). Targeted metabolite studies revealed new insights into the regulation of the biosynthesis of glutathione (GSH) and PC biosynthesis in *A. thaliana* cells (Ducruix et al. 2006) whereas MS analyses of the phloem sap of *Brassica napus* suggested a role of the thiol-peptides (GSH and PCs) in the long-distance transport of Cd (Mendoza-Cozatl et al. 2008). With the aim of understanding targets of Cd in plants, as well as general and specific defence mechanisms it induces as a response to its toxicity, researchers have taken advantage of these "omics" techniques to draw the complex signaling and metabolic network resulting from Cd contamination. The picture, however, is still incomplete, and some key aspects of the plant defence mechanisms remain unknown. We present here a summary of the different results, from "ome"-wide methodology, that participate in the establishment of the current state of the art of the plant−Cd interaction. Other chapters intend to suggest new additional directions to be considered for further improvement of our knowledge. We particularly focus on the interest and promises of combining and cross-analyzing these data in a system biology-oriented framework to decipher actions and reactions of the plant−Cd conflict.

2 Global Response of Plants to Cadmium Exposure

2.1 Overview of a Cadmium Exposure in Plants as Evaluated by Transcriptomic, Proteomic and Metabolomic Approaches

2.1.1 Primary Metabolism

Even though its manifestation and amplitude importantly differs between plant species and stress severity, the harmful effect of Cd on the photosynthetic machinery is constantly reported as a major cause of its toxicity on plants (DalCorso et al. 2008; Sanità di Toppi and Gabbrielli 1999). Observed at both macroscopic (morphological) (Semane et al. 2010) and molecular (protein) levels, Cd exposure induces a strong disorganization of the light collecting antennas (Fagioni et al. 2009), electron transfer chain (Durand et al. 2010; Kieffer et al. 2009b), Calvin cycle (Durand et al. 2010) and membranes (Semane et al. 2010), as established by proteomic approaches. Kieffer and co-workers (2008) reported for instance a down regulation of several enzymes (chloroplast ferredoxin-$NADP^+$ oxidoreductase, Cyt B6-f complex iron-sulfur subunit, oxygen-evolving enhancer protein 1 and 2) on poplar, and Hajduch et al. (2001) observed changes in RuBisCO levels as well as other enzymes of the Calvin cycle on rice leaves, later confirmed on poplar (Durand et al. 2010) or spinach leaves (Fagioni and Zolla 2009). Interestingly these changes

at the protein levels are correlated with a global down regulation of the expression of genes involved in photosynthesis, meaning that not only is this reduction of protein accumulation the result of an enhanced degradation, but also the consequence of an active repression of their synthesis. Microarray analysis performed by Herbette et al. (2006) highlighted a transcriptional repression of genes coding for subunits of the PSI (psI-G, psI-K, psI-N, psaE, psaD, etc.), PSII (psbQ, psbP, psbW), enzymes of the chlorophyll biosynthetic pathway (Mg chelatases, 4–aminobutyrate transaminases, hydroxymethylbilane synthase, etc.), and enzymes of the Calvin cycle (Transketolase, Rubisco, Ribose-5-phosphate isomerase, fructose-bisphosphate aldolase, etc.) (schematized in Fig. 1). Overall, understanding the effect of Cd on photosynthesis is of prime importance to decipher molecular mechanisms leading to Cd resistance as the ability of plants to control the damage that is done to this Achilles' heel has been proposed to be a major contributor to their sensitivity/tolerance (Bah et al. 2010). Indeed, destabilization of the photosynthetic apparatus by Cd is thought to lead to an important number of

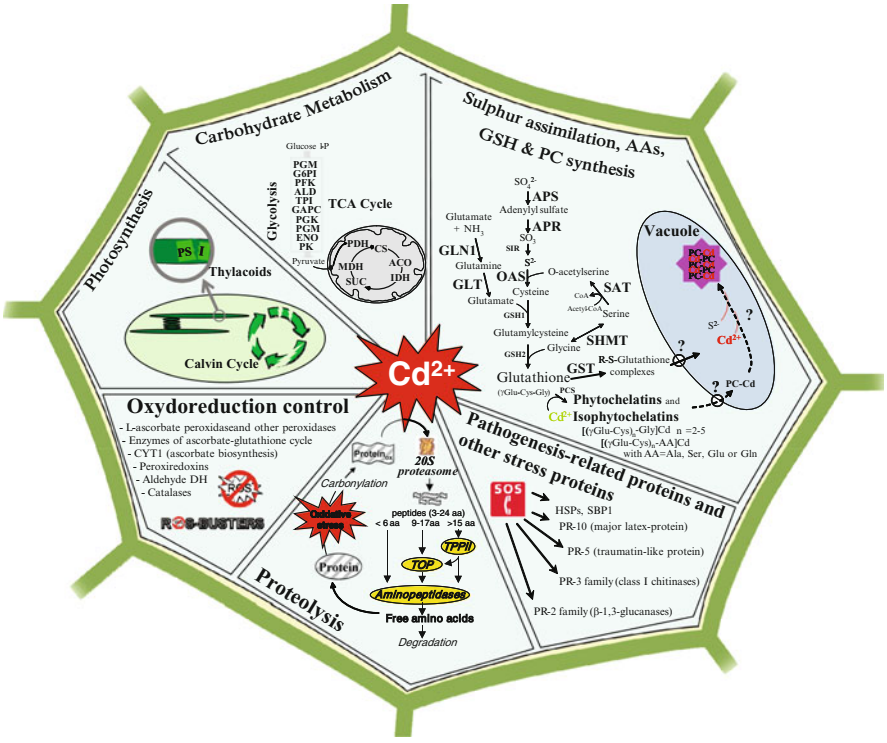

Fig. 1 *Some cellular functions and molecular mechanisms highlighted by "omics" investigations under cadmium stress*. Information concerning Cd-induced modifications of main cellular functions or metabolic pathways presented here (photosynthesis; carbohydrate metabolism, sulphur assimilation, amino acids (*AAs*), Glutathione (*GSH*) and phytochelatins (*PC*) syntheses; pathogenesis-related proteins and other stress proteins; proteolysis and oxidoreduction control) are further detailed in the text

subsequent modifications of the plant physiology, including carbohydrate metabolism re-organization or oxidative stress.

Plant response to Cd is, in contrast, less clear at the carbohydrate metabolism level, yet important modifications of its constituents are invariably mentioned. On the one hand, the shutdown of the photosynthetic machinery calls for an up-regulation of other sources of reducing power and ATP, such as glycolysis or TCA cycle; but on the other hand, supplying these pathways with necessary carbohydrates is becoming precarious as the main source of carbon has dried up. In poplar leaves, Kieffer et al. (2008) showed that an α–amylase was up-regulated, as well as a fructose kinase 2, involved in starch degradation and the phosphorylation of fructose prior to its entry in the glycolysis pathway. Similarly, genes encoding α– and β–amylases were shown to be up-regulated at the transcriptional level (Herbette et al. 2006). In parallel, numerous enzymes of this pathway (hexose kinase, fructose-bisphosphate aldolase, triose phosphate isomerase, cytosolic glyceraldehyde 3-phosphate dehydrogenase, phosphoglycerate mutase, 2-phosphoglycerate hydrolase, pyruvate kinase) were shown to be up-regulated in different proteomic works performed on *Arabidopsis* (Roth et al. 2006; Sarry et al. 2006), rice (Aina et al. 2007; Lee et al. 2010), poplar (Durand et al. 2010; Kieffer et al. 2009a, b), tomato (Rodriguez-Celma et al. 2010) or flax (Hradilova et al. 2010) (Fig. 1). Accumulation of these enzymes was also correlated with an up-regulation of several enzymes of the TCA cycle (pyruvate dehydrogenase complex enzymes 1, 2 and 3, citrate synthase, aconitase, NADP-specific isocitrate dehydrogenase, succinate dehydrogenase or malate dehydrogenase) (Kieffer et al. 2009b; Sarry et al. 2006; Semane et al. 2010; Visioli et al. 2010). However, even though some enzymes of this pathway (glucose-6-phosphate dehydrogenase, transketolase) seem to be up-regulated at the protein level (Kieffer et al. 2009b; Sarry et al. 2006), transcriptomic analysis revealed a repression of the expression for most of the enzymes, including 2-glucose-6-phosphate dehydrogenases and the transketolase, but also the 3-hydroxyisobutyrate dehydrogenase and several phosphogluconate dehydrogenases and gluconolactonases (Herbette et al. 2006). These contradictory results may reflect differences in time of exposure and concentration of Cd employed, as these pathways are, though dependent upon carbon source availability, required for preserving suitable levels of reducing molecules and carbon skeletons that are necessary for the synthesis of amino acids and molecules involved in Cd^{2+} chelation such as GSH and PC.

It is noticeable that enzymes of the sulfur assimilation pathway, all the way down to cysteine synthesis and GSH production, are actively up-regulated in order to sustain the production of PC (Figs. 1 and 2). Herbette et al. (2006) reported a transcriptional activation of 6 sulfate transporters, out of 14, in response to Cd, including the high-affinity Sultr1:1 at as early as 2 h of a 50-µM Cd exposure (Fig. 2). In addition to an increased sulfur uptake from the medium, intracellular stocks are also remobilized and Cd exposure leads to an overexpression of the vacuolar Sultr4:1 transporter, as well as others involved in long-distance transport (Sultr2:1, Sultr3:5). Genes coding for enzymes involved in the subsequent reduction of sulfur in sulfide (ATP sulfurylase, APS reductase) are also activated early. Interestingly, an ATP sulfurylase was also found up-regulated in the Cd^{2+}-hypertolerant facultative

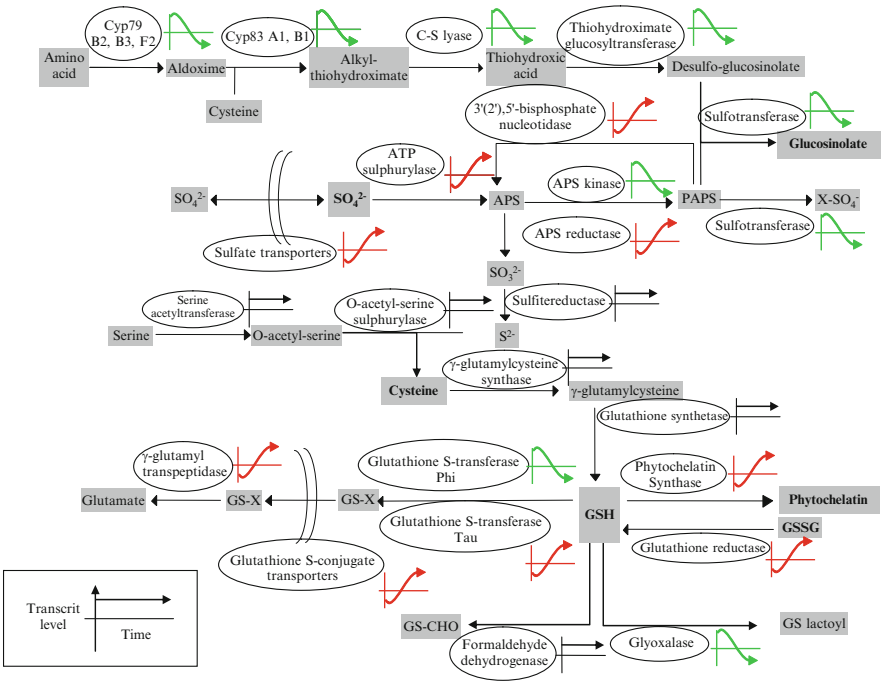

Fig. 2 *Simplified and schematic representation of transcriptional changes of genes involved in sulphur and glutathione metabolisms in roots*. The metabolites are represented in *gray boxes*; *arrows* represent enzymatic reactions; when determined, changes in gene expression are indicated schematically to the right of each gene

metallophyte *A. halleri* at 125 μM Cd^{2+} after 2 h of exposure and was present in the five genes that constitute the *A. halleri* core response (Weber et al. 2006). In *Thlaspi caerulescens*, another Zn/Cd hyperaccumulator, an ATP sulfurylase was also up-regulated under Zn-deficiency and high Cd exposure (van de Mortel et al. 2008). This gene was also strongly up-regulated in *Solanum torvum* roots stressed with a mild Cd^{2+} treatment (0.1 μM) for 3 days (Yamaguchi et al. 2010).

During Cd stress, the up-regulation of enzymes involved in the sulfur assimilation pathway, which ultimately leads to cysteine and enzymes involved in its amino acid precursor biosynthesis, was also confirmed by proteomic works with the accumulation of ATP sulfurylase, serine hydroxymethyltransferase (both mitochondrial and cytosolic forms), components of the glycine decarboxylase complex and *O*-acetylserine (thiol)lyase (Fagioni and Zolla 2009; Kieffer et al. 2009a; Roth et al. 2006; Sarry et al. 2006). In addition to these enzymes, others belonging to the amino acid metabolism and in particular to the synthesis of the other aminoacid precursors of glutathione, were also shown to be upregulated. This includes a glutamine synthetase, a glutamate synthase, an alanine aminotransferase, an aspartate aminotransferase, or the alanine:glyoxylate aminotransferase 2, which produces glycine from alanine (Aina et al. 2007; Kieffer et al. 2008, 2009a, b; Roth et al. 2006; Sarry et al. 2006; Semane et al. 2010).

Interestingly, Herbette et al. (2006) observed that Cd treatment induced an early and sustained down-regulation of many genes involved in the biosynthesis pathway of glucosinolates (Fig. 2), sulfur-rich compounds which play an important role in defense against herbivores and pathogens (Grubb and Abel 2006; Halkier and Gershenzon 2006). The down-regulation by Cd of most of the genes involved in this biosynthesis pathway observed by Herbette et al. (2006) suggests that, to support the sulfate demand for GSH and PC biosynthesis, the synthesis of glucosinolate is limited. In agreement with this hypothesis, the down-regulation of two genes encoding APS kinases and two genes encoding sulfotransferases were also observed, whereas three genes encoding 3'(2'),5'–biphosphate nucleotidase were up-regulated (Fig. 2).

2.1.2 Defence Mechanisms

Particularly highlighted in transcriptomic and proteomic studies, the deleterious effects of Cd on the plant redox status account for an important part of its toxicity. As opposed to iron, which mediates ROS production via the Haber-Weiss/Fenton reactions, Cd induces a redox imbalance by disorganizing the photosynthetic apparatus (Herbette et al. 2006) and by interfering with antioxidant enzymes and molecules (Clemens 2006; Sanità di Toppi and Gabbrielli 1999). A repression of several ROS-coping proteins, including, for instance, enzymes of the ascorbate biosynthesis pathway, can be observed (Kieffer et al. 2008), as well as the degradation of a Cu/Zn superoxide dismutase (Alvarez et al. 2009; Kieffer et al. 2008, 2009a). In contrast, a Fe-superoxide dismutase is up-regulated (Herbette et al. 2006; Kieffer et al. 2009a; Semane et al. 2010), together with different catalases and peroxidases (Alvarez et al. 2009; Herbette et al. 2006; Kieffer et al. 2008; Lee et al. 2010; Sarry et al. 2006), monodehydroascorbate reductase (Sarry et al. 2006; Alvarez et al. 2009; Herbette et al. 2006) and glutathione reductase (Herbette et al. 2006; Lee et al. 2010), transcriptional and protein levels being remarkably well correlated. In relation to this, the overexpression of numerous enzymes involved in the reduction of various oxidized molecules is particularly important. Peroxiredoxins (Ahsan et al. 2007; Durand et al. 2010; Sarry et al. 2006), phospholipid hydroperoxide glutathione peroxidase (Sarry et al. 2006), aldehyde dehydrogenases (Kieffer et al. 2009a; Sarry et al. 2006), formate dehydrogenase (Durand et al. 2010; Hradilova et al. 2010; Sarry et al. 2006), glyoxalase I (Ahsan et al. 2007), 2–nitropropane dioxygenase (Alvarez et al. 2009; Roth et al. 2006), NADPH oxidoreductase (Sarry et al. 2006), several quinone or other oxidoreductases (Kieffer et al. 2008, 2009a; Sarry et al. 2006; Semane et al. 2010; Visioli et al. 2010) were shown to be up-regulated under Cd treatment. In *Brassica juncea*, a methionine sulfoxide reductase, probably involved in the reduction of oxidized sulfur-containing residues of proteins (Davies 2005), was also reported to be induced by Cd (Alvarez et al. 2009). Interestingly, metabolite analyses performed by GC-MS or ^{1}H-NMR (Hediji et al. 2010; Sun et al. 2010) revealed a significant increase of antioxidant molecules including α-tocopherols, confirming the important role of antioxidant defences in the mechanisms of plant resistance to Cd (Collin et al. 2008).

This deleterious effect of Cd on proteins is further exemplified by the overexpression of a number of chaperones and HSPs, such as HSP70, DnaK subfamily proteins and HSP60 at the protein level (Ahsan et al. 2007; Hradilova et al. 2010; Kieffer et al. 2009b; Rodriguez-Celma et al. 2010; Sarry et al. 2006), and a disulfide isomerase (Kieffer et al. 2009b; Sarry et al. 2006), involved in protein correct folding. Up-regulation of HSPs, in particular small HSPs, was also highlighted at the transcription level (Herbette et al. 2006; Weber et al. 2006) (Fig. 1). However, and despite the overproduction of this important set of repairing enzymes, Cd-induced damages on proteins might be irreversible, as it is often the case when carbonylation occurs, for instance upon oxidative stress (Moller et al. 2007). In such a case, degradation of affected proteins is required, which importance was highlighted by in vitro assays (Polge et al. 2009). Polge et al. (2009) also showed that, in response to Cd, the 20S proteasome proteolytic pathway (i.e., the 20S proteasome and the peptidases acting sequentially downstream from the proteasome: tripeptidyl-peptidase II, thimet oligopeptidase and leucine aminopeptidase) is up-regulated at both RNA and protein activity levels in *Arabidopsis* leaves and may play a major role in degrading oxidized proteins generated by the Cd stress (Fig. 1). Experiments performed on yeast also demonstrated that mutants of the proteasome pathway are more sensitive to Cd than wt (Vido et al. 2001). Subunits of the 20S and 26S proteasomes, ubiquitin (Ub) family proteins, Ub-activating enzyme E1, Ub-conjugating enzyme E2, Ub-protein ligase E3, subtilase (serine-type endopeptidase), aspartyl protease family protein and cytosol aminopeptidase were shown to be up-regulated under Cd stress (Ahsan et al. 2007; Aina et al. 2007; Alvarez et al. 2009; Kieffer et al. 2008; Lee et al. 2010; Rodriguez-Celma et al. 2010; Roth et al. 2006; Sarry et al. 2006; Semane et al. 2010). In addition to proteins, other molecules might be affected by Cd and therefore require sequestration and degradation. The accumulation of several glutathione *S*-transferases (GST), mainly belonging to the τ- and φ-families, involved in the detoxification of toxic compounds by conjugation with GSH (Edwards et al. 2000; Marrs 1996), is often mentioned in the literature. Proteomic works performed in *Arabidopsis* revealed that AtGSTF2, 6, 7, 8, 9, 10 and AtGSTU8, 19 and 28 (Roth et al. 2006; Semane et al. 2010) were overexpressed by Cd. OsGSTU3, 4 and 12 were shown to be Cd-responsive in rice (Lee et al. 2010), and induction of various GSTs was also reported from studies conducted on soybean cells (Sobkowiak and Deckert 2006) and poplar (Kieffer et al. 2008, 2009b). Again, a good correlation exists between proteomic and transcriptomic data as several transcriptomic analyses also report up-regulated GST (Herbette et al. 2006; Kovalchuk et al. 2005; van de Mortel et al. 2008; Zhao et al. 2009) (Fig. 1).

Such detoxification processes require an important production of glutathione. While the sulfur assimilation/cysteine synthesis pathway is globally up-regulated upon Cd stress, enzymes involved in GSH synthesis (GSH1 and GSH2), in contrast, remain stable at both transcriptional and protein levels (Herbette et al. 2006; May et al. 1998; Weber et al. 2006). This strongly suggests that amino acid availability, rather than enzyme activity, is limiting in the production of GSH, used for both conjugation of toxic compounds and production of PC. While the different isoforms

of PC (defined by their C-terminal amino acid) were thought to be species specific (Zenk 1996), metabolic profiling performed on *Arabidopsis* cells actually revealed that all the known isoforms were produced in this same organism, and even specified different times and levels of production for each of them (Ducruix et al. 2006, 2008; Sarry et al. 2006). Further metabolomic approaches highlighted that, as suggested by transcriptomic and proteomic works, amino acid availability, rather than enzyme activity, was critical for GSH and PC synthesis. Indeed, severe Cd stress led to the accumulation of the GSH immediate precursor: the dipeptide γ-GluCys, immediately consumed when glycine was added to the medium (Ducruix et al. 2006). This research elegantly illustrates the meaningfulness of combining different "omics" approaches (e.g., proteomics and metabolomics), which in this case pictures strengths and weaknesses of the PC-based defence mechanism in plants.

Interestingly, transcriptomic studies have shown that genes involved in cell wall metabolism were modulated in response to Cd in roots and leaves (Herbette et al. 2006; van de Mortel et al. 2008). Genes involved in lignin biosynthesis such as cinnamoyl-coA reductases, cinamyl-alcohol dehydrogenases, 4–Coumarate: CoA ligase 2 were up-regulated (Herbette et al. 2006; van de Mortel et al. 2008) whereas several genes coding for expensins and pectinesterases involved in cell expansion were repressed (Herbette et al. 2006), suggesting that components to strengthen the cell wall were modified probably to protect the plants from Cd stress. The modification of the cell wall at the root level could be a specific detoxification mechanism that limits the entry of toxic metals in *Arabidopsis*, similar to what has been observed in *Phragmites australis* (Ederli et al. 2004). Proteomic approaches combined with genetic and biochemical analysis performed on candidate proteins that accumulates in *Arabidopsis* cells treated with Cd (Sarry et al. 2006) have highlighted potential new detoxification mechanisms that plants use to face Cd toxicity (Dutilleul et al. 2008). This was the case of the *Arabidopsis* homologue to the human selenium binding protein, SBP1. SBP1 overexpression was shown to protect *Arabidopsis* from the toxic effects of Cd and binding studies indicated direct interactions between Cd and the protein (Dutilleul et al. 2008). Expression analysis performed on *Arabidopsis SBP1::LUCIFERASE* lines and by immunodetection assays demonstrated that *SBP1* expression was not specifically induced in response to Cd stress but enhanced in response to stresses provoking a cellular sulfur demand (Hugouvieux et al. 2009). In line with these results, sulfur starvation increased SBP1 expression and SBP1 overexpression enhanced tolerance to stresses that require GSH for detoxification (Hugouvieux et al. 2009).

2.2 *Transcriptomic Analysis Allow for Large Scale Comparisons Between Species and/or Treatment*

Transcriptomics has been used to analyze the similarities and differences in the metal response among different plant species. In particular, comparative

transcriptomic analysis on *Arabidopsis thaliana* and in the Zn^{2+} and Cd^{2+}-hypertolerant facultative metallophyte *Arabidopsis halleri* were performed in order to identify response elements shared by these two species and highlight specific aspects of *A. halleri* that might lead to a better understanding of the metal-tolerance mechanisms (for more details see chapters of Marmiroli and colleagues). In pioneering works, Becher et al. (2004) and Weber et al. (2004) have shown that metal-homeostasis genes are constitutively expressed at much higher levels in *A. halleri* than in *A. thaliana* and independently from micronutrient status. It therefore appears that the effect of Cd on the *A. halleri* root transcriptome is much smaller than on that of *A. thaliana* (Weber et al. 2004). Indeed, after 2 h of treatment, only five genes were more than twofold up-regulated in *A. halleri*, versus 112 genes in *A. thaliana*. Four genes were up-regulated in the two species, constituting the "Cd^{2+} core response". A reduced Cd^{2+} uptake capacities by roots and its higher faculty to sequestrate Cd^{2+} could contribute, at least in part, to Cd^{2+} tolerance of *A. halleri* (Weber et al. 2006). More recently, Courbot et al. (2007) have shown that a QTL involved with Zn and Cd tolerance co-localizes in *A. halleri* with the plasma membrane P_{1B}-ATPase, HMA4, and it was demonstrated that the metal tolerance was due to both a triplication and altered cis regulation of the HMA4 gene (Hanikenne et al. 2008) (see also chapter on HMAs by Leonhardt et al.). Weber et al. (2006) have also shown that a short-term Cd exposure could provoke an apparent Zn^{2+} deficiency as judged by a high induction of the AtZIP9 uptake system and known as a marker of Zn deficiency. Another toxic effect of Cd could be an increased protein denaturation as illustrated by a high induction of several HSPs, and also mentioned by several proteomic data and other transcriptomic works. A transcriptomic study between *Arabidopsis thaliana* and the Zn/Cd-hyperaccumulator *Thlaspi caerulescens* exposed to different concentrations of Cd and Zn was performed by Aarts and colleagues (van de Mortel et al. 2008). Among the results they obtained, and as mentioned for *A. halleri*, a number of genes induced by Cd exposure were constitutively highly expressed in *T. caerulescens*. They also showed that Cd appears to induce Fe deficiency in *Arabidopsis*; an effect on lignin biosynthesis in both species as mentioned above and an effect on sulfate assimilation only in *T. caerulescens*. The discrepancy observed with other transcriptomic works could be explain by the difference in exposure times, since Herbette et al. (2006) mentioned that gene expression in response to Cd was more time-regulated than dose-regulated in *Arabidopsis*. Comparative transcriptomic analyses between plants exposed to different stresses were also established by other groups. Comparative microarray studies of seedlings exposed to Cd, Al, Cu and NaCl (applied at a similar degree of severity as estimated by root growth inhibition) were performed in order to identify the specific and generic responses they trigger (Zhao et al. 2009, 2010). Among the commonly up-regulated genes, they identified genes encoding ROS-scavenging enzymes such as GST and peroxidases, several Ca-binding proteins including calmodulin-like proteins, several enzymes involved in the tryptophane synthesis, the alanine amino-transferase 1 (ALAAT1), the trehalose–6–phosphate phosphatase and the arginine decarboxylase, these latter two enzymes being involved in the biosynthesis of trehalose and

polyamine synthesis, respectively, which are two stress-responsive metabolites. Interestingly, when the trehalose synthesis pathway was analyzed using the Kappa-view tool (Tokimatsu et al. 2005), an activation of the trehalose pathway was predicted, correlating with an increase of root trehalose content that was experimentally determined in all treatments (Zhao et al. 2010). In the specific group of genes that were up-regulated by Cd, Zhao et al. (2009, 2010) identified a GDP-mannose pyrophosphorylase (CYT1) involved in the ascorbate biosynthesis and critical for ROS-related stress resistance, heat shock proteins and pathogenesis-related (PR) proteins. Interestingly, several proteomic studies also revealed that some of the mechanisms involved in the plant response to a Cd stress are common, with inducible defence mechanisms activated upon pathogen attack. These include in particular the production of different PR proteins normally associated with the oxidative burst during biotic stress responses. This common feature may be due to the fact that signaling pathways are probably shared, as application of some hormones (e.g. ethylene, jasmonate and salycilic acid) are known to mimic the effect of a pathogen attack (Sels et al. 2008). Among the PR proteins induced by Cd are a major latex-protein, a PR-10 family member (Roth et al. 2006), also induced in response to salt stress and bacteria (Jiang et al. 2007; Jones et al. 2006), a traumatin-like protein, a PR-5 family member (Kieffer et al. 2008; Semane et al. 2010), class I chitinases, members of the PR-3 family and several β-1,3-glucanases belonging to the PR-2 family (Kieffer et al. 2008) (Fig. 1).

3 Future Directions

3.1 Subcellular-Level Analysis of the Cd Response

The major advantage of proteome-wide analyses of whole plants or tissues, which is a large coverage of most of the major proteins for a "no a priori" study, also constitutes a considerable drawback as peptides from minor proteins often get hidden during MS runs by other, more represented, peptides. This is particularly exemplified in the case of the vacuole, which overall protein content is suggested to represent less than 2%, in mass, of the total cellular proteome. While extremely important in the processes leading to Cd sequestration and tolerance, its protein constitution was, until recently, rather unknown and the vacuolar actors of the Cd tolerance poorly identified. The lack of exhaustiveness resulting from an under representation of a particular organelle proteome can be more or less easily circumvented by deciding to focus on this single subcellular compartment, and therefore doing a sub-fractionation of the plant material prior to protein extraction (Agrawal et al. 2011). First proteomic studies of the vacuole of *Arabidopsis* appeared between 2004 and 2007, and they led to the establishment of a list of about 1100 non-redundant proteins, including more than 120 transporters (Carter et al. 2004; Jaquinod et al. 2007a, b; Shimaoka et al. 2004; Szponarski et al. 2004).

Others have been completed, performed on barley (Endler et al. 2006) or cauliflower (Schmidt et al. 2007), which opened the way to differential proteomic analyses of this organelle under Cd stress. In this matter, Schneider et al. (2009) have used iTRAQ-labeled tonoplast samples isolated from barley subjected to 0, 20 or 200 μM Cd. Out of 56 transporters identified, 6 were found to be differentially expressed. Surprisingly, only one – an ABC-type MRP-like transporter homologous to AtMRP3 – was upregulated at 20 and 200 μM Cd, while others were only affected at 20 μM. It is interesting to notice that AtMRP3 was previously shown to be upregulated by Cd (Bovet et al. 2003), and to partly rescue the Cd-sensitive phenotype of DTY168 yeasts (Tommasini et al. 1998). A CAX1a-like transporters was found to be over-expressed at 20 μM (× 1.4), confirming that this kind of transporter might play an important role in Cd^{2+} detoxification, and a member of the natural resistance-associated macrophage protein (NRAMP) family (× 2.3), which homologous proteins AtNRAMP3 and 4 are known actors of the plant metal homeostasis (Lanquar et al. 2005, 2010). In addition, an isoform of the vacuolar H^+-pyrophosphatase was overexpressed (× 2) as well as a γ-TIP-like aquaporin (× 1.9). Such organelle-targeted differential studies were also conducted on the thylakoid membrane (Fagioni et al. 2009), where a disruption of the PSI was observed, concomitant with the diminution of chlorophyll a and b contents. In contrast, the PSII was less affected and no change was observed on the cytochrome *b6/f* and ATP-synthase complex organization. Changes at the plasma membrane level were also studied by ^{15}N labeling of cell cultures (Lanquar et al. 2007), and evidenced the overexpression by 2.5-fold of the ABC transporter AtPDR8, shown to be an extrusion pump conferring Cd resistance (Kim et al. 2007). An ammonium transporter (AtAMT1.1) was also shown to be fivefold upregulated, and seems to confirm the importance of side mechanisms, such as nutrient uptake to sustain amino acid production, as probable key players in the tolerance mechanisms. While very promising, these results did not lead to the clear identification of major, vacuolar actors of Cd tolerance, such as the ABCC1 and 2 ABC-type transporters recently characterized for their PC−arsenic transport activity in the vacuole (Song et al. 2010). The evaluation of their implication in the transport of PC−Cd complexes towards the lumen of the vacuole needs to be assessed, and may, after decades of research, finally identify the transporter responsible for this translocation. However it is particularly noticeable that, in the quest of this Holy Grail that is (or used to be?) the plant vacuolar PC−Cd transporter, authors have emphasized on the dynamic of membrane proteins. The yeast homologous *Sp*HMT1 or *Sc*YCF1, on the other hand, are not described to be up-regulated upon Cd stress, their activity being probably not limiting in the processes of vacuolar sequestration. Catalytic activities subsequent to Cd internalization in the vacuole and allowing stable and safe storage of this toxic are in contrast rather unknown, as well as a plausible recycling of the PCs, as suggested by some authors. Preliminary results of experiments aiming to identify soluble proteins differentially expressed under Cd stress revealed an over-expression of some of them, weakly expressed in control conditions, which are currently in the process of being characterized (Villiers F and Bourguignon J, unpublished data). Altogether, these results confirm an

organelle-targeted proteomic approach as very valuable, but also point out the need for researchers to expend their digging area, to soluble proteins for instance, or to activity regulation other than over-expression, such as post-translational modifications (PTM).

3.2 Post-translational Modifications: Phosphoproteomic Studies

PTM studies are an important challenge for the future and proteomic analysis of most PTM, except for protein phosphorylation, is still in the initial phases in plants (Ytterberg and Jensen 2010). Phosphoproteomic analysis of *Arabidopsis* upon various types of stimuli has tremendously increased in the literature over the last few years (Benschop et al. 2007; Kersten et al. 2009; Li et al. 2009; Niittylae et al. 2007), and has allowed the identification of numerous proteins as important actors of the processes being considered (Schulze 2010). These techniques have also been successfully applied to isolated organelles such as vacuoles (Endler et al. 2009; Whiteman et al. 2008a, b) or plasma membrane (Hem et al. 2007; Nuhse et al. 2007; Whiteman et al. 2008a), highlighting hundreds of phosphorylation sites. In parallel, other works have pointed out the phosphorylation status of different proteins such as transporters (Kobayashi et al. 2007) as essential in the regulation of aluminum tolerance in *Arabidopsis*. This should soon lead people interested in understanding the regulation of Cd tolerance to perform phosphorylation profiling of whole protein extract, as well as more tissue- or organelle-specific samples, from Cd-challenged plants.

3.3 Systems Biology and Integrated Analysis

As mentioned above, post-genomic biology has allowed advances in our understanding of plant biological systems by mining genomic data, making use of high-throughput methods (transcriptomics, proteomics, metabolomics, screening, etc.), and resources for mutants functional studies. This new era of integrative biology enables scientists to determine how the interconnected networks of genes, proteins and metabolites (substrates, other ligands, etc.) work together in complex cellular processes which can now be followed in the cell owing to revolutionary imaging technologies (Mano et al. 2009). However, the functioning of a plant as a system is not the result of a simple network, but rather a combination of multiple, intertwined, dynamic, and linear or nonlinear interactions between its components (DNA, RNA, proteins, metabolites, organelles, cell types, organs, etc.). It is now well accepted that the comprehensive knowledge of how a plant system functions in fluctuating environmental conditions cannot be achieved by the characterization of its elements one by one, or a single class of elements in isolation of the others (Ruffel et al. 2010). One of the most challenging issues for biology in this twenty-first century is deciphering the diversity and complexity of biological processes in plant science as

well as in other fields of fundamental biology. Addressing this priority is a multistep procedure as biological processes are first studied at a molecular scale, while seeking to elucidate the fine organization of the proteins involved, in order to monitor their dynamic interactions with other partners and to understand their reactivity. Then, the molecular information is integrated on a larger scale (organelle, cell and organism) and related to biological functions. A nice example of this approach is illustrated by the work of Curien and colleagues (2009). Combining expertise on molecular description of proteins, their interactions and their dynamics together with those on integrative biology, including well-thought modeling, should ultimately lead to a paradigm shift in how we understand complex biological processes, as a step towards systems biology and its technological counterpart, synthetic biology (Joyard and McCormick 2010).

3.4 Data Mining and Integration: The Bioinformatics Challenge

Because of their size, diversity and complexity, all the data generated by such multi-omics projects (DNA chips, qPCR, MS data as well as imaging and biochemical data) should be further integrated in order to search for the key information they contain. Several bottlenecks are already identified. Properly integrating data into dedicated knowledge bases (KBs) as well as designing user-oriented tools to efficiently query and visualize their content are two necessary steps towards higher level analysis, like network reconstruction or dynamic modeling of physiological states. In such a framework, standards are also necessary to ensure data quality and integrity in terms of capture, curation, verification and storage. For instance, depositing data in public repositories is necessary but not sufficient and the setting up of guidelines for reporting (namely "minimum information") and standards is an important step to ensure that submitted omics data are sufficient for non-ambiguous interpretation and querying by other scientists (Chervitz et al. 2011). Within this context, considerable efforts have been carried out to make these datasets publicly available through databases and web interfaces (for a recent list of plant database resources, see Mano et al. 2009). As such, these webtools often represent a good starting point for planning experiments or generating hypotheses in silico or exploring your own dataset by comparative approach (for review see Brady and Provart (2009)). A supplementary challenge lies in techniques of data mining for high-throughput data which require a high level of expertise and represent a constantly evolving methodological research field for functional genomics and biological networks studies. Among these resources, transcriptome datasets represent the most comprehensive of all of the large-scale data types and have led to the development of many tools for querying these both in a directed manner and correlatively. Hence, several co-expression gene databases and webtools dedicated to *Arabidopsis* have been developed for functional inference purposes, mainly by calculating standard Pearson correlation coefficients between gene pairs (for review, see Usadel et al. 2009). In addition, on the basis of a more sophisticated

methodological framework (Bansal et al. 2007), a compendium of public microarrays has been shown to be valuable either to explore the whole transcriptional network (Ma et al. 2007) or to focus on a smaller subpart such as cell wall biosynthesis (Persson et al. 2005), glucosinolate metabolic pathway (Hirai et al. 2007) or amino acid metabolism in response to abiotic stress (Less and Galili 2008). To go further, some bioinformatic programs and omics datasets can be smartly combined into a strategy to elucidate the function of a gene of interest, including modes of regulation and synthesis, and potential interacting partners, and last but not least processes that are regulated by its gene products. With the growing number of programs, webtools and various datasets available, examples of combinatory applications of bioinformatic tools that lead to the generation of testable hypotheses (sometimes with subsequent confirmation) have been recently proposed with a focus on a cross-talk between nitrate response and hormone signals for the control of the plant development (Nero et al. 2009). Regarding the field of plant response to Cd, the number of published integrated analysis remains very limited. Owing to the low amount of "omics" data produced under these experimental perturbations, a set of genes known to be deregulated in Cd stress condition was used to select co-expression graph through a large compendium of microarrays datasets encompassing various experimental conditions including biotic and abiotic stresses. The resulting transcriptional modules are assessed by GO term enrichment and promoter analysis to identify shared combinatorial control (Boyer et al. 2009). Our preliminary results highlight a potential dialog between the sulfur/nitrate assimilation pathways, the biosynthesis of glutathione amino-acid precursors and the glucosinolate biosynthesis pathway. Further studies are under investigation for confirmation and targeted experiments (Boyer et al., in preparation). This may lead to new findings of gene regulation under stress condition in plant.

4 Conclusion

Finally, if data generation is no longer the limiting factor in advancing biological research, their integration, analysis, and interpretation have become key bottlenecks and challenges that biologists conducting genomic research face daily. While advanced data analysis tools for exploiting genomic data are rapidly emerging, the narrow specialization of most current software tools forces biologists to employ many tools to analyze the data in a single biological study. Despite the improved integration in software tools, the sheer number and diversity of analytical and visualization tools available might be bewildering to biologists who wish to apply them correctly. In this regard, the setup of a sustained and open dialog between computer scientists, statisticians and biologists actually represents one of the key steps for successful collaboration in plant systems biology (Shasha 2003). By integrating selected genomic data, visualization and analysis tools into a single Web-accessible software platform, the Virtual Plant project is promising for

bridging the gap between biologists and bioinformaticians by providing an intuitive way to integrate and mine diverse data sources (Katari et al. 2010).

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References

Agrawal GK, Bourguignon J, Rolland N, Ephritikhine G, Ferro M, Jaquinod M, Alexiou KG, Chardot T, Chakraborty N, Jolivet P, Doonan JH, Rakwal R (2011) Plant organelle proteomics: collaborating for optimal cell function. Mass Spectrum Rev. doi:2010.1002/mas.20301

Ahsan N, Lee SH, Lee DG, Lee H, Lee SW, Bahk JD, Lee BH (2007) Physiological and protein profiles alternation of germinating rice seedlings exposed to acute cadmium toxicity. C R Biol 330:735–746

Aina R, Labra M, Fumagalli P, Vannini C, Marsoni M, Cucchi U, Bracale M, Sgorbati S, Citterio S (2007) Thiol-peptide level and proteomic changes in response to cadmium toxicity in *Oryza sativa* L. roots. Environ Exp Bot 59:381–392

Aloui A, Recorbet G, Gollotte A, Robert F, Valot B, Gianinazzi-Pearson V, Aschi-Smiti S, Dumas-Gaudot E (2009) On the mechanisms of cadmium stress alleviation in M*edicago truncatula* by arbuscular mycorrhizal symbiosis: a root proteomic study. Proteomics 9:420–433

Alvarez S, Berla BM, Sheffield J, Cahoon RE, Jez JM, Hicks LM (2009) Comprehensive analysis of the *Brassica juncea* root proteome in response to cadmium exposure by complementary proteomic approaches. Proteomics 9:2419–2431

Baginsky S, Hennig L, Zimmermann P, Gruissem W (2010) Gene expression analysis, proteomics, and network discovery. Plant Physiol 152:402–410

Bah AM, Sun HY, Chen F, Zhou J, Dai HX, Zhang GP, Wu FB (2010) Comparative proteomic analysis of *Typha angustifolia* leaf under chromium, cadmium and lead stress. J Hazard Mater 184:191–203

Bailey NJC, Oven M, Holmes E, Nicholson JK, Zenk MH (2003) Metabolomic analysis of the consequences of cadmium exposure in *Silene cucubalus* cell cultures via H-1 NMR spectroscopy and chemometrics. Phytochemistry 62:851–858

Bansal M, Belcastro V, Ambesi-Impiombato A, di Bernardo D (2007) How to infer gene networks from expression profiles. Mol Syst Biol 3: Article number 78. doi:10.1038/msb4100120

Becher M, Talke IN, Krall L, Kramer U (2004) Cross-species microarray transcript profiling reveals high constitutive expression of metal homeostasis genes in shoots of the zinc hyperaccumulator *Arabidopsis halleri*. Plant J 37:251–268

Benschop JJ, Mohammed S, O'Flaherty M, Heck AJR, Slijper M, Menke FLH (2007) Quantitative phosphoproteomics of early elicitor signaling in *Arabidopsis*. Mol Cell Proteomics 6:1198–1214

Bovet L, Eggmann T, Meylan-Bettex M, Polier J, Kammer P, Marin E, Feller U, Martinoia E (2003) Transcript levels of AtMRPs after cadmium treatment: induction of AtMRP3. Plant Cell Environ 26:371–381

Boyer F, Combes F, Bourguignon J, Vandenbrouck Y (2009) Extracting transcriptional regulatory modules from a large microarray compendium using a graph-based approach: application to the response of *Arabidopsis thaliana* under stress conditions. TIC Signal Process Proc Ser 48:23–27

Brady SM, Provart NJ (2009) Web-queryable large-scale data sets for hypothesis generation in plant biology. Plant Cell 21:1034–1051

Carter C, Pan SQ, Jan ZH, Avila EL, Girke T, Raikhel NV (2004) The vegetative vacuole proteorne of *Arabidopsis thaliana* reveals predicted and unexpected proteins. Plant Cell 16:3285–3303

Chervitz SA, Deutsch EW, Field D, Parkinson H, Quackenbush J, Rocca-Serra P, Sansone SA, Stoeckert CJ Jr, Taylor CF, Taylor R, Ball CA (2011) Data standards for Omics data: the basis of data sharing and reuse. Methods Mol Biol 719:31–69

Clemens S (2006) Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. Biochimie 88:1707–1719

Cobbett CS (2000) Phytochelatin biosynthesis and function in heavy-metal detoxification. Curr Opin Plant Biol 3:211–216

Cobbett C, Goldsbrough P (2002) Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Annu Rev Plant Biol 53:159–182

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

Courbot M, Willems G, Motte P, Arvidsson S, Roosens N, Saumitou-Laprade P, Verbruggen N (2007) A major quantitative trait locus for cadmium tolerance in *Arabidopsis halleri* colocalizes with HMA4, a gene encoding a heavy metal ATPase. Plant Physiol 144:1052–1065

Curien G, Bastlen O, Robert-Genthon M, Cornish-Bowden A, Cardenas ML, Dumas R (2009) Understanding the regulation of aspartate metabolism using a model based on measured kinetic parameters. Mol Syst Biol 5: Article number 271. doi:210.1038/msb.2009.1029

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 50:1268–1280

Davies MJ (2005) The oxidative environment and protein damage. Biochim Biophys Acta 1703:93–109

Ducruix C, Junot C, Fievet JB, Villiers F, Ezan E, Bourguignon J (2006) New insights into the regulation of phytochelatin biosynthesis in *A. thaliana* cells from metabolite profiling analyses. Biochimie 88:1733–1742

Ducruix C, Vailhen D, Werner E, Fievet JB, Bourguignon J, Tabet JC, Ezan E, Junot C (2008) Metabolomic investigation of the response of the model plant *Arabidopsis thaliana* to cadmium exposure: evaluation of data pretreatment methods for further statistical analyses. Chemo Intell Lab Syst 91:67–77

Durand TC, Sergeant K, Planchon S, Carpin S, Label P, Morabito D, Hausman JF, Renaut J (2010) Acute metal stress in *Populus tremula* x *P. alba* (717-1B4 genotype): leaf and cambial proteome changes induced by cadmium(2+). Proteomics 10:349–368

Dutilleul C, Jourdain A, Bourguignon J, Hugouvieux V (2008) The *Arabidopsis* putative selenium-binding protein family: expression study and characterization of SBP1 as a potential new player in cadmium detoxification processes. Plant Physiol 147:239–251

Ederli L, Reale L, Ferranti F, Pasqualini S (2004) Responses induced by high concentration of cadmium in *Phragmites australis* roots. Physiol Plant 121:66–74

Edwards R, Dixon DP, Walbot V (2000) Plant glutathione S-transferases: enzymes with multiple functions in sickness and in health. Trends Plant Sci 5:193–198

Endler A, Meyer S, Schelbert S, Schneider T, Weschke W, Peters SW, Keller F, Baginsky S, Martinoia E, Schmidt UG (2006) Identification of a vacuolar sucrose transporter in barley and *Arabidopsis* mesophyll cells by a tonoplast proteomic approach. Plant Physiol 141:196–207

Endler A, Reiland S, Gerrits B, Schmidt UG, Baginsky S, Martinoia E (2009) In vivo phosphorylation sites of barley tonoplast proteins identified by a phosphoproteomic approach. Proteomics 9:310–321

Fagioni M, Zolla L (2009) Does the different proteomic profile found in apical and basal leaves of Spinach reveal a strategy of this plant toward cadmium pollution response? J Proteome Res 8:2519–2529

Fagioni M, Damici GM, Timperio AM, Zolla L (2009) Proteomic analysis of multiprotein complexes in the thylakoid membrane upon cadmium treatment. J Proteome Res 8:310–326

Fiehn O, Kopka J, Dormann P, Altmann T, Trethewey RN, Willmitzer L (2000) Metabolite profiling for plant functional genomics. Nat Biotechnol 18:1157–1161

Fukushima A, Kusano M, Redestig H, Arita M, Saito K (2009) Integrated omics approaches in plant systems biology. Curr Opin Chem Biol 13:532–538

Gianazza E, Wait R, Sozzi A, Regondi S, Saco D, Labrac M, Agradi E (2007) Growth and protein profile changes in *Lepidium sativum* L. plantlets exposed to cadmium. Environ Exp Bot 59:179–187

Goodacre R, York EV, Heald JK, Scott IM (2003) Chemometric discrimination of unfractionated plant extracts analyzed by electrospray mass spectrometry. Phytochemistry 62:859–863

Grubb CD, Abel S (2006) Glucosinolate metabolism and its control. Trends Plant Sci 11:89–100

Hajduch M, Rakwal R, Agrawal GK, Yonekura M, Pretova A (2001) High resolution two dimensional electrophoresis separation of proteins from metal-stressed rice (*Oryza sativa* L.) leaves: drastic reductions/fragmentation of ribulose-1,5-bisphosphate carboxylase/oxygenase and induction of stress-related proteins. Electrophoresis 22:2824–2831

Halkier BA, Gershenzon J (2006) Biology and biochemistry of glucosinolates. Annu Rev Plant Biol 57:303–333

Hanikenne M, Talke IN, Haydon MJ, Lanz C, Nolte A, Motte P, Kroymann J, Weigel D, Kramer U (2008) Evolution of metal hyperaccumulation required cis-regulatory changes and triplication of HMA4. *Nature* **453**: 391–395 (15 May 2008) doi: 10.1038/nature06877; Received 20 December 2007; Accepted 28 February 2008; Published online 20 April 2008

Hediji H, Djebali W, Cabasson C, Maucourt M, Baldet P, Bertrand A, Zoghlami LB, Deborde C, Moing A, Brouquisse R, Chaibi W, Gallusci P (2010) Effects of long-term cadmium exposure on growth and metabolomic profile of tomato plants. Ecotox Environ Safe 73:1965–1974

Hem S, Rofidal V, Sommerer N, Rossignol M (2007) Novel subsets of the *Arabidopsis* plasmalemma phosphoproteome identify phosphorylation sites in secondary active transporters. Biochem Biophys Res Commun 363:375–380

Herbette S, Taconnat L, Hugouvieux V, Piette L, Magniette MLM, Cuine S, Auroy P, Richaud P, Forestier C, Bourguignon J, Renou JP, Vavasseur A, Leonhardt N (2006) Genome-wide transcriptome profiling of the early cadmium response of *Arabidopsis* roots and shoots. Biochimie 88:1751–1765

Hirai MY, Yano M, Goodenowe DB, Kanaya S, Kimura T, Awazuhara M, Arita M, Fujiwara T, Saito K (2004) Integration of transcriptomics and metabolomics for understanding of global responses to nutritional stresses in *Arabidopsis thaliana*. Proc Natl Acad Sci U S A 101:10205–10210

Hirai MY, Sugiyama K, Sawada Y, Tohge T, Obayashi T, Suzuki A, Araki R, Sakurai N, Suzuki H, Aoki K, Goda H, Nishizawa OI, Shibata D, Saito K (2007) Omics-based identification of *Arabidopsis* Myb transcription factors regulating aliphatic glucosinolate biosynthesis. Proc Nat Acad Sci U S A 104:6478–6483

Hradilova J, Rehulka P, Rehulkova H, Vrbova M, Griga M, Brzobohaty B (2010) Comparative analysis of proteomic changes in contrasting flax cultivars upon cadmium exposure. Electrophoresis 31:421–431

Hugouvieux V, Dutilleul C, Jourdain A, Reynaud F, Lopez V, Bourguignon J (2009) *Arabidopsis* putative selenium-binding protein1 expression is tightly linked to cellular sulfur demand and can reduce sensitivity to stresses requiring glutathione for tolerance. Plant Physiol 151:768–781

Ishihara T, Kobayashi E, Okubo Y, Suwazono Y, Kido T, Nishijyo M, Nakagawa H, Nogawa K (2001) Association between cadmium concentration in rice and mortality in the Jinzu River basin, Japan. Toxicology 163:23–28

Jaquinod M, Villiers F, Kieffer-Jaquinod S, Hugouvieux V, Bruley C, Garin J, Bourguignon J (2007a) A proteomics approach highlights a myriad of transporters in the *Arabidopsis thaliana* vacuolar membrane. Plant Signal Behav 2:413–415

Jaquinod M, Villiers F, Kieffer-Jaquinod S, Hugouvieux V, Bruley C, Garin J, Bourguignon J (2007b) A proteomics dissection of *Arabidopsis thaliana* vacuoles isolated from cell culture. Mol Cell Proteomics 6:394–412

Jiang Y, Yang B, Harris NS, Deyholos MK (2007) Comparative proteomic analysis of NaCl stress-responsive proteins in *Arabidopsis* roots. J Exp Bot 58:3591–3607

Jones AM, Thomas V, Bennett MH, Mansfield J, Grant M (2006) Modifications to the *Arabidopsis* defense proteome occur prior to significant transcriptional change in response to inoculation with *Pseudomonas syringae*. Plant Physiol 142:1603–1620

Joyard J, McCormick S (2010) Plant systems biology. Plant Physiol 152:401–401

Katari MS, Nowicki SD, Aceituno FF, Nero D, Kelfer J, Thompson LP, Cabello JM, Davidson RS, Goldberg AP, Shasha DE, Coruzzi GM, Gutierrez RA (2010) Virtual plant: a software platform to support systems biology research. Plant Physiol 152:500–515

Kersten B, Agrawal GK, Durek P, Neigenfind J, Schulze W, Walther D, Rakwal R (2009) Plant phosphoproteomics: an update. Proteomics 9:964–988

Kieffer P, Dommes J, Hoffmann L, Hausman JF, Renaut J (2008) Quantitative changes in protein expression of cadmium-exposed poplar plants. Proteomics 8:2514–2530

Kieffer P, Planchon S, Oufir M, Ziebel J, Dommes J, Hoffmann L, Hausman JF, Renaut J (2009a) Combining proteomics and metabolite analyses to unravel cadmium stress-response in poplar leaves. J Proteome Res 8:400–417

Kieffer P, Schroder P, Dommes J, Hoffmann L, Renaut J, Hausman JF (2009b) Proteomic and enzymatic response of poplar to cadmium stress. J Proteomics 72:379–396

Kim DY, Bovet L, Maeshima M, Martinoia E, Lee Y (2007) The ABC transporter AtPDR8 is a cadmium extrusion pump conferring heavy metal resistance. Plant J 50:207–218

Kobayashi Y, Hoekenga OA, Itoh H, Nakashima M, Saito S, Shaff JE, Maron LG, Pineros MA, Kochian LV, Koyama H (2007) Characterization of AtALMT1 expression in aluminum-inducible malate release and its role for rhizotoxic stress tolerance in *Arabidopsis*. Plant Physiol 145:843–852

Kovalchuk I, Titov V, Hohn B, Kovalchuka O (2005) Transcriptome profiling reveals similarities and differences in plant responses to cadmium and lead. Mutat Res-Fundam Mol Mech Mutagen 570:149–161

Lane TW, Saito MA, George GN, Pickering IJ, Prince RC, Morel FMM (2005) A cadmium enzyme from a marine diatom. Nature 435:42–42

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 24:4041–4051

Lanquar V, Kuhn L, Lelievre F, Khafif M, Espagne C, Bruley C, Barbier-Brygoo H, Garin J, Thomine S (2007) N-15-Metabolic labeling for comparative plasma membrane proteomics in *Arabidopsis* cells. Proteomics 7:750–754

Lanquar V, Ramos MS, 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 152:1986–1999

Lee K, Bae DW, Kim SH, Han HJ, Liu X, Park HC, Lim CO, Lee SY, Chung WS (2010) Comparative proteomic analysis of the short-term responses of rice roots and leaves to cadmium. J Plant Physiol 167:161–168

Less H, Galili G (2008) Principal transcriptional programs regulating plant amino acid metabolism in response to abiotic stresses. Plant Physiol 147:316–330

Li H, Wong WS, Zhu L, Guo HW, Ecker J, Li N (2009) Phosphoproteomic analysis of ethylene-regulated protein phosphorylation in etiolated seedlings of *Arabidopsis* mutant ein2 using two-dimensional separations coupled with a hybrid quadrupole time-of-flight mass spectrometer. Proteomics 9:1646–1661

Ma SS, Gong QQ, Bohnert HJ (2007) An Arabidopsis gene network based on the graphical Gaussian model. Genome Res 17:1614–1625

Mano S, Miwa T, Nishikawa S, Mimura T, Nishimura M (2009) Seeing is believing: on the use of image databases for visually exploring plant organelle dynamics. Plant Cell Physiol 50:2000–2014

Marrs KA (1996) The functions and regulation of glutathione S-transferases in plants. Annu Rev Plant Physiol Plant Mol Biol 47:127–158

May MJ, Vernoux T, Sanchez-Fernandez R, Van Montagu M, Inze D (1998) Evidence for posttranscriptional activation of gamma-glutamylcysteine synthetase during plant stress responses. Proc Natl Acad Sci U S A 95:12049–12054

Mendoza-Cozatl DG, Butko E, Springer F, Torpey JW, Komives EA, Kehr J, Schroeder JI (2008) Identification of high levels of phytochelatins, glutathione and cadmium in the phloem sap of Brassica napus: a role for thiol-peptides in the long-distance transport of cadmium and the effect of cadmium on iron translocation. Plant J 54:249–259

Moller IM, Jensen PE, Hansson A (2007) Oxidative modifications to cellular components in plants. Annu Rev Plant Biol 58:459–481

Nero D, Krouk G, Tranchina D, Coruzzi GM (2009) A system biology approach highlights a hormonal enhancer effect on regulation of genes in a nitrate responsive "biomodule". BMC Syst Biol 3:59. doi:10.1186/1752-0509-1183-1159

Niittylae T, Fuglsang AT, Palmgren MG, Frommer WB, Schulze WX (2007) Temporal analysis of sucrose-induced phosphorylation changes in plasma membrane proteins of *Arabidopsis*. Mol Cell Proteomics 6:1711–1726

Nuhse TS, Bottrill AR, Jones AME, Peck SC (2007) Quantitative phosphoproteomic analysis of plasma membrane proteins reveals regulatory mechanisms of plant innate immune responses. Plant J 51:931–940

Palmgren MG, Clemens S, Williams LE, Kraemer U, Borg S, Schjorring JK, Sanders D (2008) Zinc biofortification of cereals: problems and solutions. Trends Plant Sci 13:464–473

Persson S, Wei HR, Milne J, Page GP, Somerville CR (2005) Identification of genes required for cellulose synthesis by regression analysis of public microarray data sets. Proc Natl Acad Sci U S A 102:8633–8638

Pilon-Smits (2005) Phytoremediation. Ann Rev Plant Biol 56:15–29

Polge C, Jaquinod M, Holzer F, Bourguignon J, Walling L, Brouquisse R (2009) Evidence for the existence in *Arabidopsis thaliana* of the proteasome proteolytic pathway: activation in response to cadmium. J Biol Chem 284:35412–35424

Rauser WE (1990) Phytochelatins. Annu Rev Biochem 59:61–86

Rodriguez-Celma J, Rellan-Alvarez R, Abadia A, Abadia J, Lopez-Millan AF (2010) Changes induced by two levels of cadmium toxicity in the 2-DE protein profile of tomato roots. J Proteomics 73:1694–1706

Roth U, von Roepenack-Lahaye E, Clemens S (2006) Proteome changes in *Arabidopsis thaliana* roots upon exposure to Cd^{2+}. J Exp Bot 57:4003–4013

Ruffel S, Krouk G, Coruzzi GM (2010) A systems view of responses to nutritional cues in *Arabidopsis*: toward a paradigm shift for predictive network modeling. Plant Physiol 152:445–452

Sanità di Toppi L, Gabbrielli R (1999) Response to cadmium in higher plants. Environ Exp Bot 41:105–130

Sarry JE, Kuhn L, Ducruix C, Lafaye A, Junot C, Hugouvieux V, Jourdain A, Bastien O, Fievet JB, Vailhen D, Amekraz B, Moulin C, Ezan E, Garin J, Bourguignon J (2006) The early responses of *Arabidopsis thaliana* cells to cadmium exposure explored by protein and metabolite profiling analyses. Proteomics 6:2180–2198

Sato S, Soga T, Nishioka T, Tomita M (2004) Simultaneous determination of the main metabolites in rice leaves using capillary electrophoresis mass spectrometry and capillary electrophoresis diode array detection. Plant J 40:151–163

Schmidt UG, Endler A, Schelbert S, Brunner A, Schnell M, Neuhaus HE, Marty-Mazars D, Marty F, Baginsky S, Martinoia E (2007) Novel tonoplast transporters identified using a proteomic approach with vacuoles isolated from cauliflower buds. Plant Physiol 145:216–229

Schneider T, Schellenberg M, Meyer S, Keller F, Gehrig P, Riedel K, Lee Y, Eberl L, Martinoia E (2009) Quantitative detection of changes in the leaf-mesophyll tonoplast proteome in dependency of a cadmium exposure of barley (*Hordeum vulgare* L.) plants. Proteomics 9:2668–2677

Schulze WX (2010) Proteomics approaches to understand protein phosphorylation in pathway modulation. Curr Opin Plant Biol 13:280–287

Sels J, Mathys J, De Coninck BM, Cammue BP, De Bolle MF (2008) Plant pathogenesis-related (PR) proteins: a focus on PR peptides. Plant Physiol Biochem 46:941–950

Semane B, Dupae J, Cuypers A, Noben JP, Tuomainen M, Tervahauta A, Karenlampi S, Van Belleghem F, Smeets K, Vangronsveld J (2010) Leaf proteome responses of *Arabidopsis thaliana* exposed to mild cadmium stress. J Plant Physiol 167:247–254

Shasha DE (2003) Plant systems biology: lessons from a fruitful collaboration. Plant Physiol 132:415–416

Shimaoka T, Ohnishi M, Sazuka T, Mitsuhashi N, Hara-Nishimura I, Shimazaki KI, Maeshima M, Yokota A, Tomizawa KI, Mimura T (2004) Isolation of intact vacuoles and proteomic analysis of tonoplast from suspension-cultured cells of *Arabidopsis thaliana*. Plant Cell Physiol 45:672–683

Sobkowiak R, Deckert J (2006) Proteins induced by cadmium in soybean cells. J Plant Physiol 163:1203–1206

Song WY, Park J, Mendoza-Cozatl DG, Suter-Grotemeyer M, Shim D, Hortensteiner S, Geisler M, Weder B, Rea PA, Rentsch D, Schroeder JI, Lee Y, Martinoia E (2010) Arsenic tolerance in *Arabidopsis* is mediated by two ABCC-type phytochelatin transporters. Proc Natl Acad Sci U S A 107:21187–21192

Sun XM, Zhang JX, Zhang HJ, Ni YW, Zhang Q, Chen JP, Guan YF (2010) The responses of *Arabidopsis thaliana* to cadmium exposure explored via metabolite profiling. Chemosphere 78:840–845

Szponarski W, Sommerer N, Boyer JC, Rossignol M, Gibrat R (2004) Large-scale characterization of integral proteins from *Arabidopsis* vacuolar membrane by two-dimensional liquid chromatography. Proteomics 4:397–406

Tokimatsu T, Sakurai N, Suzuki H, Ohta H, Nishitani K, Koyama T, Umezawa T, Misawa N, Saito K, Shibata D (2005) KaPPA-view: a web-based analysis tool for integration of transcript and metabolite data on plant metabolic pathway maps. Plant Physiol 138:1289–1300

Tolstikov VV, Lommen A, Nakanishi K, Tanaka N, Fiehn O (2003) Monolithic silica-based capillary reversed-phase liquid chromatography/electrospray mass spectrometry for plant metabolomics. Anal Chem 75:6737–6740

Tommasini R, Vogt E, Fromenteau M, Hortensteiner S, Matile P, Amrhein N, Martinoia E (1998) An ABC-transporter of *Arabidopsis thaliana* has both glutathione-conjugate and chlorophyll catabolite transport activity. Plant J 13:773–780

Usadel B, Obayashi T, Mutwil M, Giorgi FM, Bassel GW, Tanimoto M, Chow A, Steinhauser D, Persson S, Provart NJ (2009) Co-expression tools for plant biology: opportunities for hypothesis generation and caveats. Plant Cell Environ 32:1633–1651

van de Mortel JE, Schat H, Moerland PD, Van Themaat EVL, Van der Ent S, Blankestijn H, Ghandilyan A, Tsiatsiani S, Aarts MGM (2008) Expression differences for genes involved in lignin, glutathione and sulphate metabolism in response to cadmium in *Arabidopsis thaliana* and the related Zn/Cd-hyperaccumulator *Thlaspi caerulescens*. Plant Cell Environ 31:301–324

Verbruggen N, Hermans C, Schat H (2009) Mechanisms to cope with arsenic or cadmium excess in plants. Curr Opin Plant Biol 12:364–372

Vido K, Spector D, Lagniel G, Lopez S, Toledano MB, Labarre J (2001) A proteome analysis of the cadmium response in *Saccharomyces cerevisiae*. J Biol Chem 276:8469–8474

Visioli G, Marmiroli M, Marmiroli N (2010) Two-dimensional liquid chromatography technique coupled with mass spectrometry analysis to compare the proteomic response to cadmium stress in plants. J Biomed Biotechnol. doi:567510.561155/562010/567510

Ward JL, Harris C, Lewis J, Beale MH (2003) Assessment of H-1 NMR spectroscopy and multivariate analysis as a technique for metabolite fingerprinting of *Arabidopsis thaliana*. Phytochemistry 62:949–957

Weber M, Harada E, Vess C, von 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 Cd^{2+}-hypertolerant facultative metallophyte *Arabidopsis halleri*. Plant Cell Environ 29:950–963

Whiteman SA, Nuhse TS, Ashford DA, Sanders D, Maathuis FJM (2008a) A proteomic and phosphoproteomic analysis of Oryza sativa plasma membrane and vacuolar membrane. *Plant J* 56:146–156

Whiteman SA, Serazetdinova L, Jones AME, Sanders D, Rathjen J, Peck SC, Maathuis FJM (2008b) Identification of novel proteins and phosphorylation sites in a tonoplast enriched membrane fraction of Arabidopsis thaliana. *Proteomics* 8:3536–3547

Wolfender JL, Ndjoko K, Hostettmann K (2003) Liquid chromatography with ultraviolet absorbance-mass spectrometric detection and with nuclear magnetic resonance spectroscopy: a powerful combination for the on-line structural investigation of plant metabolites. J Chromatogr A 1000:437–455

Yamaguchi H, Fukuoka H, Arao T, Ohyama A, Nunome T, Miyatake K, Negoro S (2010) Gene expression analysis in cadmium-stressed roots of a low cadmium-accumulating solanaceous plant, *Solanum torvum*. J Exp Bot 61:423–437

Ytterberg AJ, Jensen ON (2010) Modification-specific proteomics in plant biology. J Proteomics 73:2249–2266

Zenk MH (1996) Heavy metal detoxification in higher plants - a review. Gene 179:21–30

Zhao CR, Ikka T, Sawaki Y, Kobayashi Y, Suzuki Y, Hibino T, Sato S, Sakurai N, Shibata D, Koyama H (2009) Comparative transcriptomic characterization of aluminum, sodium chloride, cadmium and copper rhizotoxicities in *Arabidopsis thaliana*. BMC Plant Biol 9:32. doi:10.1186/1471-2229-1189-1132

Zhao CR, Sawaki Y, Sakurai N, Shibata D, Koyama H (2010) Transcriptomic profiling of major carbon and amino acid metabolism in the roots of *Arabidopsis thaliana* treated with various rhizotoxic ions. Soil Sci Plant Nutr 56:150–162