RESEARCH ARTICLE

Quantitative proteomic analysis of tomato genotypes with differential cadmium tolerance

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Abstract
This is a report on comprehensive characterization of cadmium (Cd)-exposed root proteomes in tomato using label-free quantitative proteomic approach. Two genotypes differing in Cd tolerance, Pusa Ruby (Cd-tolerant) and Calabash Rouge (Cd-sensitive), were exposed during 4 days to assess the Cd-induced effects on root proteome. The overall changes in both genotypes in terms of differentially accumulated proteins (DAPs) were mainly associated to cell wall, redox, and stress responses. The proteome of the sensitive genotype was more responsive to Cd excess, once it presented higher number of DAPs. Contrasting protein accumulation in cellular component was observed: Cd-sensitive enhanced intracellular components, while the Cd-tolerant increased proteins of extracellular and envelope regions. Protective and regulatory mechanisms were different between genotypes, once the tolerant showed alterations of various protein groups that lead to a more efficient system to cope with Cd challenge. These findings could shed some light on the molecular basis underlying the Cd stress response in tomato, providing fundamental insights for the development of Cd-safe cultivars.

Keywords Root proteome · Cd stress · Antioxidant · Redox · Cell wall · Metabolism

Introduction

Heavy metals can be a serious environmental problem with significant negative effects on plants (Branco-Neves et al. 2017). Cadmium (Cd) pollution is a worldwide concern that can have a major impact on plant development and yield, affecting food production and quality, apart from the risks to animal and human health caused by the consumption of Cd-contaminated products (Gratão et al. 2005; Singh et al. 2016; Carvalho et al. 2018a). Tomato genotypes contrasting for Cd tolerance showed high amounts of metal in the seeds (Carvalho et al. 2018a) and due to its toxic effects, it is very important to reduce Cd accumulation on crops aiming food safety.

Due to the increase of agricultural areas contaminated by heavy metals, the problem of Cd toxicity worsens every year, but the number of plant species that have some tolerance degree is restricted. The Cd tolerance and detoxification processes in plants are apparently organ-specific, with pathways involving the cell wall, the plasma membrane, the cytosol, and vacuolar compartments (Gratão et al. 2005; Gill and Tuteja 2010; Carvalho et al. 2018b; Soares et al. 2019). Most of Cd-tolerant plants are excluders, limiting metal accumulation and root-to-shoot transport (Gallego et al. 2012). Other useful mechanism is related to Cd sequestration in metabolically inactive parts such as root cell walls which can accumulate Cd effectively, alleviating the toxic effects of this heavy metal (Parrotta et al. 2015); however, cell wall-based protection mechanism is only one of the processes used by plants to withstand the Cd-induced stress and eventual damages (Loix et al. 2017). Investigations are still needed to identify the

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mechanisms that allows plants to extract, transport, and sequestrate heavy metals for phytoremediation purposes (Farinati et al. 2011). Most of the information available about Cd tolerance comes from studies with model plants such as *Arabidopsis halleri* (Zhao et al. 2006), *Thlaspi caerulescens* (Krämer et al. 2000), and *Solanum nigrum* (Wei et al. 2005) whereas there is a gap of information especially for important commercial crops such as tomato.

Recent studies are being targeted in order to select Cd-tolerant genotypes aiming to understand better the genetic, physiological, and biochemical characteristics of these plants (Carvalho et al. 2018c, 2019). Crop breeding programs have focused on maximizing yield instead of stress tolerance because of many reasons, amongst them the incomplete understanding of the mechanisms of stress tolerance (Gilliham et al. 2017), which makes this topic very attractive for research. Accordingly, the identification of proteins involved in responses to heavy metal stress is a fundamental step for the description of the molecular mechanisms of stress responses (Gallego et al. 2012). Genomics technologies have been useful in addressing plant abiotic stress responses, including Cd stress; however, changes in gene expression at transcript level have not always been reflected at protein level (Gygi et al. 1999). Therefore, an in-depth proteomic analysis is thus of great importance to identify targeted proteins that actively take part in Cd detoxification. Researchers use two main complementary approaches in proteomics: a gel-based, also known as protein-based approach, and a gel-free or peptide-based approach. However, gel-based approaches are limited in throughput with low peptide recovery from gels, lower quantitative reproducibility, and limitations in resolving certain classes of proteins. Most of the gel-free approaches are based on a bottom-up approach, where intact proteins are in solution digested into peptides prior to liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) (Zhang et al. 2013). This approach offers high throughput to the proteomic analyses with better coverage of the whole proteome of an organelle or cell.

Plants exposed to Cd presented variable responses at the proteome level. Although there are a large number of reports about plant responses to Cd toxicity, many aspects are still unknown and, in general, little is known about stress-elicited changes in plants at the proteome level. Moreover, these studies describes symptoms and responses of plants to Cd, while the knowledge about the overall molecular mechanisms remains unexplored (Gill and Tuteja 2010). Roots are the site of direct impact under most natural conditions of toxic metal exposure; thus, Cd tolerance mechanisms are likely to be synchronized in this tissue (Loix et al. 2017).

Previously, the tomato genotypes Pusa Ruby and Calabash Rouge were characterized as Cd-tolerant and Cd-sensitive, respectively, using the tolerance index based on the total dry weight of plants (Piotto et al. 2018). In addition, tolerant plants showed higher stress markers accumulation and enhanced enzymatic activities at 4 days of Cd exposure (Borges et al. 2018). Therefore, this time was chosen to assess here the Cd-induced effects in the root proteome of young tomato genotypes.

Material and methods

Plant growth, treatments, and sample collection

Two tomato genotypes with differential tolerance to Cd-induced stress were selected based on previous studies (Borges et al. 2018; Piotto et al. 2018): *Solanum lycopersicum* cv. Pusa Ruby (Cd-tolerant) and cv. Calabash Rouge (Cd-sensitive). The plants were grown as described by Borges et al. (2018). Briefly, 20-day-old seedlings were removed from the trays and transferred to a hydroponic system (10-L trays) containing Hoagland solution at 10% ionic strength (Hoagland and Arnon 1950), with pH = 6.5 checked daily and the total volume maintained at a constant level by using distilled-deionized water. For stress induction, a 35-μM CdCl$_2$ solution was used as recommended by Piotto et al. (2018) and plants were sampled 4 days after Cd addition. Five biological replicates composed of three plants each were sampled from treatment and control, separated into roots and shoots, dried at 55 °C until a constant weight was reached, and used for growth measurements. Five biological replicates of fresh roots were sampled from treatment and control and immediately frozen in liquid nitrogen and subsequently stored at – 80 °C for further analysis.

Quantification of stress indicators

The measurements of the malondialdehyde (MDA) and hydrogen peroxide (H$_2$O$_2$) were performed as described by Heath and Packer (1968) and Alexieva et al. (2001), respectively.

Protein extraction, quantification, and digestion

Root tissues were ground in liquid nitrogen to a fine powder in a precooled mortar with a pestle. Proteins were extracted as described by Hurkman and Tanaka (1986). Briefly, the powder (4 g) was homogenized in 10 mL of extraction solution (0.1 M Tris-HCl, 0.9 M sucrose, 10 mM EDTA, and 2% (*v/v*) β-mercaptoethanol and 2 mM PMSF) during 30 min of shaking on ice. Ten milliliters of Tris-buffered phenol (pH 8.0) was added and the mixture vortexed. Samples were incubated in a shaker for 30 min at 4 °C. After this period, the samples were centrifuged at 8000×*g* for 30 min at 4 °C and the supernatant was transferred to a fresh tube and protein precipitation was carried out by adding five volumes of 0.1 M ammonium

acetate prepared in methanol. Samples were placed at − 20 °C overnight for protein precipitation. Precipitates were collected after centrifugation at 8000×g for 30 min at 4 °C and pellets washed twice with iced 80% acetone. Protein precipitates were resuspended in 300 μL of resuspension buffer (7 M urea, 0.4% (v/v) Triton X-100, and 50 mM DTT), and protein concentration was estimated using Bradford Protein Assay Kit (Bio-Rad) and BSA as standard (Bradford 1976). Aliquots of 200 μg of the protein precipitates of each biological replicate were used for filter-aided sample preparation (FASP), as described by Loziuk et al. (2015). All solutions were prepared in 50 mM Tris-HCl, at pH 7. Protein extracts were 2-fold diluted in buffer containing 100 mM DTT and incubated for 30 min at 56 °C. After reduction, samples were alkylated for 1 h at 37 °C, adding iodoacetamide to a final concentration of 200 mM. Denatured samples were transferred into a 0.5-mL 10-kDa molecular weight cutoff centrifugation filter (EMD Millipore Billerica, MA). Samples were washed 3 times for 15 min at 14,000×g at 20 °C with buffer containing 8 M urea to remove detergents. Three extra washes for 15 min at 14,000×g at 20 °C this time using trypsin digestion buffer containing 0.5 M urea and 10 mM CaCl$_2$ were performed. Protein digestion was conducted in the filter for 12 h at 37 °C using a 1:50 enzyme:protein ratio of modified porcine trypsin (Sigma-Aldrich, St. Louis, MO). New collecting tube was used at this point. Digested peptides were eluted through centrifugation at 14,000×g at 20 °C for 15 min using 400 μL of buffer containing 1% formic acid (v/v) and 0.001 % Zwittergent 3-16 (Calbiochem, La Jolla, CA). Eluted peptides were frozen and stored at − 80 °C.

LC-MS/MS analysis

Frozen peptide samples were vacuum dried and reconstituted in 50 μL of formic acid containing 0.001% Swittergent 3-16. Peptide samples were analyzed in duplicate using a Thermo Scientific EASY nLC 1000 (Thermo Scientific, Bremen, Germany) coupled with a Q Exactive HF hybrid quadrupole-Orbitrap benchtop mass spectrometer (Thermo Scientific, Bremen, Germany). A 4-μL injection onto a PicoFrit analytical column (75 μm × 20 cm; New Objective, Woburn, MA) packed with Kinetex C18 2.6-μm particle size and 120-Å pore size (Phenomenex, Torrance, CA). A 240-min elution gradient utilizing a 5–30% B solution was performed at a flow rate of 350 nL/min. Mobile phases A and B were composed of water/acetonitrile/formic acid (98/2/0.2% and 2/98/0.2%, respectively).

For MS analysis, the peptides were analyzed in positive mode. MS spectra were acquired using data-dependent, top-12 MS/MS method using a resolving power of 70,000 for MS scans and 17,500 for MS/MS at m/z = 200. Automatic gain control (AGC) target for MS scans was set to 1E06 with a maximum ion injection time (IT) of 30 ms, and AGC target of 2E04 was used for MS/MS scans with a maximum injection time of 120 ms. The underfill ratio was set to 1%. The m/z range was set from 400 to 1600. Microscans were set to 1 for both MS and MS/MS scans. Charge state screening was enabled. Unassigned and + 1 charge states were rejected from MS/MS isolation and activation. A normalized collision energy of 27 was used with an isolation window of 2.0 m/z. Dynamic exclusion was set to 60 s. The ambient ion used for lock mass was 445.120025 m/z. A capillary voltage of 2.0 kV was used, and the temperature was set to 250 °C.

Protein identification

Database searches were performed using SEQUEST HT on Proteome Discoverer 2.1 (Thermo Scientific) platform against the *Solanum lycopersicum* database available at Phytozome website iTAG v2.3 (34,727 sequences). Search parameters were set as follows: oxidation of methionine was allowed as a variable modification and carbamidomethylation of cysteine as a static modification; enzyme, trypsin; number of allowed missed cleavages, 2; mass range, 200–2000; peptide tolerance 5 ppm; fragment ions tolerance, 0.02 Da. Percolator peptide validator was used at FDR < 1% based on peptide q value. Proteins were considered as "present" if they were detected in at least four biological replicates and if were assigned by at least two different peptides. Molecular weight (MW) and isoelectric point (pI) of proteins were obtained using Compute pI/Mw web server (http://web.expasy.org/compute_pi/).

Protein quantification and statistical analysis

Peptide-spectrum matches (PSMs) were divided by protein length defining a spectral abundance factor (SAF). SAF values were then normalized against protein levels across different runs, according to Florens et al. (2006), resulting in the NSAFs values (normalized values). The NSAF values were multiplied by 10^6 in order to deal with integers and facilitate comparisons which were performed using Perseus software v.1.4.0.17 (Tyanova et al. 2016). Briefly, NSAFs values were log2 transformed in order to estimate the extent of differential protein abundance and missed values were replaced with a constant value of zero. Student's t test was performed on Perseus for two-sample comparison (p value < 0.05, based on a 2-tailed Student's t test with the Benjamini-Hochberg correction for multiple testing at an FDR < 0.05). Proteins present in at least three biological replicates and not detected in the other genotype or condition (control and Cd treatment) were termed as "exclusives."

Protein annotation and functional classification

The protein sequences identified in the experiment were blasted against UNIPROT (ftp://ftp.uniprot.org/pub/databases/

uniprot/current_release/knowledgebase/taxonomic_divisions/) and TAIR (https://www.arabidopsis.org/download/index-auto.jsp?dir=%2Fdownload_files%2FProteins%2FTAIR10_protein_lists) databases. The functional annotation analysis of differentially accumulated proteins (DAPs) was performed based on Gene Ontology (GO) term retrieval using David Annotation database tool (Huang et al. 2007) and MapMan software version 3.5.1 (available at https://mapman.gabipd.org/web/guest/mapman-version-3.5.1). The combined graphs for biological process, molecular function, and cellular component are presented at the third level of depth. The hierarchical clustering was carried out using Perseus software loading the fold change data (control/treatment) for each genotype considering Euclidian distances to build the similarity matrix (cluster parameters: row header width = 300, column header height = 200).

Results

Cadmium impact on plant growth

A clear growth reduction was observed for both genotypes regardless tissue after Cd exposure (Fig. 1). Decreases by 40% and 50% in biomass accumulation were detected in roots of the tolerant and sensitive genotypes, respectively (Fig. 1a). When shoot growth is concerned, more pronounced inhibitory effect was detected, 70% and 60% for the sensitive and tolerant genotypes, respectively (Fig. 1b).

Cadmium increased MDA and H_2O_2 contents in tolerant roots

Higher levels of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) were observed in both genotypes when treated with Cd (Fig. 2); however, statistically significant changes were only detected in the tolerant genotype (Fig. 2a, b).

Large-scale tomato root proteome identification and quantification

A large-scale quantitative proteomic approach was employed to determine alterations in the protein profile of roots of two contrasting tomato genotypes for Cd tolerance. From the mass spectra collected, a total of 4,051 non-redundant proteins were detected in the whole experiment under a FDR lower than 1% (Fig. 3a). From the total number of proteins identified, 86% (3,477 proteins) were detected in both genotypes, being 8.6% (349 proteins) exclusively found in tolerant and 5.5% (224 proteins) exclusively detected in the sensitive genotype (Fig. 3a). The tolerant genotype exhibited a slightly higher number of identified proteins (3,826 proteins) compared with the sensitive (3,701 proteins). When proteins of tolerant genotype were analyzed (control versus treatment), 3151 common proteins were detected in both groups of plants, with 362 and 313 proteins exclusively detected in control and Cd-treated plants, respectively (Fig. 3b). The same comparison also revealed that in the sensitive genotype, 2,910 proteins were detected in both groups of plants being 408 and 383 proteins exclusively detected in control and Cd-treated plants, respectively (Fig. 3a).

Regarding the differentially accumulated proteins (DAPs) detected between control and Cd-treated plants within each genotype, the sensitive genotype showed a more pronounced response in terms of DAPs numbers. A total of 84 DAPs were detected in the tolerant genotype under Cd exposure compared with control, 55 and 29 of them showing increased and decreased abundance, respectively (Fig. 3c). In a similar comparison, the sensitive genotype revealed 358 DAPs; 165 proteins increased in abundance and 193 decreased (Fig. 3c).

The protein sequences identified by LC-MS/MS in roots of tomato genotypes were contrasted with the UNIPROT tomato database as a reference for molecular weight and isoelectric point (pI) comparisons. Both the molecular weight and pI distribution of the identified proteins were very similar to the reference, with the majority ranging between 10 and 90 kDa and with pI from 4 to 11 (Fig. 4).
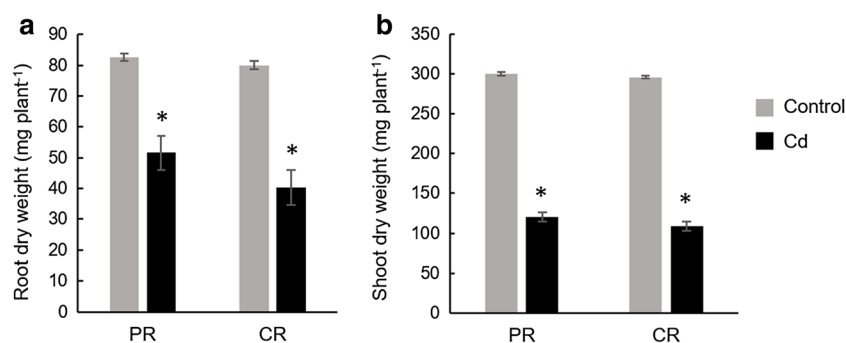

Fig. 1 Impact of Cd-induced stress on plant growth of tomato genotypes expressed as dry matter weight in root (**a**) and shoot (**b**). Values represent the means of five replicates ($n = 5$); bars are standard deviation of the mean. Asterisk means significant alteration ($p < 0.05$) in relation to respective control. Tolerant genotype Pusa Ruby (PR) and sensitive genotype Calabash Rouge (CR)
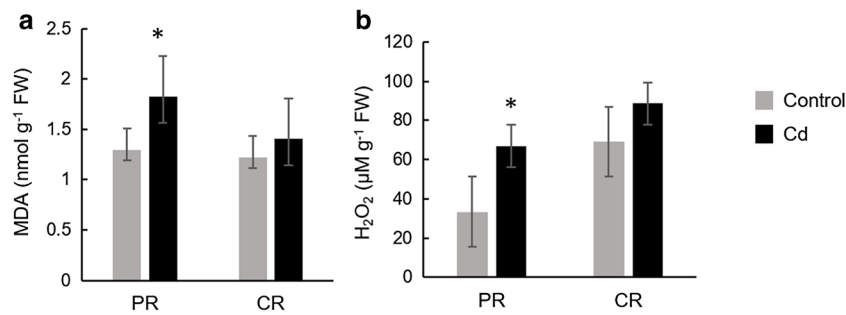
Fig. 2 Oxidative damage induced by Cd exposure in tomato roots genotypes represented as **a** malondialdehyde (MDA, nmol g^{-1} fresh weight) and **b** hydrogen peroxide (H$_2$O$_2$, μM g^{-1} fresh weight). Values represent the means of five replicates ($n = 5$); bars are standard deviation of the mean. Asterisk means significant alteration ($p < 0.05$) in relation to respective control. Tolerant genotype Pusa Ruby (PR) and sensitive genotype Calabash Rouge (CR)

Functional classification and clustering analyses of DAPs

To gain insight into the functional categories altered under Cd-induced stress, DAPs were linked to at least one GO term associated with biological process, molecular function, and cellular component. An overall similar behavior in the GO distribution was observed for DAPs in both genotypes (Fig. 5). Within the biological process distribution, the cell wall organization or biogenesis, single-organism, and metabolic cellular process categories were the most representative classes in both genotypes (Fig. 5). Regarding the cellular

Fig. 3 Protein groups identified by LC-MS/MS in roots of tomato genotypes under control and Cd-induced stress. **a** Proteins detected in each genotype. Overlapping numbers represent proteins detected in both genotypes. **b** Number of proteins detected in each condition (control and stressed) within each genotype. Overlapped numbers represent proteins identified in both conditions. **c** Differentially accumulated proteins (DAPs) detected in tomato roots under Cd-induced stress in each genotype. Numbers of proteins that increased and decreased abundance after Cd exposure are represented inside dark gray and light gray arrows, respectively. Tolerant genotype Pusa Ruby under control and stressed conditions PR and PRcd, respectively. Sensitive genotype calabash rouge under control and stressed conditions CR and CRcd, respectively

component distribution, cell part category was overrepresented in both genotypes (Fig. 5). Oxidoreductase activity and ion binding were overrepresented within the molecular function category in both genotypes (Fig. 5). Although an overall similar functional distribution between genotypes was observed, some differences can be highlighted between these plants. In the biological processes categorization, the response to biotic stimulus and response to stress categories were only represented in the sensitive genotype (Fig. 5). Among the cellular component distribution, the intracellular category was largely represented only in the sensitive genotype, while the extracellular region and envelope categories were clearly more represented in the tolerant genotype (Fig. 5). For the molecular function distribution, carbohydrate derivative binding and cofactor binding categories were only represented in the sensitive genotype while peroxidase activity was noteworthy in tolerant plants (Fig. 5).
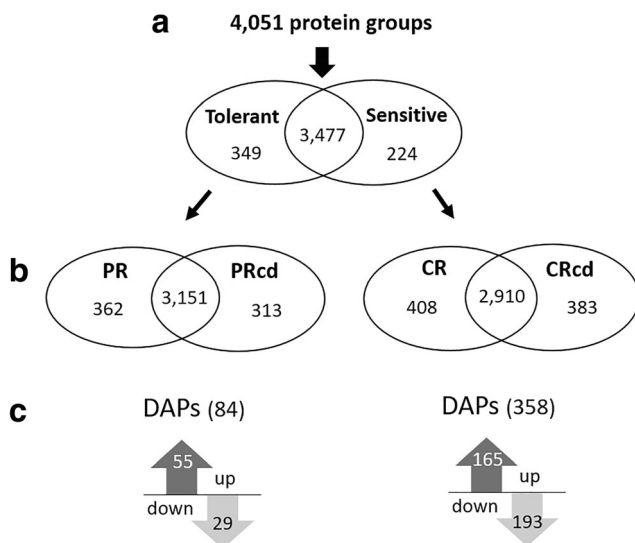
When analyzing major functional clusters of DAPs, a clear enrichment related to peroxidase, glutathione-S-transferase (GST) was found in both genotypes (Fig. 6). Under Cd stress conditions, the sensitive genotype exhibited exclusive clusters including proteins involved in cytoskeleton, S-adenosylmethionine (SAM) biosynthesis, oxidoreductase activities, nitrite/sulphite reductases, chaperone, and glycolysis (Fig. 6). The overall changes in both genotypes in terms of DAPs were associated to cell wall, redox and stress response, and carbohydrate and nitrogen metabolism (Figs. 7 and 8) in which the results will be discussed in the following sections.

Discussion

The tomato genotypes showed decreases in growth (Fig. 1) suggesting an effective stress induced by Cd. The higher production of MDA and H$_2$O$_2$ levels in the tolerant genotype (Fig. 2) suggests a ROS scavenging systems such as the antioxidant enzymes which are activated in cells contaminated with Cd, protecting them against Cd-induced oxidative stress (Gill and Tuteja 2010). According to Sharma and Dietz (2009), heavy metal–tolerant

Fig. 4 Distribution of molecular weight (**a**) and isoelectric point (pI) (**b**) of proteins identified in tomato roots. Dark gray bars represent tomato experimental proteome (4,051 proteins identified) and light gray bars represent tomato UNIPROT tomato database (33,952 proteins)
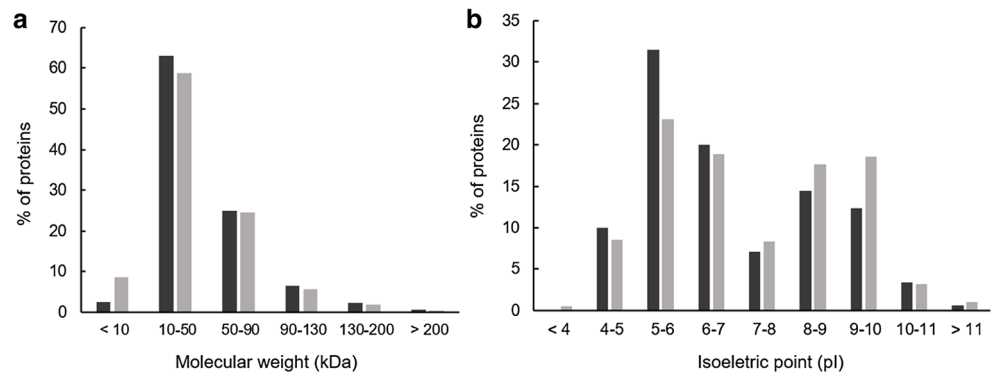
genotypes normally produce higher levels of ROS compared with the sensitive ones. Most of the proteomic research done so far on heavy metal toxicity revealed a positive correlation between tolerance and increased abundance of scavenger proteins induced by ROS (Hossain and Komatsu 2013), indicating that such condition may be due to the plant being more readily capable of dealing with the stress. In a previous report, Cd-tolerant genotype exhibited increased activity of enzymes such as ascorbate peroxidase (APX), glutathione reductase (GR), and glutathione-S-transferase (GST), diminishing the harmful effects of Cd in tomato roots (Borges et al. 2018). These results will be explored at the topic about stress responses. Furthermore, once cellular ROS production is stimulated in response to metabolic imbalances imposed by abiotic stresses (Gill and Tuteja 2010), it is possible to suggest that tolerant genotypes have a better system of signaling for defense responses when compared with the sensitive ones.

Fig. 5 Functional classification of differentially accumulated proteins (DAPs) of tomato roots using DAVID Bioinformatics tool. DAVID analysis was based on Gene Ontology (GO) term distribution: biological process, cell component, and molecular function. Tolerant genotype Pusa Ruby (PR, light gray bars) and sensitive genotype Calabash Rouge (CR, dark gray bars)
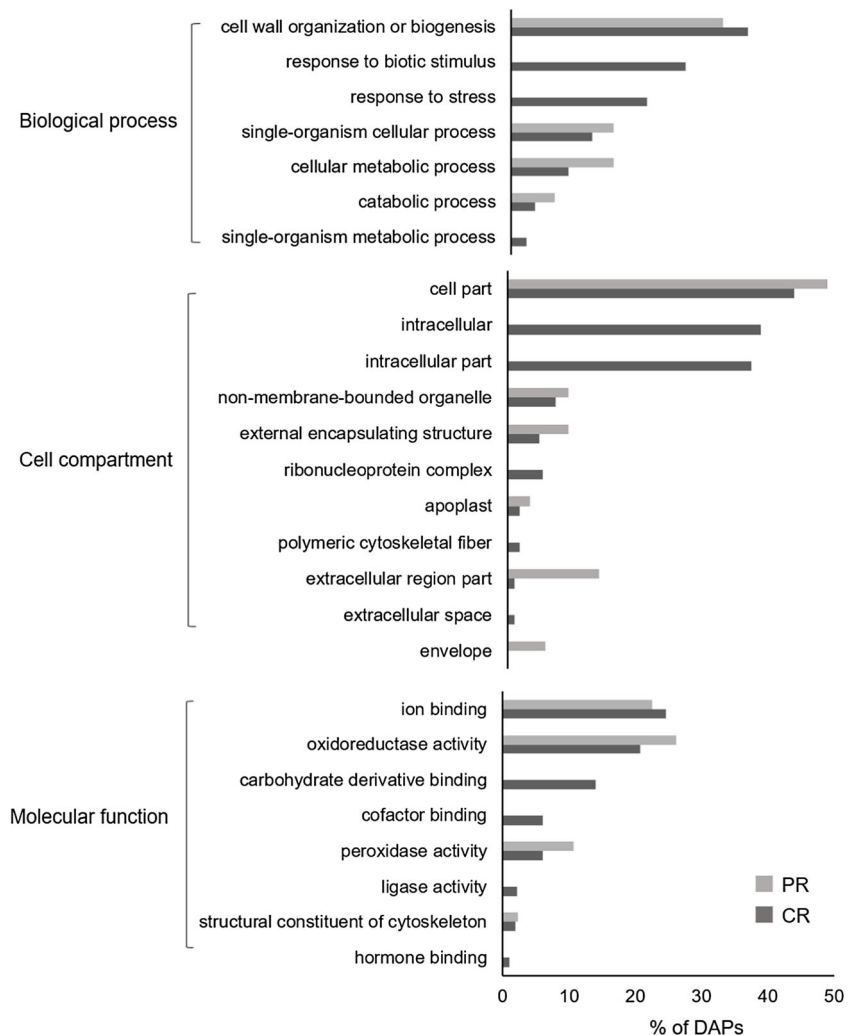
Fig. 6 Major functional clusters of differentially accumulated proteins (DAPs) enriched in tomato roots of both genotypes using DAVID Bioinformatics Resources. Tolerant genotype Pusa Ruby (PR, light gray bars) and sensitive genotype Calabash Rouge (CR, dark gray bars)
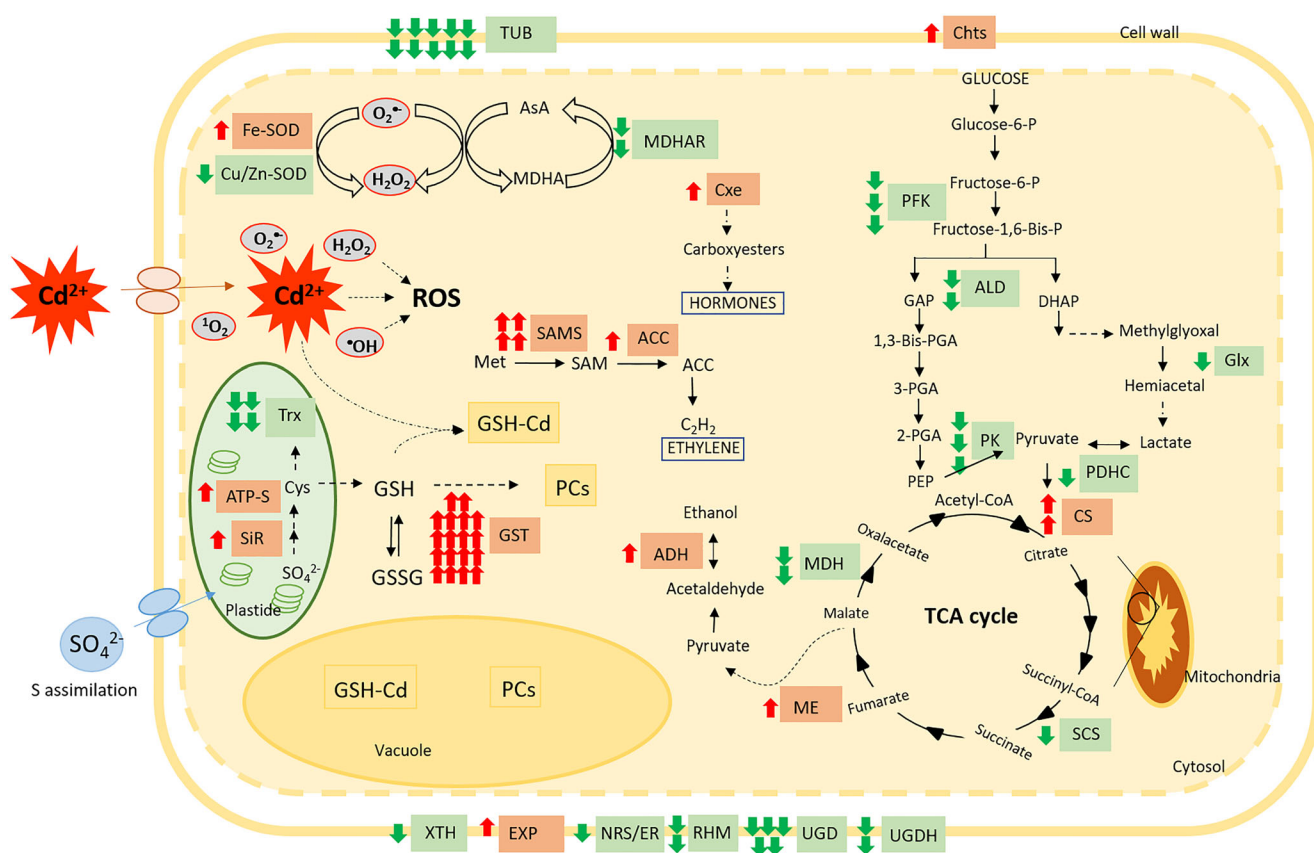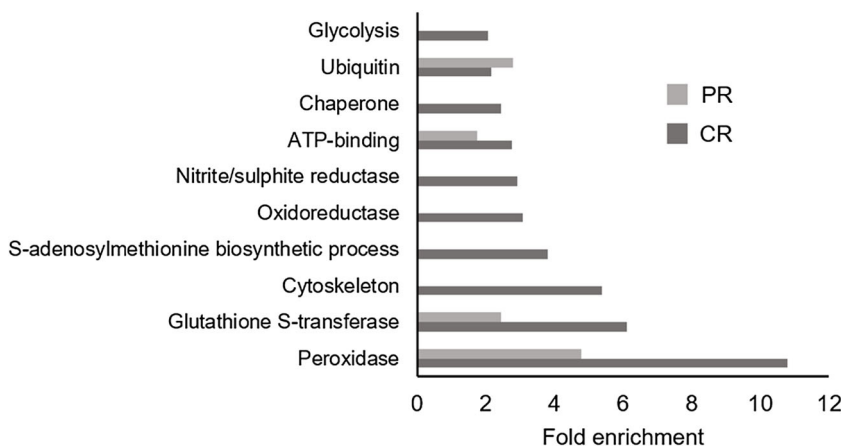

Fig. 7 Metabolism main changes in tomato-sensitive genotype after Cd exposure. Red boxes means proteins that increased abundance in Cd-treated plants compared with controls or exclusively detected in Cd-treated plants. Green boxes means reduced abundance in Cd-treated plants compared with controls or exclusively detected in control plants. Abbreviations: ACC, 1-aminocyclopropane-1-carboxylate oxidase; ADH, aldehyde dehydrogenase; ALD, aldolase; AsA, ascorbate; ATP-S, ATP-sulfurylase; Chts, chitinases; CS, citrate synthase; Cxe, carboxylesterase; Cys, cysteine; EXP, expansin; Glu, glutamine; Glx, glyoxalase; GOGAT, glutamate synthase; GS, glutamine synthase; GSH, reduced glutathione; GSH-Cd, glutathione-cadmium complex; GSSG, oxidized glutathione; GST, glutathione-S-transferase; MDH, malate dehydrogenase; MDHA, monodehydroascorbate; ME, malic enzyme; NH_4^+, ammonium ion; NiR, nitrite reductase; NO_2^-, nitrite ion; NO_3^-, nitrate ion; NRS/ER, rhamnose synthase/epimerase-reductase; PCs, phytochelatins; PDHC, pyruvate dehydrogenase complex; PFK, phosphofructokinase; PK, pyruvate kinase; RGP1, UDP-L-arabinose mutase; RHM, UDP-L-rhamnose synthase; SAMS, S-adenosylmethionine synthetase; SCS, succinyl-CoA-synthetase; SiR, sulfite reductase; SO_4^{2-}, sulfate ion; SOD, superoxide dismutase; MDHAR, monodehydroascorbate reductase; Trx, thioredoxin; TUB, tubulin; UGD, UDP-glucuronic acid decarboxylase; UGDH, UDP-glucose dehydrogenase; XTH, xyloglucan endotransglucosylases
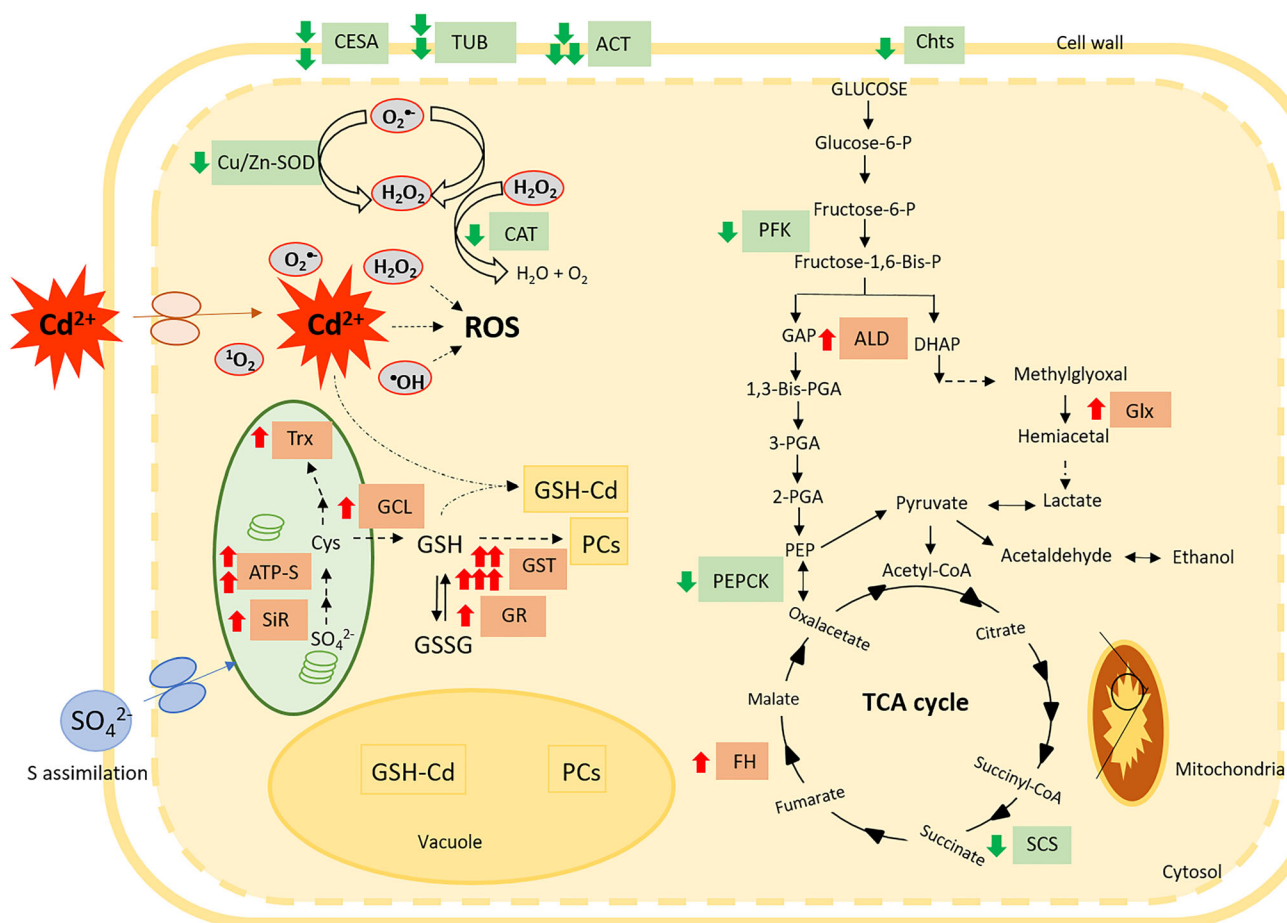
Fig. 8 Metabolism main changes in tomato-tolerant genotype after Cd exposure. Red boxes means proteins that increased abundance in Cd-treated plants compared with controls or exclusively detected in Cd-treated plants. Green boxes means reduced abundance in Cd-treated plants compared with controls or exclusively detected in control plants. Abbreviations: ACT, actin; ALD, aldolase; Glx, glyoxalase; ATP-S, ATP-sulfurylase; CAT, catalase; CESA, cellulose synthase; Chts, chitinases; Cys, cysteine; FH, fumarase; GCL, glutamate-cysteine ligase; GDH, glutamate dehydrogenase; Glu, glutamine; GR, glutathione reductase; GSH, reduced glutathione; GSH-Cd, glutathione-cadmium complex; GSSG, oxidized glutathione; GST, glutathione-S-transferase; NH_4^+, ammonium ion; NO_2^-, nitrite ion; NO_3^-, nitrate ion; PCs, phytochelatins; PEPCK, phosphoenolpyruvate carboxykinase; PFK, phosphofructokinase; SCS, succinyl-CoA-synthetase; SiR, sulfite reductase; SO_4^{2-}, sulfate ion; SOD, superoxide dismutase; Trx, thioredoxin; TUB, tubulin

Root proteome profile of tomato genotypes under Cd-induced stress

The proteomic workflow presented here was able to identify accurately more proteins than other proteomic studies (Song et al. 2013; Kieffer et al. 2009; Rodríguez-Celma et al. 2010; Roy et al. 2017) using a spectral counting proteomic approach. DAPs were identified and associated to different metabolic pathways that may be implicated in Cd stress tolerance in tomato. In a general overview, the proteome of the sensitive genotype was more responsive to excess of Cd, once the number of DAPs detected in this genotype was higher than in the tolerant (Fig. 3c). Interestingly, contrasting cellular component responses between tomato genotypes were detected: sensitive roots showed large alterations in proteins related to

intracellular components, while the tolerant exhibited increased accumulation of proteins located in the extracellular and envelope regions (Fig. 5). Extracellular mechanisms are mainly implicated in avoidance of metal entry, whereas intracellular systems aim to reduce metal burden in the cytosol (Bellion et al. 2006). Using the excluder strategy, plants try to keep the metal concentrations in the roots low, despite the elevated metal concentration in the soil solution (Gallego et al. 2012). Once tomato genotypes exhibited similar Cd accumulation in roots and shoots (Borges et al. 2018; Piotto et al. 2018), it is possible to affirm that ion excluder is not the responsible mechanism of tolerance and maybe different strategies take place in tomato to cope with the Cd challenge.

Under Cd stress, some changes in the sensitive genotype can be highlighted, such as the increased accumulation of

carboxylesterase (Cxe), responsible for selective hydrolysis of xenobiotic and trigger to hormone signaling in plants (Gershater and Edwards 2007). Four isoforms of S-adenosylmethionine synthetase (SAMS) increased in the sensitive genotype after Cd exposure (Fig. 7) and it is related to the ethylene-mediated inhibition of root growth, also involved in the alteration of cell wall structures and polymers in the roots of wheat under aluminum exposure (Fukuda et al. 2007). In tomato, increased ethylene-related proteins were detected in the sensitive genotype (Fig. 7), explaining the reduced root growth observed in these plants compared with the tolerant ones (Fig. 1).

Alterations caused by Cd exposure in redox balance and stress response

Antioxidant enzymes are the key players in ROS scavenging processes, maintaining cellular redox status and inducing stress tolerance (Gratão et al. 2005; Gallego et al. 2012). Cd exposure altered the accumulation of superoxide dismutase (SOD) in both genotypes (Figs. 7 and 8). Contradictory responses of SOD isoenzymes to Cd have been well documented (Alscher et al. 2002). Root proteome analysis of Cd-exposed *Brassica juncea* revealed an increased accumulation of Fe-SOD and decreased accumulation of Cu/Zn SOD (Alvarez et al. 2009). Accordingly, we found Cu/Zn SOD decreased in both genotypes and increased Fe-SOD in sensitive roots (Fig. 7). The Cd-induced ROS accumulation may specifically induce Fe-SOD, once Cd limits Cu and Zn uptake, thus limiting Cu/Zn SOD production and inducing Fe-SOD accumulation (Alvarez et al. 2009). The Cd-mediated repression of peroxidases and the shifts in SOD abundances indicate the possible accumulation of H_2O_2 in tolerant roots, as previously reported (Borges et al. 2018). Another aspect that should be taken into account is that the distinct isoforms of SOD are located in distinct cell compartments (Azevedo et al. 1998; Hippler et al. 2018), so the stress may differently affect the isoforms depending on how the stress affects the plant metabolism in the different organelles. In Cd-stressed poplar plants, the SOD activity decreased, while the enzymatic activity of catalase (CAT) increased in roots, suggesting that these plants trigger a response based on enhanced oxidase activity (Kieffer et al. 2008). In the regulation of H_2O_2 contents, CAT has a high reaction rate, but lower affinity to H_2O_2 when compared with APX, explaining previous findings in tomato roots (Borges et al. 2018), which demonstrated that SOD and CAT activities were unchanged in tomato genotypes treated with Cd. Our study revealed CAT decrease in tolerant roots (Fig. 8), suggesting the existence of SOD and CAT independent mechanisms to detoxify the radical superoxide and H_2O_2 in contaminated tomato cells. Decrease of monodehydroascorbate reductase (MDHAR) abundance was observed in sensitive roots exposed to Cd (Fig. 7), which

might indicate non-enzymatic disproportionation of monodehydroascorbate into ascorbate (AsA), essential for maintenance of a balanced redox status (Hossain et al. 2009).

Important in ROS scavenging, glutathione (GSH) is a protective molecule in response to abiotic stresses being their concentration and redox state very important in transducing oxidative signals originating from ROS activating the antioxidant response (Ball et al. 2004). Glutathione-S-transferase (GST) is involved in sulfur (S) and GSH metabolisms and it was found increased in various plant species when exposed to Cd stress (Kieffer et al. 2008; Zhao et al. 2011). Eighteen GST isoforms were accumulated in sensitive genotype and five isoforms in the tolerant following Cd exposure (Figs. 7 and 8), which may be involved in the direct quenching of Cd ions, forming GSH-Cd complexes, which are stored in the vacuole (Adamis et al. 2004).

Cadmium effects on S-metabolism

After Cd exposure, both tomato genotypes showed increased accumulation of S-related proteins such as ATP-sulfurylase (ATP-S) and sulfite reductases (SiR) (Figs. 7 and 8). The ATP-S catalyzes the sulfate (SO_4^{2-}) reduction to sulfide (S^{2-}) and it has been shown to be involved in plant tolerance to several abiotic stresses through different S-compounds such as Cys or GSH (Anjum et al. 2015, 2012; Gill et al. 2013). It has been recently demonstrated that SiR plays a role in protecting *Arabidopsis* and tomato plants against sulfite toxicity (Yarmolinsky et al. 2013). Further investigation showed that impaired SiR significantly decreased GSH levels and led to early leaf senescence in tomato (Yarmolinsky et al. 2014). Here, both tomato genotypes increased accumulation of S-related proteins under Cd, showing a S-dependent stress response.

Glutamate-cysteine ligase (GCL) is the first enzyme of the GSH biosynthesis, playing an important role in regulating the intracellular redox environment (Jez et al. 2004), and it was found increased only in tolerant tomato (Fig. 8) indicating that this genotype might have an improved antioxidant capacity. For cell redox regulation and signaling via thiol compounds, thioredoxins (Trx) are critical (Gelhaye et al. 2005) and it increased in the tolerant genotype (Fig. 8); conversely, it decreased in the sensitive roots together with glutaredoxins (Grx) (Fig. 7). As protein thiols, Trx and Grx are involved in protective and regulatory mechanisms (Zagorchev et al. 2013). The 13.4-fold increase in Trx was also substantially higher in rice Cu-tolerant line than in a sensitive line (4-fold) when subjected to excess copper (Song et al. 2013), and a similar correlation was reported between salt-tolerant and salt-sensitive barley genotypes (Fatehi et al. 2012). Evidences point to the participation of Trx in plant antioxidant defense mechanisms (Vieira Dos Santos and Rey 2006), reducing power necessary for detoxifying lipid hydroperoxides,

regulating the activity of enzymes involved in repair and detoxification mechanisms and finally modulating the redox status of components involved in pathways linked to oxidative stress, as we detected in the tolerant genotype.

Cadmium-induced alterations in cell wall and cytoskeleton-related proteins

Comparing tomato genotypes exposed to Cd, we found important decreases of cell wall–related proteins mainly in the sensitive genotype (Fig. 7). Two isoforms of cellulose synthase (CESA) decreased in tolerant tomato (Fig. 8). These CESAs are homologous to CESA 1, CESA 3, and CESA 6 of *Arabidopsis thaliana* which are involved in primary cell wall biosynthesis (Desprez et al. 2007), evidencing an direct effect of Cd on primary wall formation. One isoform of nucleotide-rhamnose synthase/epimerase-reductase (NRS/ER) and two isoforms of UDP-L-rhamnose synthase (RHM) decreased in the sensitive genotype; both enzymes participate in the rhamnose biosynthesis in plants. Following the decreases in cell wall–related proteins in the sensitive roots, five isoforms of UDP-glucuronic acid decarboxylase (UGD) and two isoforms of UDP-glucose dehydrogenase (UGDH) decreased (Fig. 7). UGD is a major source of UDP-xylose for the biosynthesis of xylan and xyloglucan (Zhong et al. 2017) while UGDH is a key enzyme for matrix polysaccharides in cell walls (Klinghammer and Tenhaken 2007). Based on these results, it seems evident that Cd induced a reduction of hemicellulose and pectin in cell wall, mainly in the sensitive genotype.

The tomato-sensitive genotype showed contrasting responses for proteins related to the cell wall extensibility functions such as xyloglucan endotransglucosylases/hydrolases (XTH) and expansins (EXP): XTH accumulation decreased while EXP increased (Fig. 7). In *Arabidopsis*, XTH activity was inhibited under aluminum exposure, causing reduced root growth (Zhu et al. 2007). Interestingly, transgenic poplar overexpressing XTH had a higher Cd tolerance by reducing Cd uptake and accumulation in roots, accompanied by degradation of xyloglucan in the root cell wall (Han et al. 2014). Expansins are reported as loosen plant cell walls in a non-enzymatic but pH-dependent manner (Sampedro and Cosgrove 2005). The expression of the β-expansin gene *HvEXPB1* was previously demonstrated to be associated with root hair formation in barley (Kwasniewski and Szarejko 2006) while the silencing of *OsEXPB2* was shown to affect root system architecture by inhibiting cell growth in rice (Zou et al. 2015). Although EXP increased in sensitive genotype, the changes in XTH are evidences of decreases in root growth under Cd stress, being in accordance with previous data, where the sensitive genotype showed drastic reduction in root growth under Cd exposure (Piotto et al. 2018).

Under metal stress, chitinases (Chts) are believed to act as second-line defense components, possibly by modifying the dynamics and permeability of the cell wall to metals (Mészáros et al. 2014). Here, contrasting responses of Chts were observed in tomato roots, with the sensitive genotype exhibiting an increased abundance (Fig. 7), while a decrease in tolerant Chts (Fig. 8). In poplar plants, Chts levels were correlated with sensitivity to Cd (Kieffer et al. 2008). Based on our results, it seems that in the sensitive genotype, the chitin fragments function as signal molecules for defense while in the tolerant, this mechanism is not prioritized.

Cytoskeleton dysfunction caused by Cd exposure in plant cells was previously reported where microtubules seems to be one of the main targets of Cd action (Gzyl et al. 2015). Two isoforms of tubulin (TUB) and three isoform of actin (ACT) decreased in the tolerant while ten isoforms of TUB decreased in the sensitive genotype compared with controls (Fig. 7 and 8). It is well known that tubulins are extremely sensitive to oxidative stress because their cysteine residues could be easily oxidized promoting the cross-linking between α and β-tubulin, which may prevent the assembly of microtubules in cells (Ludueña 2013). Once ROS are produced during Cd-induced stress, it seems to intensify the cellular damage by tubulins oxidation. In tomato-sensitive genotype, Cd caused the reduction of the tubulins bringing harmful consequences to the cellular organization to this genotype (Fig. 7). Taking together these results with decreases in cell wall, we can suggest that Cd induced a dramatic decrease in the protein accumulation, mainly in the sensitive genotype (Fig. 7), indicating that maybe Cd tolerance goes through the capacity of roots to limit the diffusion of Cd, with intense remodeling of cytoskeleton and cell wall–related proteins.

Cadmium-induced alterations in carbon metabolism

Proteins associated with carbon metabolism changed in abundance after Cd exposure in both tomato genotypes (Figs. 7 and 8), being glycolysis and tricarboxylic acid cycle (TCA)–related proteins strongly decreased in the sensitive genotype (Fig. 7). Three isoforms of pyrophosphate (PPi)-dependent phosphofructokinase (PFK) decreased in abundance in the sensitive genotype (Fig. 7), possibly triggering hexose phosphate accumulation in cells and preventing the glycolysis flux and ultimately, impairing the energy production. Following the glycolysis flux, aldolase (ALD) showed contrasting responses in tomato plants, decreasing accumulation in the sensitive genotype (Fig. 7) and increasing in the tolerant genotype (Fig. 8). These changes in the tolerant plants indicate that the metabolism acts enhancing the energy production, while sensitive plants had impaired energy sources under Cd-induced stress. As important energy sources, both glycolysis and TCA cycle were significantly inhibited by Cd in poplar plants (Kieffer et al. 2009) confirming the metabolic impact of Cd

toxicity (Figs. 7 and 8), which may suggest low energy demand in 4-day Cd-treated tomato due to a drastic growth inhibition (Fig. 1).

It has been observed that carbon metabolism is reprogrammed during Cd exposure (Roy et al. 2016). Three isoforms of pyruvate kinase (PK) decreased in the sensitive genotype (Fig. 7), indicating accumulation of substrate, impairing the energy production. On the other hand, the tolerant genotype decreased accumulation of phosphoenolpyruvate carboxykinase (PEPCK) possibly providing energetic sources for the respiratory pathway, with pyruvate entering the TCA cycle to support the plant metabolism even under stress (Fig. 8). Another way to feed the TCA cycle is through the generation of acetyl-CoA by pyruvate dehydrogenase complex (PDHC), which links the glycolysis metabolic pathway to the TCA cycle. PDHC decreased in abundance in the sensitive genotype, as well as other TCA-related proteins such as succinyl-CoA-synthetase (SCS), also known as succinyl-CoA-ligase (Fig. 7). In tomato roots, proteins related to carbon metabolism were strongly affected by Cd (Rodríguez-Celma et al. 2010) confirming a severe metabolic impact of Cd on the root system, as mitochondrial energy production could be negatively influenced by metal exposure. Taken together, these data suggest that tomato genotypes decreased the energy production under Cd stress (Figs. 7 and 8).

Contrasting responses for glyoxalases (Glx) were found in tomato genotypes under Cd stress, decreased in the sensitive genotype, and increased in the tolerant (Figs. 7 and 8). The Glx enzymatic pathway is involved with the detoxification of methylglyoxal, a cytotoxic byproduct released by glycolysis. In a similar manner, transgenic tobacco plants overexpressing the Glx pathway showed a tolerant phenotype against heavy metals (Singla-Pareek et al. 2006). A strong correlation between Glx and stress tolerance in plants has been carried out recently, suggesting glyoxalases as stress tolerance biomarker (Kaur et al. 2014). These findings suggest a role of Glx in tomato plants under Cd stress conditions.

Conclusions

The label-free quantitative proteomic approach performed in this study was efficient to capture the root responses of contrasting tomato genotypes under Cd-induced stress with remarkable changes in the sensitive genotype, which showed abundance reduction of important proteins related to cell wall, carbon, and nitrogen metabolism. The proteome of the sensitive genotype was more responsive to Cd excess, once it presented higher number of DAPs than the tolerant. Moreover, contrasting cellular component responses between tomato genotypes were detected: sensitive roots showed alterations in proteins related to intracellular components, while the tolerant exhibited increased accumulation of proteins located in the extracellular and envelope regions. The tomato genotypes showed contrasting responses for protective and regulatory mechanisms and the tolerant tomato showed alterations of various protein groups leading to a more efficient system to cope with Cd challenge.

References

Adamis PDB, Gomes DS, Pinto MLCC, Panek AD, Eleutherio ECA (2004) The role of glutathione transferases in cadmium stress. Toxicol Lett 154:81–88

Alexieva V, Sergiev I, Mapelli S, Karanov E (2001) The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant Cell Environ 24:1337–1344

Alscher RG, Erturk N, Heath LS (2002) Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. J Exp Bot 53:1331–1341

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

Anjum NA, Ahmad I, Mohmood I, Pacheco M, Duarte AC, Pereira E, Umar S, Ahmad A, Khan NA, Iqbal M, Prasad MNV (2012) Modulation of glutathione and its related enzymes in plants' responses to toxic metals and metalloids-a review. Environ Exp Bot 75:307–324

Anjum NA, Gill R, Kaushik M, Hasanuzzaman M, Pereira E, Ahmad I, Tuteja N, Gill SS (2015) ATP-sulfurylase, sulfur-compounds, and plant stress tolerance. Front Plant Sci 6:210

Azevedo RA, Alas RM, Smith RJ, Lea PJ (1998) Response of antioxidant enzymes to transfer from elevated carbon dioxide to air and ozone fumigation, in the leaves and roots of wild-type and a catalase-deficient mutant of barley. Physiol Plant 104:280–292

Ball L, Accotto GP, Bechtold U, Creissen G, Funck D, Jimenez A, Kular B, Leyland N, Mejia-Carranza J, Reynolds H, Karpinski S, Mullineaux PM (2004) Evidence for a direct link between glutathione biosynthesis and stress defense gene expression in Arabidopsis. Plant Cell 16:2448–2462

Bellion M, Courbot M, Jacob C, Blaudez D, Chalot M (2006) Extracellular and cellular mechanisms sustaining metal tolerance in ectomycorrhizal fungi. FEMS Microbiol Lett 254:173–181

Borges KLR, Salvato F, Alcântara BK, Nalin RS, Piotto FA, Azevedo RA (2018) Temporal dynamic responses of roots in contrasting tomato genotypes to cadmium tolerance. Ecotoxicology 27:245–258

Bradford MM (1976) A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. Anal Biochem 72:248–254

Branco-Neves S, Soares C, Sousa A, Martins V, Azenha M, Gerós H, Fidalgo F (2017) An efficient antioxidant system and heavy metal exclusion from leaves make Solanum cheesmaniae more tolerant to Cu than its cultivated counterpart. Food Energy Secur 6:123–133

Carvalho MEA, Piotto FA, Gaziola SA, Jacomino AP, Jozefczak M, Cuypers A, Azevedo RA (2018a) New insights about cadmium impacts on tomato: plant acclimation, nutritional changes, fruit quality and yield. Food Energy Secur 7:e00131

Carvalho MEA, Piotto FA, Nogueira ML, Gomes-Junior FG, Chamma HMCP, Pizzaia D, Azevedo RA (2018b) Cadmium exposure triggers genotype-dependent changes in seed vigor and germination of tomato offspring. Protoplasma 255:989–999

Carvalho MEA, Piotto FA, Franco MR, Borges KLR, Gaziola SA, Castro PRC, Azevedo RA (2018c) Cadmium toxicity degree on tomato development is associated with disbalances in B and Mn status at early stages of plant exposure. Ecotoxicology 27:1293–1302

Carvalho MEA, Piotto FA, Franco MR, Rossi ML, Martinelli AP, Cuypers A, Azevedo RA (2019) Relationship between Mg, B and Mn status and tomato tolerance against Cd toxicity. J Environ Manag 240:84–92

Desprez T, Juraniec M, Crowell EF, Jouy H, Pochylova Z, Parcy F, Höfte H, Gonneau M, Vernhettes S (2007) Organization of cellulose synthase complexes involved in primary cell wall synthesis in *Arabidopsis thaliana*. Proc Natl Acad Sci 104:15572–15577

Farinati S, DalCorso G, Panigati M, Furini A (2011) Interaction between selected bacterial strains and Arabidopsis halleri modulates shoot proteome and cadmium and zinc accumulation. J Exp Bot 62: 3433–3447

Fatehi F, Hosseinzadeh A, Alizadeh H, Brimavandi T, Struik PC (2012) The proteome response of salt-resistant and salt-sensitive barley genotypes to long-term salinity stress. Mol Biol Rep 39:6387–6397

Florens L, Carozza MJ, Swanson SK, Fournier M, Coleman MK, Workman JL, Washburn MP (2006) Analyzing chromatin remodeling complexes using shotgun proteomics and normalized spectral abundance factors. Methods 40:303–311

Fukuda T, Saito A, Wasaki J, Shinano T, Osaki M (2007) Metabolic alterations proposed by proteome in rice roots grown under low P and high Al concentration under low pH. Plant Sci 172:1157–1165

Gallego SM, Pena LB, Barcia RA, Azpilicueta CE, Lannone MF, Rosales EP, Zawoznik MS, Groppa MD, Benavides MP (2012) Unravelling cadmium toxicity and tolerance in plants: insight into regulatory mechanisms. Environ Exp Bot 83:33–46

Gelhaye E, Rouhier N, Navrot N, Jacquot JP (2005) The plant thioredoxin system. Cell Mol Life Sci 62:24–35

Gershater MC, Edwards R (2007) Regulating biological activity in plants with carboxylesterases. Plant Sci 173:579–588

Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909–930

Gill SS, Anjum NA, Hasanuzzaman M, Gill R, Trivedi DK, Ahmad I, Pereira E, Tuteja N (2013) Glutathione and glutathione reductase: a boon in disguise for plant abiotic stress defense operations. Plant Physiol Biochem 70:204–212

Gilliham M, Able JA, Roy SJ (2017) Translating knowledge about abiotic stress tolerance to breeding programmes. Plant J 90:898–917

Gratão PL, Polle A, Lea PJ, Azevedo RA (2005) Making the life of heavy metal-stressed plants a little easier. Funct Plant Biol 32:481–494

Gygi SP, Rochon Y, Franza BR, Aebersold R (1999) Correlation between protein and mRNA abundance in yeast. Mol Cell Biol 19:1720–1730

Gzyl J, Chmielowska-Bąk J, Przymusiński R, Gwóźdź EA (2015) Cadmium affects microtubule organization and post-translational modifications of tubulin in seedlings of soybean (*Glycine max* L.). Front. Plant Sci 6:937

Han Y, Sa G, Sun J, Shen Z, Zhao R, Ding M, Deng S, Lu Y, Zhang Y, Shen X, Chen S (2014) Overexpression of *Populus euphratica* xyloglucan endotransglucosylase/hydrolase gene confers enhanced cadmium tolerance by the restriction of root cadmium uptake in transgenic tobacco. Environ Exp Bot 100:74–83

Heath RL, Packer L (1968) Photoperoxidation in isoled chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125:11

Hippler FWR, Petena G, Boaretto RM, Quaggio JA, Azevedo RA, Mattos-Jr D (2018) Mechanisms of copper stress alleviation in Citrus trees after metal uptake by leaves or roots. Environ Sci Pollut Res 25:13134–13146

Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil. California Agricultural Experiment Station, Berkeley, p 32

Hossain Z, Komatsu S (2013) Contribution of proteomic studies towards understanding plant heavy metal stress response. Front Plant Sci 3: 12

Hossain Z, López-Climent MF, Arbona V, Pérez-Clemente RM, Gómez-Cadenas A (2009) Modulation of the antioxidant system in citrus under waterlogging and subsequent drainage. J Plant Physiol 166: 1391–1404

Huang DW, Sherman BT, Tan Q, Collins JR, Alvord WG, Roayaei J, Stephens R, Baseler MW, Lane HC, Lempicki RA (2007) The DAVID Gene Functional Classification Tool: a novel biological module-centric algorithm to functionally analyze large gene lists. Genome Biol 8:R183

Hurkman WJ, Tanaka CK (1986) Solubilization of plant membrane proteins for analysis by two-dimensional gel electrophoresis. Plant Physiol 81:802–806

Jez JM, Cahoon RE, Chen S (2004) *Arabidopsis thaliana* glutamate-cysteine ligase: functional properties, kinetic mechanism, and regulation of activity. J Biol Chem 279:33463–33470

Kaur C, Singla-Pareek SL, Sopory SK (2014) Glyoxalase and methylglyoxal as biomarkers for plant stress tolerance. Crit Rev Plant Sci 33:429–456

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

Klinghammer M, Tenhaken R (2007) Genome-wide analysis of the UDP-glucose dehydrogenase gene family in Arabidopsis, a key enzyme for matrix polysaccharides in cell walls. J Exp Bot 58:3609–3621

Krämer U, Pickering IJ, Prince RC, Raskin I, Salt DE (2000) Subcellular localization and speciation of nickel in hyperaccumulator and non-accumulator *Thlaspi* species. Plant Physiol 122:1343–1354

Kwasniewski M, Szarejko I (2006) Molecular cloning and characterization of beta-expansin gene related to root hair formation in barley. Plant Physiol 141:1149–1158

Loix C, Huybrechts M, Vangronsveld J, Gielen M, Keunen E, Cuypers A (2017) Reciprocal interactions between cadmium-induced cell wall responses and oxidative stress in plants. Front Plant Sci 8:1867

Loziuk PL, Parker J, Li W, Lin CY, Wang JP, Li Q, Sederoff RR, Chiang VL, Muddiman DC (2015) Elucidation of xylem-specific transcription factors and absolute quantification of enzymes regulating cellulose biosynthesis in *Populus trichocarpa*. J Proteome Res 14: 4158–4168

Ludueña RF (2013) A hypothesis on the origin and evolution of tubulin. In: Jeon KW (ed) International Review of Cell and Molecular Biology. Academic, Cambridge, pp 41–185

Mészáros P, Rybanský Ľ, Spieß N, Socha P, Kuna R, Libantová J, Moravčíková J, Piršelová B, Hauptvogel P, Matušíková I (2014) Plant chitinase responses to different metal-type stresses reveal specificity. Plant Cell Rep 33:1789–1799

Parrotta L, Guerriero G, Sergeant K, Cai G, Hausman JF (2015) Target or barrier? The cell wall of early- and later-diverging plants vs cadmium toxicity: differences in the response mechanisms. Front Plant Sci 6:133

Piotto FA, Carvalho MEA, Souza LA, Rabêlo FHS, Franco MR, Batagin-Piotto KD, Azevedo RA (2018) Estimating tomato tolerance to heavy metal toxicity: cadmium as study case. Environ Sci Pollut Res 25:27535–27544

Rodríguez-Celma J, Rellán-Álvarez R, Abadía A, Abadía J, López-Millán AF (2010) Changes induced by two levels of cadmium toxicity in the 2-DE protein profile of tomato roots. J Proteome 73: 1694–1706

Roy SK, Cho SW, Kwon SJ, Kamal AHM, Kim SW, Oh MW, Lee MS, Chung KY, Xin Z, Woo SH (2016) Morpho-physiological and proteome level responses to cadmium stress in sorghum. PLoS One 11: e0150431

Roy SK, Cho SW, Kwon SJ, Kamal AHM, Lee DG, Sarker K, Lee MS, Xin Z, Woo SH (2017) Proteome characterization of copper stress responses in the roots of sorghum. BioMetals 30:765–785

Sampedro J, Cosgrove DJ (2005) The expansin superfamily. Genome Biol 6:242

Sharma SS, Dietz KJ (2009) The relationship between metal toxicity and cellular redox imbalance. Trends Plant Sci 14:43–50

Singh S, Singh A, Bashri G, Prasad SM (2016) Impact of Cd stress on cellular functioning and its amelioration by phytohormones: an overview on regulatory network. Plant Growth Regul 80:253–263

Singla-Pareek SL, Yadav SK, Pareek A, Reddy MK, Sopory SK (2006) Transgenic tobacco overexpressing glyoxalase pathway enzymes grow and set viable seeds in zinc-spiked soils. Plant Physiol 140: 613–623

Soares C, Carvalho MEA, Azevedo RA, Fidalgo F (2019) Plants facing oxidative challenges - a little help from the antioxidant networks. Environ Exp Bot 161:4–25

Song Y, Cui J, Zhang H, Wang G, Zhao FJ, Shen Z (2013) Proteomic analysis of copper stress responses in the roots of two rice (*Oryza sativa* L.) varieties differing in Cu tolerance. Plant Soil 366:647–658

Tyanova S, Temu T, Sinitcyn P, Carlson A, Hein MY, Geiger T, Mann M, Cox J (2016) The Perseus computational platform for comprehensive analysis of proteomics data. Nat Methods 13:731–740

Vieira Dos Santos C, Rey P (2006) Plant thioredoxins are key actors in the oxidative stress response. Trends Plant Sci 11:329–334

Wei SH, Zhou QX, Wang X, Zhang KS, Guo GL, Ma QYL (2005) A newly-discovered Cd-hyperaccumulator *Solanum nigrum* L. Chin Sci Bull 50:33–38

Yarmolinsky D, Brychkova G, Fluhr R, Sagi M (2013) Sulfite reductase protects plants against sulfite toxicity. Plant Physiol 161:725–743

Yarmolinsky D, Brychkova G, Kurmanbayeva A, Bekturova A, Ventura Y, Khozin-Goldberg I, Eppel A, Fluhr R, Sagi M (2014) Impairment in sulfite reductase leads to early leaf senescence in tomato plants. Plant Physiol 165:1505–1520

Zagorchev L, Seal CE, Kranner I, Odjakova M (2013) A central role for thiols in plant tolerance to abiotic stress. Int J Mol Sci 14:7405–7432

Zhang Y, Fonslow BR, Shan B, Baek MC, Yates JR (2013) Protein analysis by shotgun/bottom-up proteomics. Chem Rev 113:2343–2394

Zhao FJ, Jiang RF, Dunham SJ, McGrath SP (2006) Cadmium uptake, translocation and tolerance in the hyperaccumulator *Arabidopsis halleri*. New Phytol 172:646–654

Zhao L, Sun YL, Cui SX, Chen M, Yang HM, Liu HM, Chai TY, Huang F (2011) Cd-induced changes in leaf proteome of the hyperaccumulator plant *Phytolacca americana*. Chemosphere 85: 56–66

Zhong R, Teng Q, Haghighat M, Yuan Y, Furey ST, Dasher RL, Ye ZH (2017) Cytosol-localized UDP-xylose synthases provide the major source of UDP-xylose for the biosynthesis of xylan and xyloglucan. Plant Cell Physiol 58:156–174

Zhu J, Alvarez S, Marsh EL, LeNoble ME, Cho IJ, Sivaguru M, Chen S, Nguyen HT, Wu Y, Schachtman DP, Sharp RE (2007) Cell wall proteome in the maize primary root elongation zone. II. Region-specific changes in water soluble and lightly ionically bound proteins under water deficit. Plant Physiol 145:1533–1546

Zou H, Wenwen Y, Zang G, Kang Z, Zhang Z, Huang J, Wang G (2015) OsEXPB2, a β-expansin gene, is involved in rice root system architecture. Mol Breed 35:41