Modulation of plant proteome composition is an inevitable process to cope with the environmental challenges including heavy metal (HM) stress. Soil and water contaminated with hazardous metals not only cause permanent and irreversible health problems, but also result substantial reduction in crop yields. In course of time, plants have evolved complex mechanisms to regulate the uptake, mobilization, and intracellular concentration of metal ions to alleviate the stress damages. Since, the functional translated portion of the genome plays an essential role in plant stress response, proteomic studies provide us a finer picture of protein networks and metabolic pathways primarily involved in cellular detoxification and tolerance mechanism. In the present review, an attempt is made to present the state of the art of recent development in proteomic techniques and significant contributions made so far for better understanding the complex mechanism of plant metal stress acclimation.

INTRODUCTION

High-throughput OMICS techniques are extensively being exploited in recent times to dissect plants molecular strategies of heavy metals (HMs) stress tolerance. Plants growing in HMs contaminated environment have developed coordinated homoeostatic mechanisms to regulate the uptake, mobilization, and intracellular concentration of toxic metal ions to alleviate stress damages. As the functional translated portion of the genome play a key role in plant stress response, proteomic studies provide us a finer picture of protein networks and metabolic pathways primarily involved in cellular detoxification and tolerance mechanism against HM toxicity.

By definition, elements having specific gravity above five are considered as HMs. Nevertheless, the term HM commonly refers to toxic metals, e.g., cadmium (Cd), copper (Cu), chromium (Cr), lead (Pb), zinc (Zn) as well as hazardous metalloids viz., arsenic (As), boron (B), which exert negative effects on plant growth and development (Hossain et al., 2012a). However, changes in gene expression at transcript level allow the entry of Cd2+, Pb2+ (Welch and Norvell, 1999; Perfus-Barbeoch et al., 2002). Once HM ions enter the cell, cellular functions are affected by a wide range of actions. The negative impact of HM includes binding of HM ions to sulfhydryl groups of proteins, replacement of essential cations from specific binding sites, leading to enzyme inactivation and production of reactive oxygen species (ROS), resulting in oxidative damages to lipids, proteins and nucleic acids (Sharma and Dietz, 2009).

Over the last decade, extensive research on plants response to HM stress has been conducted to unravel the tolerance mechanism. Genomics technologies have been useful in addressing plant abiotic stress responses including HM toxicity (Bohnert et al., 2006). However, changes in gene expression at transcript level have not always been reflected at protein level (Coggi et al., 1999). An in-depth proteomic analysis is thus of great importance to identify target proteins that actively take part in HM detoxification mechanism.

Plant response to HM stress has been reviewed extensively over the past decade (Sanita Di Toppo and Gabbrielli, 1999; Cobbett, 2006; Ma et al., 2001; Cobbett and Goldbrough, 2002; Hall, 2002; Maksymiec, 2007; Sharma and Dietz, 2009; Verbruggen et al., 2009; Yang and Chu, 2011; Hossain et al., 2012a). However, review articles on application of proteomics in analyzing cellular mechanism for HM tolerance are limited (Ahsan et al., 2009; Luque-Garcia et al., 2011; Villeris et al., 2011). Current review represents the state of art of recent developments in proteomic techniques and significant contributions made so far to strengthen our knowledge about plants HM-stress tolerant strategies.
response cascade at protein level. Special emphasis is given to highlight the role of metal stress-related proteins engage in HM ions sequestration, antioxidant defense system, and primary metabolism for deeper understanding of coordinated pathways involve in detoxification of HM ions within plant cells. Furthermore, future applications of proteome study of subcellular organelles are discussed to get the new insights into the plant cell response to HMs.

**QUANTITATIVE PROTEOMIC TECHNIQUES USED FOR ANALYSIS OF HM-RESPONSIVE PROTEINS**

Conventional two-dimensional gel electrophoresis (2-DE) approach coupled with protein identification by mass spectrometry (MS) has been the most widely used proteomic technique for investigation of HM-induced alteration of plant proteome composition (Table 1). Protein extraction and purification from the HM-stressed tissue is the most crucial step in 2-DE approach, as the amount and quality of the extracted proteins ultimately determine the protein spot number, resolution, and intensity. Phenolic compounds, proteolytic and oxidative enzymes, terpenes, pigments, organic acids, inhibitory ions, and carbohydrates are some common interfering substances present in recalcitrant plant tissues. Inferior 2-D separation results due to proteolytic breakdown, streaking, and charge heterogeneity. Proteomic studies on plant response against HM stress have revealed that trichloroacetic acid/acetone precipitation (Patterson et al., 2007; Zhen et al., 2007; Kieffer et al., 2008; Alves et al., 2011; Hossain et al., 2012b,c) and phenol-based (Bona et al., 2007; Alves et al., 2009; Vannini et al., 2009; Lee et al., 2010; Ritter et al., 2010; Rodriguez-Celma et al., 2010; Ahsan et al., 2012; Sharmin et al., 2012) protocols are the effective protein extraction methods for obtaining high quality proteome map. Nevertheless, phenol-based method is the most appropriate in extracting glycoproteins, and produce high-resolution proteome map for recalcitrant plant tissues (Saravanan and Rose, 2004; Komatsu and Ahsan, 2009).

As compared to classical staining procedure of 2-DE gel using CBB or silver staining, advanced fluorescence two-dimensional difference gel electrophoresis (2-D DIGE) approach is now being used which allows comparison of the differentially expressed proteins of control and HM-stressed tissue on one single gel (Kieffer et al., 2008; Alves et al., 2009). DIGE is basically a gel-based method where proteins were labeled with fluorescent dyes (CyDyes – Cy2, Cy3, and Cy5) prior to electrophoresis. With the advancement of technology multiplexed isobaric tagging (iTRAQ) of peptides has allowed comparative, quantitative analysis of multiple samples. This second generation gel free proteomic approach has been well exploited for gaining comprehensive understanding of plants response to Cd and B (Patterson et al., 2007; Alves et al., 2009; Schneider et al., 2009).

**PLANT STRATEGIES OF HM TOLERANCE**

In course of time, higher plants have evolved sophisticated mechanisms to regulate the uptake, mobilization, and intracellular concentration of HM ions (Figure 1). Apart from the plasma membrane exclusion method, the most common way to protect the cell from the adverse effects of HMs includes synthesis of membrane transporters and thiol-containing chelating compounds for vacuolar sequestration. Furthermore, increased abundance of defense proteins for effective ROS scavenging and molecular chaperones for re-establishing normal protein conformation help HM-stressed plants to maintain redox homeostasis. Modulations of vital metabolic pathways – photosynthesis and mitochondrial respiration – further help the stressed plant to produce more reducing power to compensate high-energy demand of HM challenged cells.
| Metal | Plant (tissue) | Protein extraction buffer + precipitation | Protein solubilization/lysis buffer | Proteomic methodologies | IP | Major findings | Reference |
|-------|---------------|-----------------------------------------|-----------------------------------|------------------------|-----|----------------|----------------|
| Cd    | G. max L. cvs. Harosoy (H), Fukuyutaka (F), CDH-80 C (leaf, root) | 10% TCA, 0.07% 2-ME in acetone | 8 M urea, 2 M Thiourea, 5% CHAPS, 2 mM TBP, ampholytes (pH 3–10) | IPG, 2-DE, nani,C-MS/MS, MALDI-TOF MS | 32 (HL), 26 (FL), 44 (CL), 16 (R) | Activation of SOD, APX, and CAT ensures cellular protection from ROS mediated damages under cadmium stress; enhanced expression of molecular chaperones help in stabilizing protein structure and function; thus maintain cellular homeostasis. | Hossain et al. (2012b) |
|       | G. max L. cv. Enrei (leaf) | 10% TCA, 0.07% 2-ME in acetone | 8 M urea, 2 M Thiourea, 5% CHAPS, 2 mM TBP, ampholytes (pH 3–10) | IPG, 2-DE, nani,C-MS/MS, MALDI-TOF MS | 78 | High abundance of Hsp70 helps BABA-primed plants to maintain normal protein functions; higher abundance of Pn1 indicates BABA potential antioxidant defense system to combat Cd stress. | Hossain et al. (2012c) |
| Cd    | G. max L. cv. Enrei, Harosoy (root microsome) | 0.5 M Tris-HCl (pH 8.0), 2 mM EDTA, 2 mM DTT, 2% ME | 8.5 Murea, 2.5 M thiourea, 5% CHAPS, 1% DTT, 1% Triton X-100, 0.5% Bovine (pH 5–6) | IPG, 2-DE, nani,C-MS/MS, MALDI-TOF MS | 22 | Upregulation of proteins associated with Cd-chelating pathways and increased lignification of xylem vessels lead to lower root to shoot location of Cd in cv. Enrei. | Ahsan et al. (2012) |
| Cd    | L. esculentum Mill cv. Tres Cantos (root) | phenol-saturated Tris-HCl 0.1 M (pH 8.0), 5 mM ME | 8 M urea, 2 % (w/v) CHAPS, 50 mM DTT, 2 mM PMSE, 0.2 % (w/v) 3–10 ampholytes | IPG, 2-DE, MALDI-TOF/MS, LIFT TOF-TOF | 27 (low Cd), 33 (high Cd) | Low Cd treatment (10 μM) activates glycolysis, TCA cycle and respiration; at high Cd (100 μM) major decreases in growth, a shutdown of carbohydrate metabolism and decreases in respiration takes place. | Rodríguez-Celma et al. (2010) |
| Cd    | O. sativa L. cv. Dongjin (root, leaf) | 0.5 M Tris-HCl (pH 8.0), 50 mM EDTA, 900 mM sucrose, 100 mM KO, 2% ME, 1 mM PMSE + iTRAQ dissolution buffer | 7 M urea, 2 M Thiourea, 4% CHAPS, 0.5% PVME, 50 mM DTT, 0.5% IPG buffer | IPG, 2-DE, MALDI-TOF/MS | 18 (R), 19 (L) | Low Cd treatment (10 μM) activates glycolysis, TCA cycle and respiration; at high Cd (100 μM) major decreases in growth, a shutdown of carbohydrate metabolism and decreases in respiration takes place. | Lee et al. (2010) |
| Cd    | H. vulgare L. var. Baraka (leaf mesophyll tonoplast) | 0.5 M Tris-HCl (pH 8.0), 50 mM EDTA, 100 mM KCl, 1% ME, 1 mM PVME + ITRAQ dissolution buffer | 8 M urea, 2 M Thiourea, 4% CHAPS, 0.5% PVME, 50 mM DTT, 0.5% IPG buffer | IPG, 2-DE, MALDI-TOF/MS | 56 | Candidate proteins like CAXI and MRP like ABC transporter play significant role in vacuolar Cd²⁺ transport, hence Cd²⁺ detoxification. | Schneider et al. (2009) |

(Continued)
| Metal | Plant (tissue) | Protein extraction buffer + precipitation | Protein solubilization/lysis buffer | Proteomic methodologies | IP | Major findings | Reference |
|-------|---------------|-------------------------------------------|-----------------------------------|------------------------|-----|----------------|-----------|
| B. juncea L. (Acc: PI 173874) (root) | Tris-buffered phenol (pH 8.8) and 600 mL of 0.1 M Tris–HCl with 10 mM EDTA, 0.4% w/v 2-ME, 0.9 M sucrose | DIGE solubilization buffer (7 M urea, 2 M thiourea, 4% w/v CHAPS, 0.2% w/v SDS, 10 mM Tris, pH 8.9), and 0.5 M bicine pH 9.4 with 0.09% w/v SDS (for iTRAQ Label) | IPG, 2D DIGE, ITAQ, nanoLC-ESI-MS/MS | 102 | Up-regulation of mitochondrial respiration provides energy and reducing power to cope with metal stress, photosynthesis comparatively less affected. | Alvarez et al. (2009) |
| P. tremula L. (twig) | 20% TCA and 0.1% (w/v) DTT in ice-cold acetone | Labeling buffer | IPG, 2D DIGE, MALDI-TOF-TOF MS | 125 | | Kieffer et al. (2008) |
| Cu | E. siliculosus strains | 15% w/v PVP, 0.7 M sucrose, 0.1 M KO, 0.5 M Tris–HCl (pH 7.5), 250 mM EDTA, protease inhibitor, 2% w/v ME, 0.5% w/v CHAPS + phenol saturated Tris–HCl (pH 7.5) | 7 M urea, 2 M thiourea, 4% w/v CHAPS, 60 mM DTT, 20 mM Tris–HCl (pH 8.8), Biolytes (pH 3–10) | IPG, 2D DIGE, MALDI-TOF MS | 10 | | Ritter et al. (2010) |
| O. sativa L. | Wuyunjing (germinating embryos) | 10% TCA in ice-cold acetone | 8 M urea, 4% CHAPS, 65 mM DTT, 0.2% w/v Biolytes (pH 3–10) | IPG, 2D DIGE, MALDI-TOF MS | 16 | First proteomic evidence that metallothionein and CYP90D2 (a putative small cytochrome P450) are Cu-responsive proteins in plants. | Zhang et al. (2009) |
| C. sativa Var. Felina 34 (root) | 0.5 M Tris–HCl (pH 7.5), 0.7 M sucrose, 10 mM EDTA, 0.1 M KO, 10 mM thiourea, 2 mM PMSF/EDTA/ME + ice-cold acetone | 9 M urea, 4% w/v CHAPS, 50 mM Tris–HCl, 0.5% Triton X-100, 20 mM DTT, 2% w/v IPG Buffer | IPG, 2D DIGE, LC-MS/MS | 20 | Copper-induced alterations to reduce ascorbic acid promote PCs-mediated vacuolar transport; Suppression in change in ROS scavenging enzymes. | Bona et al. (2007) |
| O. sativa L. cv. Hwayeong | 0.5 M Tris–HCl (pH 8.3), 2% w/v NP-40, 0 mM MgCl, 2% w/v ME, 1 mM PMSF, 1% w/v PVP + aconite | 9.5 M urea, 2% w/v NP-40, and 3% w/v ME, 1 mM PMSF, 1% w/v PVP + aconite | IPG gel (tube gel), 2D DIGE, MALDI-TOF MS | 25 | Excess Cu induces oxidative stress thus hampering metabolic processes; up-regulation of antioxidant and stress-related regulatory proteins (glyoxalase I, per oxidase) help to maintain cellular homeostasis. | Ahsan et al. (2007b) |
Table 1 | Continued

| Metal | Plant (tissue) | Protein extraction/lysis buffer + precipitation | Protein solubilization/lysis buffer | Proteomic methodologies | IP | Major findings | Reference |
|-------|----------------|-----------------------------------------------|-----------------------------------|--------------------------|----|----------------|-----------|
| B     | L. albus cv. Rio Mar (root) | 0.06 M DTT, 10% (w/v) TCA in cold acetone with 0.06 M DTT | 2 M thiourea, 7 M urea, 4% (w/v) CHAPS, 0.4% (w/v) Triton X-100, 0.06 M DTT, and 1% (v/v) IPG buffer 3–10NL | IPG, 2-DE, LC-MALDI-TOF and LC-MS | 128 | Proteins associated with energy (glycolysis, TCA cycle, oxidation-reduction), cell division, protein metabolic processes suppressed under B deficiency. | Alves et al. (2011) |
|       | H. vulgare cvs. GP Sp, Sh, ShDH (Root, leaf) | 50 mM phosphate buffer (pH 7.8), 20 mM KO, 0.5 M Suc, 10 mM DTT, 0.2 mM PMSE, 10 mM EDTA, 10 mM EGTA + 10% (w/v) TCA in acetone | 0.5 M TEAB (pH 8.5) containing 0.1% SDS | iTRAQ peptide tagging, MS/MS | 139 | Higher abundance of iron deficiency sensitive (IDS2), IDS3, and methylthio-ribose kinase observed in B tolerant barley is linked to siderophore production | Patterson et al. (2007) |
| As    | Anabaena sp. PC7120 (algal cells) | 10 mM Tris-HCl (pH 8.0), 1.5 mM MgCl₂, 10 mM KCl + 10% (w/v) TCA in acetone | 7 M urea, 2 M thiourea, 4% (w/v) CHAPS, 40 mM DTT and 10% (v/v) IPG buffer 4–7 | IPG, 2-DE, MALDI-TOF and LC-MS | 45 | Upr-regulations of PKG, PFK II, FBPase, TK, ATP synthase, Px, Pxi, oxidoreductase help to maintain normal glycolysis, PPP and turnover rate of Calvin cycle, protect cells from oxidative stress, thereby helping As-stress acclimation. | Pandey et al. (2012) |
|       | O. sativa L. cv. Dongjin (leaf) | 0.5 M Tris-HCl (pH 8.3), 2% (v/v) NP40, 20 mM MgCl₂, 2% (w/v) ME, 1 mM PMSE, 0.7 M sucrose + acetone precipitation | 8 M urea, 1% CHAPS, 0.5% (v/v) IPG buffer pH 4–7 | IPG, 2-DE, MALDI-TOF MS, ESI-MS/MS | 12 | Energy and metabolism related proteins over expressed indicating higher energy demand under As stress; down-regulation of RuBisCO and chloroplast 23 kDa ribulose-1,5-bisphosphate carboxylase/oxygenase are led to decreased photosynthesis. | Ahsan et al. (2010) |
|       | A. tenuis (leaf) | Glacial acetone containing 0.07% (v/v) 2-ME, 0.34% (w/v) plant protease inhibitor, and 4% (w/v) PVPP | 4% (w/v) CHAPS, 7 M urea, 2 M thiourea, 2% (w/v) DTT, 1% (w/v) bisulfate pH 3–10, 1% (w/v) mercaptoethanol pH 6–9.5 | IPG, 2-DE, MALDI-TOF MS, ESI-MS/MS | 31 | As treatment resulted in partial disruption of the photosynthetic processes with prominent attenuation of the Rubisco. | Duquesney et al. (2008) |
| Mn    | V. unguiculata L. | 0.5 M Tris-HCl (pH 8.3), 2% (v/v) NP40, 20 mM MgCl₂, 2% (w/v) ME, 1 mM PMSE, 0.7 M sucrose + acetone precipitation | 8 M urea, 1% CHAPS, 0.5% (v/v) IPG buffer pH 4–7 | IPG, 2-DE, MALDI-TOF MS, ESI-MS/MS | 23 | Energy, primary metabolic pathways suppressed under stress; higher GS content coupled with enhanced expressions of GR, SAMS, GSTs, CS, GR mitigate As-induced oxidative stress. | Ahsan et al. (2010) |
|       | Wap, v/v Tvu PR, Tvu, 1997 (leaf) | 700 mM sucrose, 900 mM Tris, 50 mM EDTA, 100 mM KO, and 2% (v/v) ME + water saturated phenol | 8 M urea, 2% (v/v) CHAPS, 0.5% (v/v) IPG buffer pH 3–11, 50 mM DTT | IPG, 2-DE, MALDI-TOF MS, ESI-MS/MS | 8 | Lower abundance of chloroplastic proteins involved in CO₂ fixation and photosynthesis indicate channeling metabolic energy to combat the Mn stress, coordinated interplay of apoptotic and symbiotic responses essential for stress response. | Fuhr et al. (2008) |
| Metal | Plant (tissue) | Protein extraction buffer + precipitation | Protein solubilization/lysis buffer | Proteomic methodologies | IP | Major findings | Reference |
|-------|---------------|------------------------------------------|-----------------------------------|------------------------|----|----------------|-----------|
| Cr    | M. sinensis cv. Kosung (root) | 0.5 M Tris-HCl, pH 8.3, 2% (v/v) NP-40, 20 mM MgCl₂, 1 mM PMSF, 2% (v/v) ME, and 1% (v/v) PVP | 8 M Urea, 1% CHAPS, 0.5% (v/v) PG buffer pH 4–7, 20 mM DTT | PG, 2-DE, MALDI-TOF MS, MALDI-TOF/TOF MS | IPG 36 | Novel accumulation of chromium-responsive proteins (e.g., IMPase, nitrate reductase, adenine phosphoribosyl transferase, formate dehydrogenase, putative dihydrolipoamide dehydrogenase) observed; Cr toxicity is linked to heavy metal tolerance and senescence pathways. | Sharmin et al. (2012) |
| Cr    | P. subcapitata strain Hindák (algal cells) | 500 mM Tris-HCl (pH 8), 700 mM sucrose, 10 mM EDTA, 4 mM acetic acid, 0.4% ME, 0.2% Triton X-100, 10% (v/v) PMSF, 1 μM Leupeptin, 0.1 mg/mL AFA lactic + water saturated phenol | 7 M Urea, 2 M thiourea, 4% CHAPS, 50 mg/mL DTT | IPG, 2-DE, LC-ESI-MS/MS | IPG 16 | 16 Cr-stress target photosynthetic proteins (Rubisco, Rubisco activase, Light Harvesting Chlorophyll protein complex, stress-related Chl a/b binding protein) identified; Cr also induces modulation of proteins involved in amino acids metabolism. | Vannini et al. (2009) |
| Al    | G. max (L.) Merr cv. Bai 10, BenDi 2 (root) | 10% (v/v) TCA in acetone containing 0.07% (w/v) DTT, 1% PVP, and 0.07% (w/v) DTT | 7 M Urea, 2 M thiourea, 2% (v/v) CHAPS, 1% (v/v) DTT, and 2% Pharmalyte pH 3–10 | IPG, 2-DE, MALDI-TOF MS | IPG 30 | Chlorophylls, PR 10, phytochrome B, GT-binding protein, ABC transporter ATP binding protein either newly induced or up-regulated, facilitate stress defense, signal transduction, transport, protein folding, gene regulation, primary metabolisms. | Zhen et al. (2007) |
| Al    | O. sativa L. cv. Xiangnuo 1 (KN1) (root) | 40% (v/v) TCA, 5 M Urea, 2 M thiourea, 2% (v/v) CHAPS, 5% (w/v) PVP and 50 mM DTT + ice-cold acetone with 0.07% (v/v) DTT | 5 M Urea, 2 M thiourea, 4% w/v CHAPS, 2% w/v PG buffer, 40 mM DTT | PG, 2-DE, MALDI-TOF/TOF MS, MALDI-TOF/TOF MS | PG 17 | Antioxidation and detoxification lead by up regulation of Al-responsive proteins (Cu–Zn SOD, GST, SAMs 2, ultimately related to sulfur metabolism). | Yang et al. (2007) |

BABA, (-)-aminobutyric acid; CS, cysteine synthase; CHAPS, 3-[2-hydroxyethyl]trimethylammonium]propane sulfonate; Cp, Clipper; FBA II, fructose bisphosphate aldolase 2; FBPase, fructose 1,6-bisphosphatase; GIP, Golden Promise; IP, number of identified proteins; PPR, pentatricopeptide pathway; Ps, p35S::35S; PXX, phosphoglucoisomerase; SIMS, Simmondsia chinensis synthase; Sh, Sahara; Tk, transketolase; Tk, thiol-reduct.; TBE, tris/borate/EDTA; TGA, triethyiaminum bicarbonate; TP, tyrosine-specific protein phosphatase; vBMP, vanadium-dependent bromoperoxidase.
COMPLEXATION, CHELATION, AND COMPARTMENTATION OF HMs WITHIN CELL

One of the important plant strategies of detoxifying HMs within cell is to synthesize low molecular weight chelators to minimize the binding of metal ions to functionally important proteins (Verbruggen et al., 2009). The thiol-containing chelating compounds strongly interact with HM, thus reducing free HM ions from cytosol and hence limiting HM toxicity (Cobbett and Goldsborough, 2002). The phytochelatins (PCs) and metallothioneins (MTs), the two best characterized cysteine-rich HM binding protein molecules, play crucial roles in HM tolerance mechanism (Cobbett and Goldsborough, 2002).

Phytochelatins synthesized from glutathione (GSH) by the enzyme PC synthase readily form complexes with HM in the cytosol and to facilitate their transport into vacuoles (Grill et al., 1989; Figure 1). Although PCs synthesis found to be induced in presence of most of the studied HMs, modulation of proteins, amino acids involved in PC biosynthesis have been the most widely studied in response to Cd. Our recent comparative proteome analysis of high and low Cd accumulating soybeans has revealed enhanced expression of glutamine synthetase (GS) under Cd stress. The enzyme GS is involved in the synthesis of GSH through glutamate biosynthesis pathway (Sarry et al., 2006; Semane et al., 2010). The enhanced expression of GS leads to more GSH formation (Hossain et al., 2012b). Induction of GSH synthesis implies higher metal binding capacity as well as enhanced cellular defense mechanism against oxidative stress (Verbruggen et al., 2009). Since GSH is the precursor of PC, enhanced expression of GS helps the cell to synthesize and accumulate more PC under Cd stress. Since GSH is the precursor of PC, enhanced expression of GS is involved in the synthesis of GSH through glutamate biosynthesis pathway (Sarry et al., 2006; Semane et al., 2010; Ahsan et al., 2012). In contrast, sharp decline in GS abundance has been reported in Cd-stressed rice roots (Lee et al., 2010). Ahsan et al. (2012) exploited proteomic technique in combination of metabolomics for deeper understanding of PC-mediated detoxification of Cd\(^{2+}\) in soybean roots. Comparative analysis revealed that proteins (GS beta 1) and amino acids (glycine, serine, glutamic acid) associated with Cd chelating pathways are highly active in low root-to-shoot Cd translocating cultivar. In addition, proteins involved in lision biosynthesis were shown to be increased under stress. Proteomic findings suggest that translocation of Cd ions from the root to the aerial parts might be prevented by the increased xylem lignifications.

The PC biosynthetic pathway has been finely dissected in Cd-exposed Arabidopsis thaliana cells using protein and metabolite profiling (Sarry et al., 2006). At high Cd concentration global pool of GSH decreased dramatically with the increase in dipeptide γGlu-Cys, suggesting high cellular demand of GSH for sustaining PC [(γ-Glu-Cys)n-Gly] synthesis. Alvarez et al. (2009) implemented two quantitative proteomics approaches – 2-D DIGE and iTRAQ – to find out the relation between Cd\(^{2+}\) sequestration and thiol metabolism. Both techniques identified an increased abundance of proteins involved in sulfur metabolism. Sulfite reductase and O-acetylserine sulfhydrylase, involved in reduction of sulfate to cysteine, were found to be overexpressed in Cd-treated Brassica juncea roots. Authors suggested that under Cd-stress, sulfate availability for synthesis of PCs and GSH may limit Cd tolerance. Significant inductions of GSH and PCs (PC\(_3\)) in Cd-stressed rice roots further confirm the role of thiol-peptides in HM tolerance mechanism (Aina et al., 2007). Another proteomic study by Pandey et al. (2012) revealed higher abundance of cysteine synthase (CS) with higher contents of PCs and higher transcript of PC synthase in arsenic stressed Anabaena indicating their positive roles in As sequestration. Arsenic induced increases in GSH and PCs were also recorded in fronds of arsenic hyperaccumulator Pteris vittata (Bona et al., 2011). Interestingly, no such increase was evident in roots under As treatment. Proteomic results indicate that PCs could play role in As detoxification in P. vittata fronds only, but overall PC mediated detoxification is not the primary mechanism of As-tolerance in As hyperaccumulator, but to other adaptive mechanism. Upr egulation of proteins (CS and GSHs) and GSH pool involved in As detoxification has also been documented in proteomic study of As-stressed rice roots (Ahsan et al., 2008). Apart from Cd and As stress, CS and GSH also play essential role in Al adaptation for rice (Tang et al., 2007) and soybean (Zhen et al., 2007).

Unlike PCs, proteomics-based report on HM-induced alterations of MTs is very limited. Zhang et al. (2009) for the first time identified MT-like proteins from Cu-stressed germinating rice seed embryos. A number of gene expression studies have shown that MT genes are involved in Cu homeostasis and tolerance in Arabidopsis (Murphy and Taiz, 1995) and Silene vulgaris (van Hoof et al., 2001). Plant MTs not only play vital role in chelating Cu through the Cys\(\beta\)Cys groups but are also considered as a potent scavenger of ROS (Cobbett and Goldsborough, 2002; Wang et al., 2004). The final step of HM detoxification involves sequestering of either free HMs or PCs-HMs complexes into cell vacuoles (Hall, 2002). This accumulation is mediated by tonoplast-bound cation/proton exchanger, P-type ATPase and ATP-dependent ABC transporter (Salt and Rassner, 1995; Hall, 2002). Transporters are also situated in plasma membrane and facilitate transport of HMs into apoplasm. As the vacuoles or apoplasts have limited metabolic activity, accumulations of HMs in these compartments reduce the toxic effects of HMs (Schneider et al., 2009). The iTRAQ analysis of Cd-exposed barley leaf mesophyll tonoplast protein led to the identification of ~50 vacuolar transporters, that include vacuolar ATPase subunits, MRP-like ABC transporter and two novel CAX transporters (CAX1a and CAX5) and one Al-activated malate transporter protein (Schneider et al., 2009). Induction of these transporters especially cation/proton exchanger 1a and ABC transporter assure Cd\(^{2+}\) transport into vacuole (Aina et al., 2007). Further proteomic study by Lee et al. (2010) revealed induction of vacuolar proton-ATPase in rice roots and leaves indicating their positive role in Cd detoxification through vacuolization.

HM-INDUCED OXIDATIVE STRESS AND ALTERATION OF REDOX HOMEOASTASIS

Cellular ROS generation gets accelerated upon exposure to HM stress. HMs (Cu, Fe, Cr) that are directly involved in cellular redox reaction lead to ROS generation known as redox active, while redox inactive HMs (Cd, Al, As, Ni) trigger oxidative stress by depleting cells major thiol-containing antioxidants and enzymes, disrupting
electronic transport chain or by inducing lipid peroxidation (Ercal et al., 2001; Hossain et al., 2012a). The excess intracellular ROS level alters protein structure by inducing oxidation of both protein backbone and amino acid side chain residues (Villiers et al., 2011).

To counter stress, plants have evolved robust antioxidant defense mechanism comprised of both enzymatic and non-enzymatic components (Hossain et al., 2012d). Most of the proteomic research done so far on HM-related toxicity revealed positive correlation between tolerance and increased abundance of scavenger proteins. Within plant cells, SOD constitutes the first line of defense against ROS. It plays pivotal role in cellular defense against oxidative stress, as its activity directly modulates the amount of $O_2^-$ and $H_2O_2$, the two important Haber-Weiss reaction substrates. The excess $O_2^-$ generated under HM-stress usually disproportionate into $H_2O_2$ by the action of SOD, which is then metabolized by the components of the ascorbate-GSH cycle. Higher expressions of SOD isoforms (Cu/Zn-SOD, Fe-SOD) have been documented in plants exposed to excess Cd (Kieffer et al., 2008, 2009; Alvarez et al., 2009; Farinati et al., 2009; Semane et al., 2010; Hossain et al., 2012b) and Al (Yang et al., 2007). Interestingly, root proteome analysis of Cd-exposed B. juncea has shown decreased expression of MDAR in response to ascorbate (Alvarez et al., 2009). In contrary, shoot proteome analysis of Cd treatment or B deficiency (Alves et al., 2011) lead to decreased abundance of POD and FAD. The detected suppression of POD is in accordance with the decrease in POD reported in maize roots treated with Al (Wang et al., 2011).

The abundance of another antioxidant enzyme of ascorbate-GSH cycle, monodehydroascorbate reductase (MDAR) was found to be increased in response to Cd (Sarry et al., 2006; Alvarez et al., 2009). MDAR helps to scavenge monodehydroascorbate radical and by doing this it generates dehydroascorbate (DHA), the oxidized form of ascorbate. Up-regulation of MDAR assures production of DHA, the substrate of dehydroascorbate reductase (DHAR) enzyme that catalyzes reduction of DHA to AsA (reduced ascorbate). In contrary, shoot proteome analysis of Arabidopsis halleri has shown decreased expression of MDAR in response to Cd, Zn, and rhizosphere microorganisms (Farinati et al., 2009). This down-regulation is also evident in roots of Lupinus albus undergoing long-term B deficiency (Alves et al., 2011). Decreased MDAR abundance in HM-stressed plants might indicate non-enzymatic disproportionation of monodehydroascorbate into AsA, essential for maintenance of balanced redox status (Hossain et al., 2009). Yet another well documented antioxidant found to be up-regulated under HM stress is peroxiredoxin (Prx). The Prx is basically a thiol peroxidase with multiple functions. It (a) detoxifies hydroperoxides; (b) plays essential role in enzyme activation and redox sensing; (c) acts as molecular chaperone similar to HSPs; (d) induces cell signaling (Dietz, 2003; Dietz et al., 2006; Jang et al., 2004; Barranco-Medina et al., 2009). Prx was found to be induced under Cd (Sarry et al., 2006; Ahsan et al., 2007a; Hossain et al., 2012b) and As (Quejaso and Tena, 2006; Pandey et al., 2012) stress.

Plants are also equipped with some additional defense proteins, shown to be up-regulated by HM stress. This group includes thioredoxin (Trx), Trx-dependent peroxidase, NADP(H)- oxidoreductase and glyoxylase I (Gly I). Trx is known to suppress apoptosis as well as supplies reducing equivalents to antioxidants (Hishiya et al., 2008). Excess Cu treatment seems to down-regulate the abundance of Trx and Trx-POD in germinating rice embryo (Zhang et al., 2009) and Catharina sativa roots (Bona et al., 2007) respectively. However, enhanced expression of Trx was found to be helpful in mitigating oxidative stress in As-treated Arabidopsis (Pandey et al., 2012).

Methylglyoxal (MG), a cytotoxic by-product of glycolysis generally accumulated in cell in response to environmental stresses including HM (Espanet et al., 1995). MG readily interacts with nucleic acids and proteins causing alteration of function (Yadav et al., 2005). Detoxification of MG through glyoxalase pathway involves active participation of GSH and Gly I and Gly II enzymes. Up-regulation of Gly I was found to help the germinating rice seedlings in detoxifying MG under Cd (Ahsan et al., 2007a) and Cu (Ahsan et al., 2007b). Higher Gly I abundance was also reported in Cd + Zn + microorganisms treated A. halleri shoots (Farinati et al., 2009). Proteomic study also highlighted enhanced expression of NADP(H)-oxidoreductase by Cd (Sarry et al., 2006; Lee et al., 2010) and As (Pandey et al., 2012) toxicity. Interestingly, excessive Ca (Bona et al., 2007), Cu (Sharrin et al., 2012) treatments or B deficiency (Alves et al., 2011) lead to decreased abundance of POD and FAD. The detected suppression of POD is in accordance with the decrease in POD reported in maize roots treated with Al (Wang et al., 2011).

The abundance of another antioxidant enzyme of ascorbate-GSH cycle, monodehydroascorbate reductase (MDAR) was found to be increased in response to Cd (Sarry et al., 2006; Alvarez et al., 2009). MDAR helps to scavenge monodehydroascorbate radical and by doing this it generates dehydroascorbate (DHA), the oxidized form of ascorbate. Up-regulation of MDAR assures production of DHA, the substrate of dehydroascorbate reductase (DHAR) enzyme that catalyzes reduction of DHA to AsA (reduced ascorbate). In contrary, shoot proteome analysis of Arabidopsis halleri has shown decreased expression of MDAR in response to Cd, Zn, and rhizosphere microorganisms (Farinati et al., 2009). This down-regulation is also evident in roots of Lupinus albus undergoing long-term B deficiency (Alves et al., 2011). Decreased MDAR abundance in HM-stressed plants might indicate non-enzymatic disproportionation of monodehydroascorbate into AsA, essential for maintenance of balanced redox status (Hossain et al., 2009). Yet another well documented antioxidant found to be up-regulated under HM stress is peroxiredoxin (Prx). The Prx is basically a thiol peroxidase with multiple functions. It (a) detoxifies hydroperoxides; (b) plays essential role in enzyme activation and redox sensing; (c) acts as molecular chaperone similar to HSPs; (d) induces cell signaling (Dietz, 2003; Dietz et al., 2006; Jang et al., 2004; Barranco-Medina et al., 2009). Prx was found to be induced under Cd (Sarry et al., 2006; Ahsan et al., 2007a; Hossain et al., 2012b) and As (Quejaso and Tena, 2006; Pandey et al., 2012) stress.

Plants are also equipped with some additional defense proteins, shown to be up-regulated by HM stress. This group includes thioredoxin (Trx), Trx-dependent peroxidase, NADP(H)- oxidoreductase and glyoxylase I (Gly I). Trx is known to suppress apoptosis as well as supplies reducing equivalents to antioxidants (Hishiya et al., 2008). Excess Cu treatment seems to down-regulate the abundance of Trx and Trx-POD in germinating rice embryo (Zhang et al., 2009) and Catharina sativa roots (Bona et al., 2007) respectively. However, enhanced expression of Trx was found to be helpful in mitigating oxidative stress in As-treated Arabidopsis (Pandey et al., 2012).

Methylglyoxal (MG), a cytotoxic by-product of glycolysis generally accumulated in cell in response to environmental stresses including HM (Espanet et al., 1995). MG readily interacts with nucleic acids and proteins causing alteration of function (Yadav et al., 2005). Detoxification of MG through glyoxalase pathway involves active participation of GSH and Gly I and Gly II enzymes. Up-regulation of Gly I was found to help the germinating rice seedlings in detoxifying MG under Cd (Ahsan et al., 2007a) and Cu (Ahsan et al., 2007b). Higher Gly I abundance was also reported in Cd + Zn + microorganisms treated A. halleri shoots (Farinati et al., 2009). Proteomic study also highlighted enhanced expression of NADP(H)-oxidoreductase by Cd (Sarry et al., 2006; Lee et al., 2010) and As (Pandey et al., 2012). This protein is a vital component of plants second line of defense, protecting cells from HM-induced oxidative damages.

Plants tolerance against HMs is often attributed to steady state of GSH pool for its multifunctional activities in PC synthesis, MG detoxification, ROS scavenging through ascorbate-GSH cycle, GSTs mediated decomposition of toxic compounds as well as stress signaling (Figure 1). Within GSH cycle, glutathione reductase (GR) acts as a rate limiting enzyme that catalyzes reduction of oxidized glutathione (GSSG) to GSH (reduced glutathione) and with the help of DHAR it maintains high AsA/DHA ratio necessary for tight control of HM-induced ROS scavenging. The delicate balance between GSH and GSSG is critical for keeping a favorable redox status for the detoxification of $H_2O_2$. Higher abundance of GSTs has been observed in response to Cd (Alves et al., 2009; Lee et al., 2010), As (Ahsan et al., 2008; Pandey et al., 2012), Cu (Zhang et al., 2009). Findings of Ahsan et al. (2008) revealed increased activity of GST-omega in rice roots following exposure of AsV, indicating the probable role of GST-omega in inorganic arsenic biotransformation and metabolism. The authors also suggested that depletion in GSH content may be associated with high rate of PCs synthesis thus detoxification of As through compartmentalization or due to down-regulation of enzymes of GSH biosynthetic pathways such as GR and CS. The HM-induced PCs synthesis coupled with GSH depletion is in agreement with earlier studies by Sarry et al. (2006) and Di Bacco et al. (2005).

Proteomic analyses strongly indicate that accumulation of defense proteins chiefly enzymatic components of ascorbate-GSH cycle, POD, CAT, GSTs, Gly I, Prx, Trx help cells to mitigate HM-induced oxidative stress by scavenging ROS.
MOLECULAR CHAPERONES

Protein dysfunction is an inevitable consequence of a wide range of adverse environmental conditions including HM toxicity. Molecular chaperones/heat-shock proteins (HSPs) are responsible for protein stabilization, proper folding, assembly, and translocation under both optimum and adverse growth conditions (Wang et al., 2004). In our study, enhanced abundance (>2-fold) of HSP70 protein was detected in leaves of high Cd-accumulating soybean cultivar Harosoy while low Cd-accumulating cv. Fukuyu-taka exhibited decreased expression (Hossain et al., 2012b). Cd-induced up-regulation of HSP70 is also evident in response to various HMs including Cd (Kieffer et al., 2009; Hadidzova et al., 2010; Rodriguez-Celma et al., 2010), Sr (Shaerin et al., 2012), and B deficiency (Alves et al., 2011). Albus et al. (2007a) reported increased expression of DnaK-type molecular chaperone BiP and chaperone protein Hca in germinating rice seedlings exposed to acute Cd toxicity. Al-B stress is also known to induce one LMW-HSP and three DnaJ-type proteins in Al-stressed soybean (Zhen et al., 2007). To sum up, HSPs/chaperones play pivotal role in combating HM stress by re-establishing normal protein conformation and hence, cellular homeostasis.

HM-INDUCED ALTERATION OF PROTEINS INVOLVED IN PHOTOSYNTHESIS AND ENERGY METABOLISM

Down-regulation of photosynthetic machinery is a known phenomenon of Cd stress. Low abundance of proteins involved in photosynthesis electron transport chain and Calvin cycle has been reported in Cd-exposed Populard (Kieffer et al., 2008, 2009; Durand et al., 2010) and Thlaspi (Tuomainen et al., 2006). Pioneer proteomic work by Hajduch et al. (2001) of rice leaves exposed to HMs revealed drastic reduction in abundance/fragmentation of large and small subunits of RuBisCO (LSU and SSU), suggesting complete disruption of photosynthetic machinery by HM stress. This decrease in RuBisCO has also been documented in other HMs toxicity like As (Duquesnoy et al., 2009) and Cd (Kieffer et al., 2008). Proteomic analysis for other toxic HMs like As-exposed leaf proteome of Agrostis tenuissimus has shown total disruption of RuBisCO LSU and SSU along with oxygen-evolving enhancer protein 1 and oxygen evolving protein 2 in response to 134 μM As(V) treatment (Duquesnoy et al., 2009). Potassium dichromate treatment had similar effects on algal Chl a/b protein complex. However, Vaninni et al. (2009) reported higher abundance of RuBisCO activase in Parakirchnera subcapitata under chromate treatment. Interestingly, in our proteomic experiment with Cd-exposed soybean, increased abundance of RuBisCO LSU-binding protein subunits alpha and beta, RuBisCO activase, oxygen-evolving enhancer protein 1 and 2, NAD(P)H-dependent oxido-reductase, photosystem I and II-related proteins were evident (Hossain et al., 2012b). Enhanced expressions of proteins involved in photosystem I, II, and Calvin cycle might be an adaptive feature to overcome the Cd injury in soybean. This increased abundance is in accordance with the findings of Semane et al. (2010), who also reported increased expression of photosynthetic protein abundance in leaves of Arabidopsis treated with mild Cd stress. In our opinion, contribution of high photosynthetic assimilates into respiration would help plants to yield more energy needed to combat the Cd+ stress.

To maintain the normal growth and development under stressed environment, plants need to up regulate metabolic pathways such as glycolysis and tricarboxylic acid (TCA) cycle. Detailed analysis of HM toxicity-related proteome works has shown higher abundance of glycolytic enzymes phosphoglycerate mutase (PGM), glucose-6-phosphate isomerase (G6PI), triose phosphate isomerase (TPI), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), enolase (ENO), and pyruvate kinase (PK) in response to Cd (Sarry et al., 2006; Kieffer et al., 2008; Rodriguez-Celma et al., 2010; Hossain et al., 2012b), Cr (Labra et al., 2006). However, down-regulation of GAPDH was reported in As-treated rice roots (Albus et al., 2008) and roots of Lupinus albus under B deficiency (Alves et al., 2011). Similarly, Cu-treated Cannabis roots exhibited down-regulation of another glycolytic enzyme ENO, the metalloenzyme that catalyzes penultimate step of glycolysis – conversion of 2-phosphoglycerate to phosphoenolpyruvate (Bona et al., 2007).

Like glycolysis, enzymes of TCA cycle citrate synthase (CS), succinate dehydrogenase (SD), malate dehydrogenase (MDH), aconitase (ACO), aconitate hydratase (AHC) were found to be up-regulated under Cd stress (Sarry et al., 2006; Kieffer et al., 2009; Rodriguez-Celma et al., 2010; Semane et al., 2010; Hossain et al., 2012b; Figure 1). In contrast, suppressions of several AH isozymes were evident in long-term B deficiency (Alves et al., 2011). Overall, up-regulation of glycolysis and TCA cycle might help the stressed plant to produce more reducing power to compensate high-energy demand of HM challenged cell.

ACCUMULATION OF PR PROTEINS IN RESPONSE TO HM STRESS

Plant cells trigger some common defense machineries whenever they encounter a biotic or abiotic stress. Accumulation of PR proteins is one of such plant defense strategies and often associated with systemic acquired resistance (SAR) against a wide range of pathogens (Van Loon, 1997; Durrant and Dong, 2004). Using the 2-DE approach, Elvira et al. (2008) successfully identified different PR protein isoforms (viz. PR-1, β-1,3-glucanases PR-2, chitinases PR-3, osmotin-like protein PR-5, peroxidases PR-9, germin-like protein PR-16, and NPPrp27-like protein PR-17) in Capsicum chinense leaves and additionally resolved their specific accumulation pattern in both the compatible and incompatible PMMoV–C. chinense interactions. Apart from the assigned role in plant defense against pathogenic constraints, PR proteins also play key role in adaptation to stressful environments including HM toxicity (Hensel et al., 1999; Rakwal et al., 1999; Van Loon and Van Strien, 1999; Hajduch et al., 2001; Aoyama et al., 2004; Edeva, 2005). Kieffer et al. (2008) documented marked increase in abundance of PR proteins class I chitinases (PR-3 family), several β-1,3-glucanases (PR-2 family), and thaumatin-like protein (PR-5 family) in Cd-exposed poplar leaves. Endo-1,3-beta-glucanase, a class 2 PR protein, also found to be induced in rice roots under short-term Cd stress (Lee et al., 2010). Higher abundance of PR proteins under HM as documented in many proteomic studies is in accordance with previous transcriptomic analysis of mercuric chloride-treated Zea mays leaves (Diderjean et al., 1996). Like Cd stress, PR-10 and LIR18β protein (both belong to PR-10 family),
and an acidic chitinase (PR-8 family) were de novo expressed under B deficiency (Alves et al., 2011). Stress-induced increase in ROS level has been shown to induce PR protein accumulation (Iwai et al., 2006). Treatment with excess Cu increased abundance of two PR proteins (PR-10a and putative PR proteins) in germinating rice embryos (Zhang et al., 2009). Analysis of the Vigna unguic- slata leaf apoplast proteome using 2-DE and LC-MS/MS also revealed accumulation of several PR-like proteins glucanase, chitinase, and thaumatin-like proteins in response to excess Mn supply (Fecht-Christoffers et al., 2003). Transgenic tobacco overexpressing pepper gene CABPR1 encoding basic PR-1 protein showed enhanced resistance against HMs as well as pathogen stresses (Sanower et al., 2005). These transgenic lines exhibited significant decline in total POD activity, suggesting that overexpression of CABPR1 in tobacco cells altered redox balance. Although, the precise role of PR proteins in combating HM stress is not yet clearly understood, the authors suggested that the induced redox imbalance might lead to H2O2 accumulation, triggering stress tolerance cascade. Several in vitro experiments have demonstrated that PR proteins display additional functions related to growth and development by modulating signal molecules (Kasprowzykwska, 2003; Liu and Ekramoddoullah, 2006). However, further proteomic investigations need to be undertaken to resolve the underlying molecular mechanism of PR proteins mediated plants HM tolerance.

CONCLUSION AND FUTURE PROSPECTS

The present review outlines the impact of HMs stresses on plant proteome constituents. Most of the investigations done so far primarily highlighted the differential expression of proteins involved in plant defense and detoxification pathways, namely ROS scavenging, chelation, and compartmentalization. In addition, accumulation of PR proteins and modulation of plants vital metabolic pathways CO2 assimilation, mitochondrial respiration in maintaining the rate of reducing power and energy required for combating HM-induced stress has been discussed in detail. Careful analysis of published proteomic works on HM toxicity has revealed that classical 2-DE coupled with MS-based protein identification has been the most widely used proteomic technique in investigating plant HM tolerance at organ/whole plant level. These proteomic findings have enriched us for deeper understanding plants HM tolerance mechanism.

The cellular mechanism of sensing stress and transduction of stress signals into the cell organelle represent the initial reaction of plant cells toward any kind of stress including HM. Communication through intracellular compartments plays a significant role in stress signal transduction process that finally activates defense gene cascade (Hossain et al., 2012d). To dissect the underlying molecular mechanism of how a plant cell modulates its protein signature to cope with stress, in depth study on organellar proteome would be of great contribution toward development of HM-tolerant crops.

As the PCs mediated HM-ion detoxification pathway ends in sequestration of PC-HM complexes into vacuole through various transporter proteins present in tonoplast membrane, more research on vacuole proteome needs to be undertaken for identification and characterization of novel metal transporter proteins responsible for cytoplasmic efflux of transition metal cations into vacuole. Legendary work by Schneider et al. (2009) on quantitative detection of changes in barley leaf mesophyll tonoplast proteome using advanced gel free ITRAQ method has enriched our knowledge about contribution of vacuolar transporters to Cd2+ detoxification. Plasma membrane proteome should be another target of future proteomic research on HM stress, as it acts as a primary interface between the cellular cytoplasm and the extracellular environment, thus playing a vital role in stress signal perception and transduction. Furthermore, transporter proteins present in cell membrane have importance in up-taking HM-ions into the cell. As most of the organelle membrane proteins are hydrophobic in nature, MS-based gel free system would be the most promising technique for identification of such proteins.

Plants response to multiple HMs would be another interesting area of future proteomic research (Sharma and Dietz, 2009). This could shed some light on cross talk between different HM stress signal pathways.

Heavy metal-induced protein oxidation study through redox proteomic approach has been the focus of much interest. More initiatives in this topic need to be taken as PDT/oxidation modification of proteins provides fundamental information about HM toxicity mechanism and biomarker discovery (Dowling and Sheehan, 2006; Bronaci et al., 2011).

In summary, we believe that more research on sub-proteome based HM approach would provide new insights into plants HM-stress response mechanism. HM-induced novel marker proteins would further enable us to design HM-tolerant transgenic crops.

ACKNOWLEDGMENTS

The authors thankfully acknowledge support from the Department of Science and Technology, Government of India, through DST-BOYSCAST Fellowship Programme and National Agricultural and Food Research Organization, Japan.
Alvarez, S., Berla, B. M., Sheffield, J., Calvo, E. R., Jot, J. M., and Halko, L. S. (2009). Comprehensive analysis of the Brassica juncea root protein in response to cadmium exposure by complementary proteomic approaches. Proteomics 9, 2435–2455.

Alviti, A., Moro, S., Jones, P., Pinheiro, C., Passeier, J., and Rincón, C. P. (2013). The analysis of Arabidopsis root metalloenvelope revealed cytokinin-aldol features due to long-term brassinosteroid deficiency. J. Proteomics 74, 1551–1565.

Barber, D. J. W., and Thomas, J. K. (1978). Reactions of radicals with lecithin bilayers. Radiat. Res. 74, 52–65.

Barrett-Medina, S., Latorre, J. J., and Díaz, K. J. (2009). The oligomeric conformation of peroxisomal leads redox state to function. FEBS Lett. 583, 1890–1896.

Bolhuis, H. J., Gong, Q., and Ma, S. (2006). Unraveling abiotic stress tolerance mechanisms – getting genomics going. Curr. Opin. Plant Biol. 9, 180–188.

Bona, E., Marsano, F., Massa, N., Carbone, C., Gusso, P., Arpegio, D., et al. (2011). Protective analysis in a tool for investigating anemic stress in Pteris vittata roots coloured or not by heavy metal treatment. J. Proteomics 74, 1353–1360.

Bona, E., Marsano, F., Carbone, C., and Berta, G. (2007). Protective characterization of copper stress response in Glycine max roots proteomes. J. Proteomics 7, 1121–1130.

Braccioni, D., Bernardini, G., and Sanseverino, W. (2011). Linking protein oxidation to environmental pollution: proteomic analysis of Puccinia arachidica infected Arabidopsis thaliana. J. Proteomics 74, 2234–2257.

Bulld, J. M., and Loner, J. N. (2011). The impact of heavy metals on land use and the implications for man and the environment. Sci. Total Environ. 408, 203–227.

Coppens, C. S. (2008). Phytocelatins and their roles in heavy metal detoxification and homeostasis. Ann. Rev. Plant Biol. 59, 159–182.

Di Rocco, D., Koptera, S., Sebastiani, L., and Rennopp, H. (2007). Does glutathione metabolism have a role in the defence of pomegranate against arsenic stress? New Physiol. 57, 75–80.

Dudeja, L., Freudoi, P., Naeon, W., Genet, G., Mattei, J., and Barbard, G. (1996). Heavy-metal-responsive genes in maize: identification and comparison of their expression upon various forms of abiotic stress. Plant Sci. 110, 1–11.

Díaz, K. J., Jacob, S., Oelze, M. L., Luna, M., Tognetti, V., de Miranda, S. M., et al. (2006). The function of peroxiredoxins in plant organelle redox modulation. J. Exp. Bot. 57, 1697–1709.

Dowling, V. A., and Shawhan, D. (2006). Proteomic in a route to identification of toxicity targets in environmental toxicology. Proteomics 6, 5507–5604.

Durand, T. C., Sergent, K., Francillon, S., Carpin, S., Libal, P., Morabito, D., et al. (2010). Acute metal stress in Pseudomonas aeruginosa: P. aeruginosa (717-1B genotype) leaf and canabial proteomic changes induced by cadmium stress. J. Proteomics 73, 349–368.

Durrant, W. E., and Dong, X. (2004). Systemic acquired resistance. Annu. Rev. Phytopathol. 42, 185–209.

Dumas, J., Goepf, F., Nadalou, L., Bruslard, G., Piguet-Bousquet, A., and Ledigot, G. (2009). Identification of Agrostis stolonifera leaf proteins in response to Au(III) and Au(III) induced stress using a proteomics approach. Plant Cell Physiol. 48, 216–230.

Felle, A. (2001). Pathogenesis-related proteins. Progress in the last 5 years. Gev. Appl. Plant Physiol. 51, 109–124.

Felton, M. I., Galindo-Murillo, M. M., Gálvez, P., García-Luque, I., and Serra, M. (2012a). Proteomic analysis of phosphatase protein expression in cadmium-stressed Brassica juncea L. leaves: leaf and cambial proteomic altered features due to long-term cadmium exposure. J. Proteomics 75, 5757–5770.

Feldmann, C. A., Braconi, D., Bernardini, G., and Sanseverino, W. (2010). The analysis of Pteris vittata stress response in heavy metal-catalysed root proteome by arbuscular mycorrhizal symbiosis. J. Proteomics 73, 1651–1662.

Fehlmann, I., Ruhlka, F., Bhalkar, H., Vlekor, M., Grig, M., and Brabosky, B. (2010). Comparative analysis of proteomic changes in contrasting flax cultivars under cadmium exposure. Euphytica 171, 421–433.

Hossain, M. A., Piyatida, P., Teixeira da Silva, J. A., and Fujita, M. (2012a). Molecular mechanism of heavy metal detoxification and tolerance J. Exp. Bot. 53, 145–111.

Holmedal, R., Rakvå, R., Agaival, G. K., Yonekura, M., and Prostova, A. (2001). High-resolution two-dimensional electrophoresis separation of proteins from metal-stressed rice (Oryza sativa L.) leaves. Plant proteomics. Proteomics 22, 2844–2851.

Hill, I. J. L. (2002). Cellular mechanisms for heavy metal detoxification and tolerance J. Exp. Bot. 53, 1–11.

Hosny, G., Kunze, G., and Kunze, I. (1999). Expression of the tobacco gene CPR20 in Arabidopsis thaliana up-regulates development stage, wounded, alkaline acid and heavy metals. Plant Sci. 146, 165–174.

Hosny, G., El-Nakeem, G., Moustafa, M., and Ledoigt, G. (2009). Screening of heavy metal-resistant Pichia pastoris strains displaying oxidative stress tolerance. J. Proteomics 72, 325–336.

Kafkafi, P., Demoule, J., Hoffmann, L., Hausman, J. E. Y., and Rentz, J. (2008). Quantitative changes in protein expression of cadmium-exposed poplar plants. Proteomics 8, 2514–2520.

Kafkafi, P., Phlhum, O., Ould, M., Zaidel, J., Demoule, J., Hoffmann, L., et al. (2009). Comparative proteomic and metabolic analysis to unravel cadmium stress response in poplar leaves. J. Proteomics 70, 400–417.

Komatsu, S., and Alonso, A. (2008). Soybean proteomics and its application to functional analysis. J. Proteomics 72, 699–712.

Kaszprzewski, A. (2003). Plant cell cultures – regulation and function. Curr. Opin. Plant Biol. 6, 809–814.

LaPointe, M. A., Gansz, E., Wieth, R., Lerner, L., Sonni, A., and Begoni, S., et al. (2008). Zos f. protein changes in response to potassium deficient treatments. Chromosome Res. 16, 1234–1244.

Lee, K., Lee, W., Kim, S. H., Han, H. J., Liu, S., Park, K. C., et al. (2010). Comparative proteomic analysis of high and low cadmium accumulating soybeans under cadmium stress. Amino Acids 41, 2593–2616.

Hosny, L., Lopez-Clement, M. F., Arbore, V., Perez-Clemente, B. M., and Gomez-Cadenas, A. (2009). Modulation of the antioxidants system in citrus under wounding and subsequent drainage. J. Plant Physiol. 166, 1391–1394.

Hossain, Z., Makino, T., and Komatsu, S. (2012a). Proteomic study of f-aminoacrylic acid mediated-cadmium stress alleviation in soybean. J. Proteomics 75, 4751–4764.

Hossain, Z., Nouri, M. Z., and Komatsu, S. (2012b). Plant cell organelle proteomics in response to abiotic stress: contrasting flax cultivars under cadmium exposure. Euphytica 179, 421–433.

Jung, H. H., Lee, K. O., Che, Y. H., Jung, B. G., Park, S. K., Park, J. H., et al. (2004). Two enzymes in one; two year proteomic displays oxidative stress-dependent switching from a peroxidase to a molecular chaperone function. FEBS Lett. 542, 455–455.

Iwa, N. S., Agrawal, G. K., Tamagami, S., Yonekura, M., Han, O., Jeschke, H., et al. (2006). Role of defense-related marker genes, proteins and secondary metabolites in defining rice self-defense mechanisms. Plant Physiol. 144, 261–270.

Kafkafi, P., Demoule, J., Hoffmann, L., Hausman, J. E. Y., and Rentz, J. (2008). Quantitative changes in protein expression of cadmium-exposed poplar plants. Proteomics 8, 2514–2520.

Kafkafi, P., Phlhum, O., Ould, M., Zaidel, J., Demoule, J., Hoffmann, L., et al. (2009). Comparative proteomic and metabolic analysis to unravel cadmium stress response in poplar leaves. J. Proteomics 70, 400–417.

Komatsu, S., and Alonso, A. (2008). Soybean proteomics and its application to functional analysis. J. Proteomics 72, 699–712.

Kaszprzewski, A. (2003). Plant cell cultures – regulation and function. Curr. Opin. Plant Biol. 6, 809–814.
risky root and leaves to cadmium. J. Plant Physiol. 167, 161–168.
5 Liu, J. J., and Ekramoddoullah, A. (2006). The family 10 of plant proteomics-related proteins: their structure, regulation, and function in response to biotic and abiotic stresses. Physiol. Mol. Plant Pathol. 68, 1–15.
6 Losque-Garcín, J. L., Cabezas-Sanchez, P., and Camara, C. (2015). Proteomics as a tool for examining the toxicity of heavy metals. Trends Anal. Chem. 37, 703–716.
7 Mu, J. F., Ryan, P. R., and Delhaize, E. (2001). Aluminum tolerance in plants and the compelling role of organic acids. Plant Sci. 167, 273–278.
8 Makymiec, W. (2007). Signaling responses in plants to heavy metal stress. Acta Physiol. Plant. 29, 177–187.
9 Murphy, A., and Tait, L. (1995). Comparison of metalloenzyme gene expression and protein levels in two Arabidopsis ecotypes. Correlation with copper tolerant. Plant Physiol. 109, 945–954.
10 Pansky, S., Rao, R., and Rai, L. C. (2012). Proteomics combines morphological, physiological and biochemical attributes to unveil the survival strategy of Anabaena sp. PC72120 under arsenic stress. J. Proteomics 75, 921–937.
11 Patterson, J., Ford, K., Causon, A., Nickson, S., and Bucci, A. (2007). Increased abundance of proteins involved in photosynthetic processes in barley-tolerant barley. Plant Physiol. 144, 1612–1621.
12 Perkins, N. D., Pappin, D. J., Creasy, D. M., and Cottrell, J. S. (2000). The use of probabilistic scoring to enhance the throughput and accuracy of protein identification. J. Proteome Res. 9, 1442–1452.
13 Sarowar, S., Kim, Y. J., Kim, E. N., Kim, K., Dheung, B. K., Islam, B. R., et al. (2005). Osmoprotection of a poplar basic and hydrophilic proteins 1 gene enhances plants tolerance to heavy metal and pathogen stress. Mol. Cell. Proteomics 4, 218–228.
14 Tann, D., E, T., Bohn, L., Dusein, C., Lafaye, A., Junot, C., Hugouvieux, V., et al. (2010). Leaf proteome analysis of differentially expressed proteins induced by Al toxicity in soybean. Plant Physiol. 151, 542–554.
15 Wilson, J. W., Li, W., Viers, J. R., Geng, J., Zhang, S., et al. (2007). Comparative proteome analysis of two strains of the model brown alga Pseudokirchneriella subcapitata. J. Proteomics 6, 2180–2198.
16 Semane, B., Dupae, J., Cuypers, A., Lafaye, A., Junot, C., Hugouvieux, V., et al. (2010). Leaf proteome analysis of recalcitrant plant species. Proteomics 10, 113–126.