Stress response of lettuce (*Lactuca sativa*) to environmental contamination with selected pharmaceuticals: A proteomic study

Author links open overlay panelInêsLeitão[a]Céline C.Leclercq[b]David M.Ribeiro[a]JennyRenaut[b]André M.Almeida[a]Luisa L.Martins[a]Miguel P.Mourato[a]

https://doi.org/10.1016/j.jprot.2021.104291Get rights and content

Highlights

- Lettuce plants are able to cope with pharmaceutical contaminants in short period of exposure.
- Aquaporins and heat shock proteins are affected by the presence of carbamazepine and acetaminophen.
- Metformin was the contaminant which showed less differences in relation to control plants.
- Differences among contaminated roots and leaves were reported for the same pathways.

Abstract

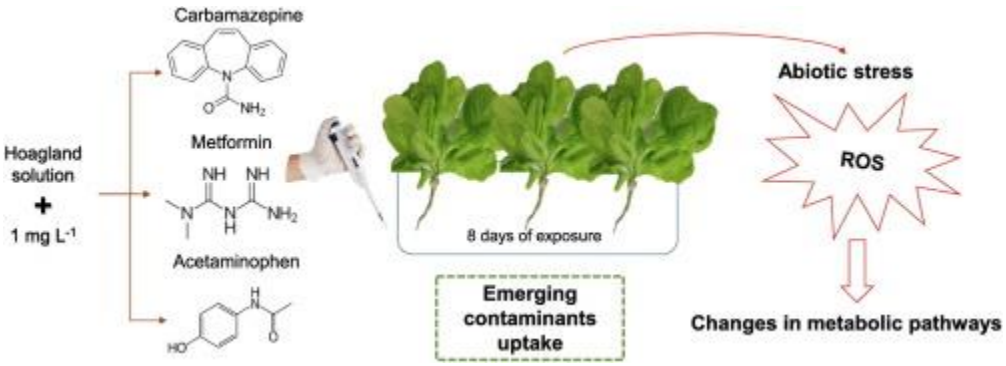
Pharmaceutical compounds have been found in rivers and treated wastewaters. They often contaminate irrigation waters and consequently accumulate in edible vegetables, causing changes in plants metabolism.

The main objective of this work is to understand how lettuce plants cope with the contamination from three selected pharmaceuticals using a label free proteomic analysis.
A lettuce hydroponic culture, grown for 36 days, was exposed to metformin, acetaminophen and carbamazepine (at 1 mg/L), during 8 days, after which roots and leaves were sampled and analysed using a liquid chromatography-mass spectrometry proteomics-based approach.
In roots, a total of 612 proteins showed differentially accumulation while in leaves 237 proteins were identified with significant differences over controls. Carbamazepine was the contaminant that most affected protein abundance in roots, while in leaves the highest number of differentially accumulated proteins was observed for acetaminophen. In roots under carbamazepine, stress related protein species such as catalase, superoxide dismutase and peroxidases presented higher abundance. Ascorbate peroxidase increased in roots under metformin. Cell respiration protein species were affected by the presence of the three pharmaceuticals suggesting possible dysregulation of the Krebs cycle. Acetaminophen caused the main differences in respiration pathways, with more emphasis in leaves. Lettuce plants revealed different tolerance levels when contaminants were compared, being more tolerant to metformin presence and less tolerant to carbamazepine.

Significance

The significant increase of emerging contaminants in ecosystems makes essential to understand how these compounds may affect the metabolism of different organisms. Our study contributes with a detailed approach of the main interactions that may occur in plant metabolism when subjected to the stress induced by three different pharmaceuticals (acetaminophen, carbamazepine and metformin).

Graphical abstract



1. Download : Download high-res image (169KB)
2. Download : Download full-size image

- **Previous** article in issue
- **Next** article in issue

Keywords

Lettuce
Proteomics
Stress proteins
Pharmaceuticals

Abbreviations

ACT
: Acetaminophen

APX
: ascorbate peroxidase

AsA
: ascorbate

BP
: Biological Process

CBZ
: Carbamazepine

CAT
: catalase

Ctrl
: Control

CYP450
: Cytochromes P450

GABA
: gamma-aminobutyric acid

GAD
: Glutamate decarboxylase

G6PDH
: Glucose-6-phosphate 1-dehydrogenase

GSH
: Glutathione

GSSG
: Oxidized glutathione

GR
: glutathione reductase

HSP
: Heat Shock Protein

IDH
: isocitrate dehydrogenase

MTF
: Metformin

MF
: Molecular function

PAL
: phenylalanine ammonia-lyase

POX
: peroxidases

ROS
: reactive oxygen species

SOD
: superoxide dismutase

UGHD
: UDP-glucose 6-dehydrogenase

1. Introduction

Lettuce (*Lactuca sativa*) is one of the most widely consumed leafy vegetables worldwide and may easily be subjected to different abiotic stresses like water deficit, pathogens, heavy metals, salinity and pharmaceutical products, among other factors [[1], [2], [3], [4]]. It is also considered a model plant and has thus been used as a bioindicator or sentinel species in studies concerning the effect of different contaminants [[5], [6], [7]].

Although several references may be found in the literature regarding oxidative stress induced by contaminants in lettuce [2,8,9], there is still a generalized lack of information concerning the detoxification response of plants to specific pharmaceutical products. The mechanisms related to this metabolic response are in fact very diverse and depend on different factors, such as plant species, tolerance to contaminant, developmental stage, type of contaminant and its persistence [2], among many others.

Metformin (MTF) is used as therapeutic treatment for type 2 diabetes adult patients. The occurrence of this pharmaceutical in the environment has already been reported, for instance, in Canada [10] and in Germany [11], where MTF was detected in surface and drinking water. The uptake of this pharmaceutical by plants was addressed by Eggen et al. [12] who described the translocation of MTF to the edible part of different cultures (beans, potatoes, tomato and carrots). Acetaminophen (ACT) is an extensively used analgesic and antipyretic. Regarding plant uptake, literature described the translocation of ACT from roots to shoots of radish [13]. Additionally, Chuang et al. [14] described the accumulation of this pharmaceutical in lettuce tissues. Carbamazepine (CBZ) is an anticonvulsant reported already as a

pharmaceutical with high occurrence in environment. Chuang et al. [14] showed the effects of CBZ uptake in lettuce plants.

These compounds have been reported as being present in different ecosystems (rivers, soils, groundwater) in different European countries such as Portugal, Germany, France, Luxembourg, Spain and Poland [15,16]. As such, the exposure of edible vegetables to this type of contaminants will likely become a problem, not only to the food chain but also to the environment. Fram et al., [17] analysed 1231 groundwater samples used for drinking water where a maximum concentration of 1.89 μg/L of acetaminophen was found. The occurrence of metformin in the environment has been reported not only in wastewater treatment plants influents (101–129 μg/L) and effluents (2.2–21 μg/L), but also in surface waters (1.7 μg/L) and sewage sludge (500–1600 μg/kg DW) [12]. In Canada metformin was detected in 48% of the samples from surface water with a maximum concentration of 1487 ng/L [10]. Carbamazepine was also detected in treated wastewaters in the USA in concentrations of 1.2 μg/L [18]. Aquatic compartments are the most affected by the presence of pharmaceuticals, in particular carbamazepine that was detected in concentrations ranging from 0.03 to 11.6 μg/L in several EU countries [19,20].

Numerous tools have been developed regarding the study of abiotic stresses, particularly oxidative stress in plants as a consequence of the imbalance of reactive oxygen species (ROS) and the resulting oxidative damage. The main regulators of oxidative stress are antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), peroxidases (POX) and glutathione (GSH) and ascorbate (AsA) as non-enzymatic antioxidants [[21], [22], [23]]. In fact, plant stress is a dynamic process with different phases according to the adaptation level of the plant: first an alarm phase occurs, followed by acclimation, maintenance and exhaustion phases [24].

To study plant stress, different methods have been recently addressed, mainly using omics-based approaches. In particular, a proteomics-based approach with liquid chromatography coupled to a mass spectrometer (LC-MS/MS) may provide detailed information about abundance and distribution of a plethora of protein species. As such, the identification and quantification of proteins involved in different metabolic pathways will provide detailed information about the changes during the response to environmental factors, such as abiotic or biotic stresses [25], being able to highlight

the major metabolic pathways triggered by such response and therefore contributing to understand the toxic effects of these compounds.

Under exposure to contaminants, plants activate their defence mechanisms against oxidative stress, which will result in the production of signalling molecules and stress related protein species, namely heat shock proteins (HSP) [26] and other protein species responsive to ROS, such as catalases and peroxidases. For instance, Freitas et al., [27] studied the exposure of lettuce to cylindrospermopsin and a microcystin-LR/cylindrospermopsin mixture showing some variations on HSP70 and 90. Dordio et al. [28] reported an increase in the activity of SOD and CAT in *Typha* spp. plants contaminated with carbamazepine, confirming the occurrence of oxidative stress. In another example, Alkimin et al. [29] studied the toxicity of three different pharmaceuticals (diclofenac, acetaminophen and chlorpromazine) on *Lemna minor* and *Lemna gibba* plants, showing an increase in CAT activity for all the three pharmaceuticals. Similarly, Bartha et al., [30] also reported significant increases on peroxidases activity in *Brassica juncea* after 1 week of treatment with 1 mM of acetaminophen. Finally, other examples include the proteome analysis of plants (e.g. tomato, maize, poplar, salicornia, grapevine) under mineral stress, water deficit, heavy metals content, salinity, herbicides contamination have been reported by several authors using different approaches [[31], [32], [33], [34], [35]].

This work aims to study the main metabolic pathways in lettuce tissues contaminated with three different pharmaceuticals (metformin, carbamazepine and acetaminophen) using a proteomic analysis. The main focus of this analysis will be the relation among stress related protein species and specific metabolic pathways regarding defence mechanisms. This work will contribute to improve current knowledge regarding the effect of lettuce contamination with these pharmaceutical compounds and for a better understanding of plant defence mechanisms against oxidative stress. These three pharmaceuticals were chosen for two main reasons: 1) their occurrence in the environment has been reported in several studies as previously mentioned; 2) their high levels of consumption in Portugal and in the rest of Europe (Portuguese National Pharmaceutical Agency – INFARMED).

2. Material and methods

2.1. Experimental design and sample collection

To obtain the plant material used in this experiment, a hydroponic experiment was carried out. The plants were placed in a controlled environment, with temperatures between 20 and 25 °C, a relative humidity of 60–65%, a light period of 12 h with irradiance of 250 mmol m^{-2} s^{-1} at leaf level (Aralab *Fitoclima D1200* growth chamber). Afterwards, seeds of the cultivar *Lactuca sativa* L. *Maravilha das Quatro Estações* were germinated in peat substrate and kept for 15 days until the development of the first leaf. Afterwards young lettuce plants were transferred to hydroponic culture with Hoagland solution [36] and developed in this medium an additional period of 21 days. The Hoagland solution was renewed every 5 days. On the 36th day, plants were divided in the following groups: control or contaminated with 1 mg/L of one of the three pharmaceuticals under study (MTF, ACT and CBZ). This concentration was selected in order to induce a measurable stress response but not high enough to cause the death of the plant and also as these would be the concentration indicative of what may be found in the literature. Indeed, the range of concentrations in the literature is wide and therefore, we intended to select an average concentration for our work. Shenker et al. [37] studied the effects of CBZ in cucumber subjected to concentrations higher than 10 mg/L. ACT contamination was already studied in plants of *Brassica juncea* (150 mg/L) [38]. Kotyza et al. [39] studied the effects of acetaminophen in plants of *Armoratia rusticana*, *Linum usitatissimum* (both at 30 mg/L), *Hordeum vulgare*, *Lupinus luteolus*, *Phragmites autralis* (all in a range of 15–181 mg/L). Eggen et al. [40] carried out a study with carrots subject to MTF contaminant, with a concentration of 10 mg kg^{-1} in spiked soil, revealing some negative effects in plants development. The same authors conducted a different study to test the uptake of MTF by *Brassica napus* and *Brassica rapa* as well as by tomato and three different cereals (wheat, barley and oats). Besides, due to previous work the concentration of 1 mg/L showed to cause a certain level of oxidative damage in lettuce plants.

Groups were designated as Ctrl, MTF, ACT and CBZ. A total of eight plants was used per treatment. After 8 days, eight lettuce plants were harvested. The biomass of roots and leaves was measured. Roots and aerial parts (leaves) were separated, snap-frozen

in liquid nitrogen and kept at −80 °C until further analysis. A scheme of experimental design is available in supplementary data (S1).

2.2. Protein extraction and sample preparation

To carry out the proteomics analysis, 8 samples of lettuce from each experimental group (4 leaves and 4 roots each) were analysed for a total of 32 samples (16 per tissue). The sample extraction procedure followed the method described by Ribeiro et al. [41]. First, approximately 0.3–0.4 g of fresh tissue were weighted and ground in order to homogenize the sample. Protein extracts were obtained by adding 1.5 mL of extraction buffer of 20% *w*/*v* Trichloroacetic Acid (TCA) and 0.1% w/v of Dithiothreitol (DTT) in acetone. The crushed samples were placed at −20 °C overnight to ensure protein precipitation. A centrifugation at 30,000*g* during 45 min at 4 °C was performed for all sample extracts. After centrifugation, the pellet was collected and washed three times with rinsing buffer of 0.1% *w*/*v* DTT in acetone and vacuum dried. Finally, the protein pellet was well solubilized with 115 µL of lysis buffer (7 M urea, 2 M thiourea and 0.5% (*w*/*v*) CHAPS).
To estimate the protein amount obtained, the method of Reducing Agent Compatible/Detergent Compatible quantification (Bio-Rad RC DC™ Protein Assay Kit II) was performed following the manufacturer's instructions. For protein quantification, 3 biological replicates and 2 analytical replicates were performed.
A calibration curve was prepared throughout the dilution of 2 µg/µL BSA (0, 0.04, 0.4, 0.8, 1.2, and 1.6 µg/µL).
For sample preparation, 20 µg of total proteins were loaded according to manufacturer instructions on a Criterion™ XT precast 1D gel (12% Bis-Tris SDS-PAGE, 18 wells, Bio-Rad, USA). A short migration was achieved for 10 min. The protein gels were stained in a bath with Instant Blue™ (Expedeon, UK). Afterwards, the gels were divided in two equal parts, for each sample, and cut into small pieces. Each part was placed in a microplate well. Then, the proteins were reduced with DTT, alkylated with iodoacetamide and destained. Thereafter, trypsin (5 ng/µl, mass grade Promega) was added to carry on in-gel protein digestion. The samples were then placed in an ice bath for 30 min followed by incubation for 5 h at 37 °C after which the peptides were

extracted from the gels. The extracted peptides were dried and stored at −20 °C until LC-MS/MS analysis.

2.3. LC-MS/MS

The LC-MS/MS analysis followed a classical MS analysis for bottom-up approach [[42], [43], [44], [45]]. Samples were analysed with Eksigent NanoLC 425 system (SCIEX, Belgium) coupled with SCIEX TripleTOF® 6600 MS system. Peptides were loaded onto a C18 trap column (5 × 0.5 mm YMC triart) using a loading buffer (2% acetonitrile (ACN), 0.1% formic acid (FA)) at a flow rate of 10 μL/min and were separated on a C18 LC column (Luna® Omega 3 μm polar C18 100 Å 150 × 0.3 mm, Phenomenex) using a linear binary gradient with a mobile phase A composed with 0.1% FA in LC-MS grade water and mobile phase B of ACN with 0.1% FA. Peptides were eluted at a flow rate of 5 μL/min with 3% B, followed by an increase from 3 to 30% during 45 min, increase to 40% in 7 min, then reaching 80% to wash the column. Prior to the next injection the column was re-equilibrated for a few minutes with 3% B.

The mass range of full scan MS spectra was set to 400–1250 Da. The maximum accumulation time for precursor ions was set for 250 ms and a dynamic exclusion was set to 10s. The 30 most intense ions with a charge state of 2^+ to 4^+ were selected and fragmented using the rolling collision energy. The mass range of MS/MS scans was set to 100–1500 Da with an accumulation time of 50 ms.

2.4. Protein identification

MS data were processed with Progenesis QI for Proteomics software (version 4.2, Nonlinear Dynamics, Waters, UK).

The protein and peptide identification were performed via Mascot Daemon interface (version 2.6.0. Matrix Science, UK) searching against *Lactuca sativa* database on UniprotKB (released 27th of November 2019, 39366 sequences - https://www.uniprot.org/proteomes/UP000235145). Different search parameters were used: trypsin enzyme, a maximum of 2 missed cleavages, a peptide tolerance of 20 ppm, and a fragment mass tolerance of 0.3 Da. Carbamidomethylation at cysteine was set as fixed modification and the variable modifications included oxidation at

methionine, N-terminal protein acetylation and conversion of tryptophan to kynurenine. The identified proteins with a significant Mascot-calculated confidence of 95% were selected for further analysis.

2.5. Data analysis and interpretation

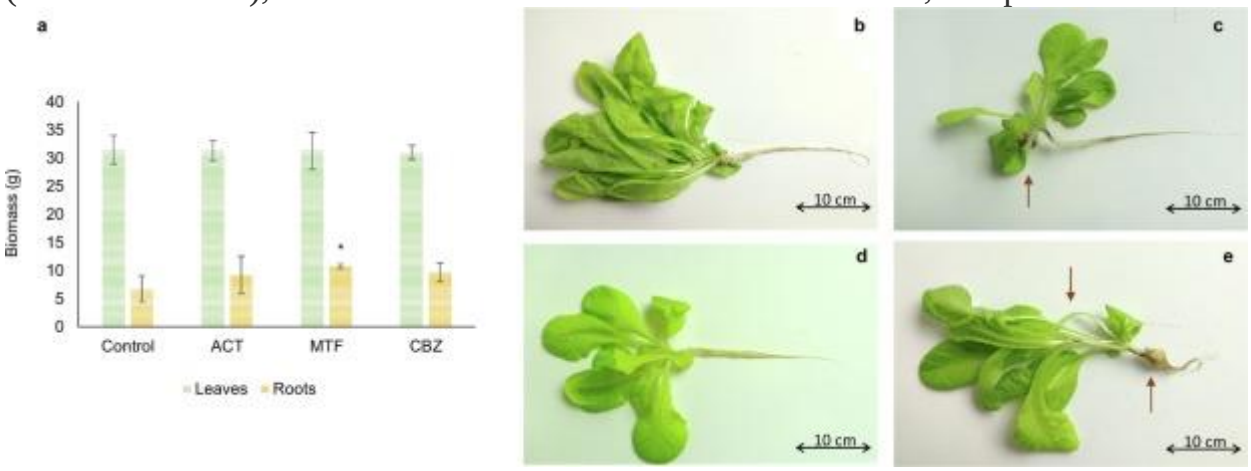
To ensure the significant differences between conditions in Progenesis QIP, the following filters were applied on the identified protein list: an ANOVA *p*-value < 0.05, at least 1 unique and 2 significant peptides per protein, and a fold change higher than 1.5. Data analysis and visualization were carried out using Progenesis QIP.
Protein species showing differential abundance were selected for each condition and the normalized abundance obtained on Progenesis QIP was applied to comparisons between control and pharmaceutical contamination (Control vs MTF, Control vs ACT, Control vs CBZ).

After data analysis, the list of significant proteins was imported to Blast2GO in order to blast and obtain more information about "uncharacterized proteins", and to complete proteome characterization of *Lactuca sativa*. Default parameters were applied. The protein sequences were mapped and annotated with sequence identity against existing functional annotation from yet uncharacterised sequences. The differentially identified proteins were classified based on Gene Ontology (GO) identifications according to their biological processes, cellular component and molecular functions.
To obtain protein information about metabolic functions and biological processes, differentially significant proteins from contaminations vs control condition, were loaded in the PANTHER tool (http://www.pantherdb.org/). The proteins differentially expressed (in comparison to control) for roots and leaves of each condition were displayed in a Venn diagram using the bioinformatics and evolutionary genomics tool (Gent University, Belgium): http://bioinformatics.psb.ugent.be/webtools/Venn/. In order to obtain a relation of protein species abundance among contaminants for both tissues, heatmaps were obtained in R Studio 3.6.3 software applying the packages "tidyverse", "bitops", "heatmap.plus", and "RColorBrewer".

The MS proteomics data has been deposited to the ProteomeXchange Consortium [46] via the PRIDE [47] partner repository with the dataset identifier PXD021474 and https://doi.org/10.6019/PXD021474.

3. Results

Regarding lettuce growth and development, no significant differences between control and MTF, CBZ or ACT contaminations were found in leaf biomass, while in roots, only a small increase in root mass in plants growing under MTF was detected (Fig. 1a). Some changes in the development of both the leaves and the roots were detected (Fig. 1b, c, d, e). Under CBZ contamination, plants revealed darker and smaller roots as well as etiolated leaves. Plants exposed to ACT were the most similar to control (leaves and roots), while the ones with MTF showed less leaves, compared to control.



1. Download : Download high-res image (127KB)
2. Download : Download full-size image

Fig. 1. Biomass of roots and leaves (a) of lettuce after 8 days of experiment: Control (b), 1 mg/ L MTF (c), 1 mg/ L ACT (d) and 1 mg/ L CBZ (e).

3.1. Differential protein abundance

The list of selected protein species that were differentially accumulated in contaminated plants are presented in Table 1, Table 2, Table 3. Comparisons between control and contaminated plants were established in order to understand how proteins abundance changed under stress conditions. The main criteria for comparison between control and contaminant groups was the Max Fold Change, and *p*-value as described.

Table 1. Abundance of identified protein species in roots and leaves contaminated with MTF. Legend: MTF: Metformin, MFC: Max Fold Change, which reveals the trend observed between contamination and control roots and leaves for each protein species, Control mean: mean of protein species abundance for replicates of control; MTF mean: mean of protein species abundance for replicates of MTF.

Accession number	SeqName	Protein name	GO Cellular Component	Peptide count	Unique peptides
Metformin: proteins abundance in Roots					
A0A2J6KT66_LACSA	tr|A0A2J6KT66|A0A2J6KT66_LACSA	Isocitrate dehydrogenase [NADP]	Mithocondrion	34	2
A0A2J6LQ77_LACSA	tr|A0A2J6LQ77|A0A2J6LQ77_LACSA	Heat shock 70 kDa protein 3*	Cytoplasm	23	5
A0A2J6LYZ4_LACSA	tr|A0A2J6LYZ4|A0A2J6LYZ4_LACSA	Leucine–tRNA ligase, cytoplasmic*		3	2
A0A2J6MG26_LACSA	tr|A0A2J6MG26|A0A2J6MG26_LACSA	Coatomer subunit beta’	Golgi apparatus, golgi membrane	4	1
A0A2J6KXJ0_LACSA	tr|A0A2J6KXJ0|A0A2J6KXJ0_LACSA	Ras-related protein Rab11C*	Endosome	7	1
Metformin: proteins abundance in Leaves					
A0A2J6L886_LACSA	tr|A0A2J6L886|A0A2J6L886_LACSA	Histone H2B	Nucleosome, nucleus	18	1
A0A2J6LX58_LACSA	tr|A0A2J6LX58|A0A2J6LX58_LACSA	Histone H2A	Nucleosome, nucleus	3	2
A0A4D5XX70_ULVLA	tr|A0A4D5XX70|A0A4D5XX70_ULVLA	Photosystem II protein D1	Photosystem II, chloroplast thylakoid membrane, integral component of membrane	7	1
A0A2J6K5S3_LACSA	tr|A0A2J6K5S3|A0A2J6K5S3_LACSA	Aquaporin TIP1-1*	Endoplasmic reticulum, golgi apparatus, plasma membrane, plant-type cell	2	2

Accession number	SeqName	Protein name	GO Cellular Component	Peptide count	Unique peptides
			wall, plasmodesm, chloroplast envelope, central vacuole		
A0A2J6K8E6_LACSA	tr|A0A2J6K8E6|A0A2J6K8E6_LACSA	Sulfurtransferase	Mitochondrion, cytosol, chloroplast	6	1
A0A2J6KJN7_LACSA	tr|A0A2J6KJN7|A0A2J6KJN7_LACSA	ATP synthase subunit beta	Chloroplast thylakoid membrane, proton-transporting ATP synthase complex, catalytic core F(1)	62	1
A0A2J6KL66_LACSA	tr|A0A2J6KL66|A0A2J6KL66_LACSA	UDP-glucose 6-dehydrogenase	Cell wall, cytosol, nucleus	10	2
A0A2J6LII2_LACSA	tr|A0A2J6LII2|A0A2J6LII2_LACSA	Pectinesterase	Vacuolar membrane, cytosol, plasma membrane, plant-type cell wall, apoplast	8	2
S5RCK4_9CHLO	tr|S5RCK4|S5RCK4_9CHLO	Ribulose bisphosphate carboxylase large chain (Fragment)	Chloroplast	29	1

Table 2. Abundance of identified protein species in roots and leaves contaminated with ACT. Legend: MTF: Metformin, MFC: Max Fold Change, which reveals the trend observed between contamination and control roots and leaves for each protein species, Control mean: mean of protein species abundance for replicates of control; ACT mean: mean of protein species abundance for replicates of ACT.

Accession number	SeqName	Protein name	GO cellular component	Peptide count	Unique peptides
Acetaminophen: proteins abundance in Roots					
A0A2J6JUU9_LACSA	tr|A0A2J6JUU9|A0A2J6JUU9_LACSA	Peroxidase	Cell Wall, plant-type cell wall,	10	3

Accession number	SeqName	Protein name	GO cellular component	Peptide count	Unique peptides
			Extracellular region or secreted extracellular region, plasmodesma		
A0A2J6JWK1_LACSA	tr|A0A2J6JWK1|A0A2J6JWK1_LACSA	Succinate–CoA ligase	Mithocondrion, succinate-CoA ligase complex (ADP-forming)	7	1
A0A2J6K261_LACSA	tr|A0A2J6K261|A0A2J6K261_LACSA	Peroxidase	Extracellular region or secreted	8	2
A0A2J6K8N7_LACSA	tr|A0A2J6K8N7|A0A2J6K8N7_LACSA	UDP-glucose 6-dehydrogenase	Cytosol, nucleus	8	1
A0A2J6KFP0_LACSA	tr|A0A2J6KFP0|A0A2J6KFP0_LACSA	Proteasome subunit beta	Nucleus, cytoplasm, proteasome core complex	6	1
A0A2J6KNL8_LACSA	tr|A0A2J6KNL8|A0A2J6KNL8_LACSA	L-ascorbate oxidase*	Extracellular region or secreted	12	2
A0A2J6KQP9_LACSA	tr|A0A2J6KQP9|A0A2J6KQP9_LACSA	Ribosomal_S13_N domain-containing protein	Cytosolic small ribosomal subunit, nucleolus	9	1
A0A2J6KTF4_LACSA	tr|A0A2J6KTF4|A0A2J6KTF4_LACSA	GLOBIN domain-containing protein		13	1
A0A2J6LK16_LACSA	tr|A0A2J6LK16|A0A2J6LK16_LACSA	ML domain-containing protein	Vacuole	2	1
A0A2J6LSU9_LACSA	tr|A0A2J6LSU9|A0A2J6LSU9_LACSA	Sod_Fe_C domain-containing protein	Mithocondrion	3	1
A0A2J6L4F3_LACSA	tr|A0A2J6L4F3|A0A2J6L4F3_LACSA	Iso_dh domain-containing protein	Mithocondrion	11	1
A0A2J6M212_LACSA	tr|A0A2J6M212|A0A2J6M212_LACSA	Aquaporin PIP1–2*	Membrane, membrane protein	6	2
A0A2J6MB56_LACSA	tr|A0A2J6MB56|A0A2J6MB56_LACSA	Cytochrome c domain-containing protein	mitochondrial respiratory chain complex III, integral	5	1

Accession number	SeqName	Protein name	GO cellular component	Peptide count	Unique peptides
			component of membrane		
A0A2J6JN51_LACSA	tr|A0A2J6JN51|A0A2J6JN51_LACSA	BZIP domain-containing protein	Nucleus	8	1
A0A2J6JQH6_LACSA	tr|A0A2J6JQH6|A0A2J6JQH6_LACSA	Heat shock cognate 70 kDa protein	Cytoplasm	18	1
A0A2J6LDA2_LACSA	tr|A0A2J6LDA2|A0A2J6LDA2_LACSA	ABC transporter C family member 5*	Plant-type vacuole, vacuolar membrane, integral component of membrane, membrane	24	3
A0A2J6LKE3_LACSA	tr|A0A2J6LKE3|A0A2J6LKE3_LACSA	DUF3591 domain-containing protein	Nucleus	2	1
Acetaminophen: proteins abundance in Leaves					
A0A2J6JT18_LACSA	tr|A0A2J6JT18|A0A2J6JT18_LACSA	Plasma membrane ATPase	Cell, nucleus, Golgi apparatus, plasma membrane, vacuole organization, plasmodesma, plant-type vacuole membrane, integral component of membrane	5	3
A0A2J6JUD5_LACSA	tr|A0A2J6JUD5|A0A2J6JUD5_LACSA	Aconitase_C domain-containing protein	Chloroplast stroma	3	1
A0A2J6KBC0_LACSA	tr|A0A2J6KBC0|A0A2J6KBC0_LACSA	PKS_KS domain-containing protein	Mitochondrion, cytosol, chloroplast stroma	5	2
A0A2J6M8C0_LACSA	tr|A0A2J6M8C0|A0A2J6M8C0_LACSA	DEAD-box ATP-dependent RNA helicase 56*	Transcription export complex, chromosome, telomeric	2	2

Accession number	SeqName	Protein name	GO cellular component	Peptide count	Unique peptides
			region, transcription factor complex, cytosol		
A0A2J6MFR3_LACSA	tr|A0A2J6MFR3|A0A2J6MFR3_LACSA	RuBisCO_large domain-containing protein	Chloroplast	12	5
A0A2J6JJY7_LACSA	tr|A0A2J6JJY7|A0A2J6JJY7_LACSA	Malate dehydrogenase	Integral component of membrane	11	1
A0A2J6JKZ8_LACSA	tr|A0A2J6JKZ8|A0A2J6JKZ8_LACSA	HATPase_c domain-containing protein	Choroplast, endoplasmic reticulum, perinuclear region of cytoplasm	19	1
A0A2J6JRK6_LACSA	tr|A0A2J6JRK6|A0A2J6JRK6_LACSA	Heat shock 70 kDa protein. Mitochondrial*	Mithocondrion, vacuolar membrane, cytoplasm	26	1
A0A2J6JSS3_LACSA	tr|A0A2J6JSS3|A0A2J6JSS3_LACSA	ATP synthase subunit beta	Chloroplast thylakoid membrane mitochondrial proton-transporting ATP synthase complex, proton-transporting ATP synthase complex, catalytic core F(1)	6	1
A0A2J6K617_LACSA	tr|A0A2J6K617|A0A2J6K617_LACSA	Aquaporin PIP2–2*	Nucleus, vacuole, plasma membran, plasmodesma, integral component of membrane, protein-containing complex	7	2

Accession number	SeqName	Protein name	GO cellular component	Peptide count	Unique peptides
A0A2J6L1W8_LACSA	tr|A0A2J6L1W8|A0A2J6L1W8_LACSA	Cytochrome P450 89A9*	Integral component of membrane	3	1
A0A2J6L8C5_LACSA	tr|A0A2J6L8C5|A0A2J6L8C5_LACSA	Chlorophyll a-b binding protein, chloroplastic	Photosystem I, photosystem II antenna complex, chloroplast thykaloid membrane, plastoglobule	16	2
A0A2J6L8J8_LACSA	tr|A0A2J6L8J8|A0A2J6L8J8_LACSA	Pyruvate dehydrogenase E1 component subunit beta	Mitochondrial matrix, chloroplast stroma, plastid pyruvate dehydrogenase complex	6	1
A0A2J6LBI7_LACSA	tr|A0A2J6LBI7|A0A2J6LBI7_LACSA	Fructose-bisphosphate aldolase	Mitochondrion, cytosol, plasmodesma, chloroplast thylakoid, chloroplast envelope, plastoglobule, protein-containing complex	35	20
A0A2J6LKG3_LACSA	tr|A0A2J6LKG3|A0A2J6LKG3_LACSA	Sulphite reductase 1*	Chloroplast nucleoid, chloroplast stroma, sulphite reductase complex (NADPH)	20	8
A0A2J6LRA7_LACSA	tr|A0A2J6LRA7|A0A2J6LRA7_LACSA	Mitochondrial outer membrane protein porin 2*	Cell wall, nucleolus, vacuolar membrane, plasma membrane, mithocondrial outer membrane	10	1

Accession number	SeqName	Protein name	GO cellular component	Peptide count	Unique peptides
A0A2J6LWI3_LACSA	tr|A0A2J6LWI3|A0A2J6LWI3_LACSA	Cytochrome P450 704C1*	Vacuole, endoplasmic reticulum, endoplasmic reticulum membrane, plastid	12	3
A0A2J6M9H3_LACSA	tr|A0A2J6M9H3|A0A2J6M9H3_LACSA	Thioredoxin reductase	Cell, mitochondrial intermembrane space, mitochondrial matrix, cytosol, chloroplast envelope	6	3
A0A2J6MB66_LACSA	tr|A0A2J6MB66|A0A2J6MB66_LACSA	Cytochrome c domain-containing protein	Mitochondrial respiratory chain complex III, vacuolar membrane, cytosol, integral component of membrane	8	1
A0A2J6MBG4_LACSA	tr|A0A2J6MBG4|A0A2J6MBG4_LACSA	MADS-box transcription factor 18*	Nucleus	2	2
Q9SEC1_LACSA	tr|Q9SEC1|Q9SEC1_LACSA	Cytosolic fructose-1,6-bisphosphate (Fragment)	Nucleoplasm, cytosol, plasma membrane	10	1
A0A2J6JWH7_LACSA	tr|A0A2J6JWH7|A0A2J6JWH7_LACSA	FBPase domain-containing protein	Nucleoplasm, cytosol, plasma membrane	16	6

Table 3. Abundance of identified protein species in roots and leaves contaminated with CBZ. Legend: MTF: Metformin, MFC: Max Fold Change, which reveals the trend observed between contamination and control roots and leaves for each protein species, Control mean: mean of protein species abundance for replicates of control; CBZ mean: mean of protein species abundance for replicates of CBZ.

Accession number	SeqName	Protein name	GO cellular component	Peptide count	Uniqu peptic
Carbamazepine: proteins abundance in Roots					

Accession number	SeqName	Protein name	GO cellular component	Peptide count	Uniqu peptic
ACCD_LACSA	sp|Q332W9|ACCD_LACSA	Acetyl-coenzyme A carboxylase carboxyl transferase subunit beta, chloroplastic	Chloroplast stroma	8	1
A0A1V0JFJ8_9ASTR	tr|A0A1V0JFJ8|A0A1V0JFJ8_9ASTR	Glutamine synthetase	Cell wall, mitochondrion, plasma membrane, plasmodesma, apoplast	16	6
A0A2J6JKN6_LACSA	tr|A0A2J6JKN6|A0A2J6JKN6_LACSA	Ferredoxin–nitrite reductase*	Mitochondrion, cytosol, chloroplast stroma, apoplast	25	7
A0A2J6JNJ9_LACSA	tr|A0A2J6JNJ9|A0A2J6JNJ9_LACSA	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A beta isoform	Cytoplasm, protein phosphatase type 2A complex, protein serine/threonine phosphatase complex	2	2
A0A2J6JWG1_LACSA	tr|A0A2J6JWG1|A0A2J6JWG1_LACSA	FAD-binding FR-type domain-containing protein	Integral component of membrane	7	1
A0A2J6KGI4_LACSA	tr|A0A2J6KGI4|A0A2J6KGI4_LACSA	NOT2_3_5 domain-containing protein	CCR4-NOT core complex	4	1
A0A2J6KWR2_LACSA	tr|A0A2J6KWR2|A0A2J6KWR2_LACSA	TITAN-like protein	Nucleus	12	1
A0A2J6L401_LACSA	tr|A0A2J6L401|A0A2J6L401_LACSA	Phytocyanin domain-containing protein	Plasma membrane, integral component of membrane	3	1
A0A2J6L551_LACSA	tr|A0A2J6L551|A0A2J6L551_LACSA	Probable aquaporin PIP2–2*	Membrane, membrane protein	6	2
A0A2J6L5S6_LACSA	tr|A0A2J6L5S6|A0A2J6L5S6_LACSA	Epimerase domain-containing protein	Integral component of membrane	9	1

Accession number	SeqName	Protein name	GO cellular component	Peptide count	Uniqu peptic
A0A2J6LR00_LACSA	tr|A0A2J6LR00|A0A2J6LR00_LACSA	FAD-binding PCMH-type domain-containing protein	Endoplasmic reticulum, plant-type cell wall, plasmodesma	11	1
A0A2J6M2H0_LACSA	tr|A0A2J6M2H0|A0A2J6M2H0_LACSA	Aquaporin PIP1–2*	Plasma membrane, integral component of membrane	6	1
A0A0K0M234_9ASTR	tr|A0A0K0M234|A0A0K0M234_9ASTR	ATP synthase subunit alpha	Plastid, proton-transporting ATP synthase complex, catalytic core F(1)	15	1
A0A2J6JJ32_LACSA	tr|A0A2J6JJ32|A0A2J6JJ32_LACSA	HATPase_c domain-containing protein	Chloroplast, endoplasmic reticulum, perinuclear region of cytopasm	15	4
A0A2J6JJ68_LACSA	tr|A0A2J6JJ68|A0A2J6JJ68_LACSA	Peroxidase	Extracellular region or secreted	26	3
A0A2J6JSR2_LACSA	tr|A0A2J6JSR2|A0A2J6JSR2_LACSA	Peroxidase	Cell wall, plant-type cell wall, plasmodesma	16	1
A0A2J6JVK2_LACSA	tr|A0A2J6JVK2|A0A2J6JVK2_LACSA	Heat shock 70 kDa protein 16*	Cytosol, nucleus	19	4
A0A2J6JY36_LACSA	tr|A0A2J6JY36|A0A2J6JY36_LACSA	Glucose-6-phosphate 1-dehydrogenase	Nucleus, peroxisome, cytosol, chloroplast stroma, cytoplasmic side of plasma membrane	23	1
A0A2J6JZN8_LACSA	tr|A0A2J6JZN8|A0A2J6JZN8_LACSA	Cytochrome P450 81Q32*	Endoplasmic reticulum, membrane	10	2
A0A2J6JZS6_LACSA	tr|A0A2J6JZS6|A0A2J6JZS6_LACSA	Beta-galactosidase	Extracellular region or	7	2

Accession number	SeqName	Protein name	GO cellular component	Peptide count	Uniqu peptic
			secreted, apoplast		
A0A2J6K6E5_LACSA	tr|A0A2J6K6E5|A0A2J6K6E5_LACSA	PPM-type phosphatase domain-containing protein	Cytosol, plasma membrane, intracellular membrane-bounded organelle	11	1
A0A2J6K758_LACSA	tr|A0A2J6K758|A0A2J6K758_LACSA	Xanthotoxin 5-hydroxylase CYP82C4*	Membrane, membrane protein	9	1
A0A2J6K7D2_LACSA	tr|A0A2J6K7D2|A0A2J6K7D2_LACSA	Alpha-galactosidase	Cell wall, plant-type cell wall	4	1
A0A2J6KAN3_LACSA	tr|A0A2J6KAN3|A0A2J6KAN3_LACSA	Superoxide dismutase	Mitochondrion	4	2
A0A2J6KD90_LACSA	tr|A0A2J6KD90|A0A2J6KD90_LACSA	Heat shock 70 kDa protein 1*	Cytoplasm	14	1
A0A2J6KP89_LACSA	tr|A0A2J6KP89|A0A2J6KP89_LACSA	Ultraviolet-B receptor UVR8*	Nucleus, cytoplasm, microtubule organizing center	16	1
A0A2J6KV41_LACSA	tr|A0A2J6KV41|A0A2J6KV41_LACSA	Probable serine/threonine protein kinase IREH1*	Cytosol, plasma membrane, root hair	28	2
A0A2J6L7K5_LACSA	tr|A0A2J6L7K5|A0A2J6L7K5_LACSA	Isocitrate dehydrogenase [NAD] subunit, mitochondrial	Mitochondrion	16	4
A0A2J6L8E9_LACSA	tr|A0A2J6L8E9|A0A2J6L8E9_LACSA	ATPase_AAA_core domain-containing protein	Cell wall, nucleus, chloroplast thylakoid membrane, chloroplast envelope, apoplast	7	1
A0A2J6LNF5_LACSA	tr|A0A2J6LNF5|A0A2J6LNF5_LACSA	Serine/threonine-protein phosphatase	Nucleus	16	6

Accession number	SeqName	Protein name	GO cellular component	Peptide count	Uniqu peptic
A0A2J6MA69_LACSA	tr|A0A2J6MA69|A0A2J6MA69_LACSA	Serine/threonine-protein phosphatase	Nucleus	18	1
A0A2J6MBU6_LACSA	tr|A0A2J6M5I7|A0A2J6M5I7_LACSA	Protein disulfide isomerase-like 1–6	Endoplasmic reticulum	6	1
Carbamazepine: proteins abundance in Leaves					
A0A2J6JKN6_LACSA	tr|A0A2J6JKN6|A0A2J6JKN6_LACSA	Ferredoxin–nitrite reductase. Chloroplastic*	Mitochondrion, cytosol, chloroplast stroma, apoplast	30	12
A0A2J6JXX4_LACSA	tr|A0A2J6JXX4|A0A2J6JXX4_LACSA	Pyruvate dehydrogenase E1 component subunit beta	Nucleoplasm, vacuolar membrane, Golgi apparatus, cytosol, mitochondrial pyruvate dehydrogenase complex	8	3
A0A2J6JZV2_LACSA	tr|A0A2J6JZV2|A0A2J6JZV2_LACSA	Ribosomal protein L19	Cytosolic large ribosomal subunit	18	3
A0A2J6K7W1_LACSA	tr|A0A2J6K7W1|A0A2J6K7W1_LACSA	Heat shock cognate 70 kDa protein 2*	Extracellular region, cell wall, vacuolar membrane; Golgi apparatus, plasma membrane	21	1
A0A2J6KH56_LACSA	tr|A0A2J6KH56|A0A2J6KH56_LACSA	Malate dehydrogenase	Mitochondrial matrix, cytosol, chloroplast	23	5
A0A2J6LDE7_LACSA	tr|A0A2J6LDE7|A0A2J6LDE7_LACSA	DNA helicase	Nucleus, MCM complex	15	4
A0A2J6LLM6_LACSA	tr|A0A2J6LLM6|A0A2J6LLM6_LACSA	Pyruvate dehydrogenase E1 component subunit alpha-3. chloroplastic*	Nucleus, cytosol, plasma membrane	19	4
A0A0F6VYH0_ULVLA	tr|A0A0F6VYH0|A0A0F6VYH0_ULVLA	Ribulose bisphosphate	Chloroplast	24	2

Accession number	SeqName	Protein name	GO cellular component	Peptide count	Uniqu peptic
		carboxylase large chain (Fragment)			
A0A2J6JGI3_LACSA	tr|A0A2J6JGI3|A0A2J6JGI3_LACSA	Helicase ATP-binding domain-containing protein	Mitochondrial outer membrane, peroxisome	9	1
A0A2J6JKZ1_LACSA	tr|A0A2J6JKZ1|A0A2J6JKZ1_LACSA	Chlorophyll a-b binding protein, chloroplastic	Vacuolar membrane, photosystem I, photosystem II;, chloroplast envelope, chloroplast thykaloid membrane, plastoglobule	13	15
A0A2J6JVT9_LACSA	tr|A0A2J6JVT9|A0A2J6JVT9_LACSA	L-ascorbate oxidase homolog	Vacuolar membrane, plant-type cell wall, plasmodesma, apoplast	10	5
A0A2J6JXU8_LACSA	tr|A0A2J6JXU8|A0A2J6JXU8_LACSA	PKS_ER domain-containing protein	Mitochondrion, cytosol, membrane, cytoplasm	9	1
A0A2J6K3X3_LACSA	tr|A0A2J6K3X3|A0A2J6K3X3_LACSA	SCP domain-containing protein	Extracellular space, cytoplasm	7	2
A0A2J6KG99_LACSA	tr|A0A2J6KG99|A0A2J6KG99_LACSA	Glutathione reductase	Cell, mitochondrion, cytosol, chloroplast stroma, cytoplasm	19	7
A0A2J6KYJ5_LACSA	tr|A0A2J6KYJ5|A0A2J6KYJ5_LACSA	Chlorophyll a-b binding protein, chloroplastic	Photosystem II, photosystem I, chloroplast thylakoid membrane	3	1
A0A2J6L8H4_LACSA	tr|A0A2J6L8H4|A0A2J6L8H4_LACSA	Chlorophyll a-b binding protein, chloroplastic	Photosystem I, photosystem II, chloroplast thylakoid membrane,	20	2

Accession number	SeqName	Protein name	GO cellular component	Peptide count	Uniqu peptic
			chloroplast envelope, plastoglobule		

In Table 1(a,b), the most relevant proteins in relation to our study are presented in roots and leaves when subjected to MTF contamination. It is possible to identify some proteins of interest in roots, such as Ras-related protein Rab11C, isocitrate dehydrogenase (IDH) and heat shock 70 kDa protein 3 (HSPs). The last two revealed higher normalized abundance in control roots. On the other hand, the leaves showed differentially accumulated protein species such as histones H2A and H2B, and photosystems II protein D1 (highest abundance in the control), while sulfurtransferase, aquaporin PIP1, pectinesterase, ATP synthase subunit beta and rbsL (RuBisCo) were more abundant in MTF contaminated leaves. In total, only 8 protein species were identified as significantly different in roots and 32 protein species in leaves, revealing different trends in stress response to MTF. The contamination with ACT revealed differences between roots and leaves tissues. A total of 47 protein species were identified with significant differences concerning the roots of Control and ACT conditions. Once again, different protein species were selected as being of interest to our study. In the case of roots, differentially accumulated protein species include proteasome subunit beta, GLOBIN domain-containing protein and others such as aquaporin PIP1-2, ribosomal_S13_N domain-containing protein, cytochrome *c* domain-containing protein, -succinate-CoA ligase and UDP-glucose 6-dehydrogenase. BZIP domain-containing protein, ABC transporter C family member 5, DUF3591 domain-containing protein and Hsc70 protein prevail in ACT contamination over control (Table 2). In leaves, among those 17 protein species (Table 2), some proteins of interest such as malate dehydrogenase, diverse cytochrome P450 proteins, HSPs, aquaporin PIP2–2 or thioredoxin reductase showed statistically higher abundance in ACT than control.

In terms of number of identified protein species (statistically significant), CBZ was the contaminant that most affected roots protein species abundance. Indeed, 181 protein species revealed significant differences between the control and CBZ conditions. Data regarding the number of differentially accumulated protein species are presented in S1. On the other hand, in leaves only 43 protein species were

identified. Several HSPs and peroxidases, as well as IDH [NAD] subunit and SOD were identified as differentially accumulated in roots under CBZ. All protein species were linked to oxidative stress response. In leaves, several protein species revealed higher abundance in leaves under CBZ contamination within a range of MFC between 1.5 and 36.3. Among them, chlorophyll *a*-*b* binding proteins, ruBisCo large chain, glutathione reductase (GR) and ascorbate L-oxidase were more abundant.

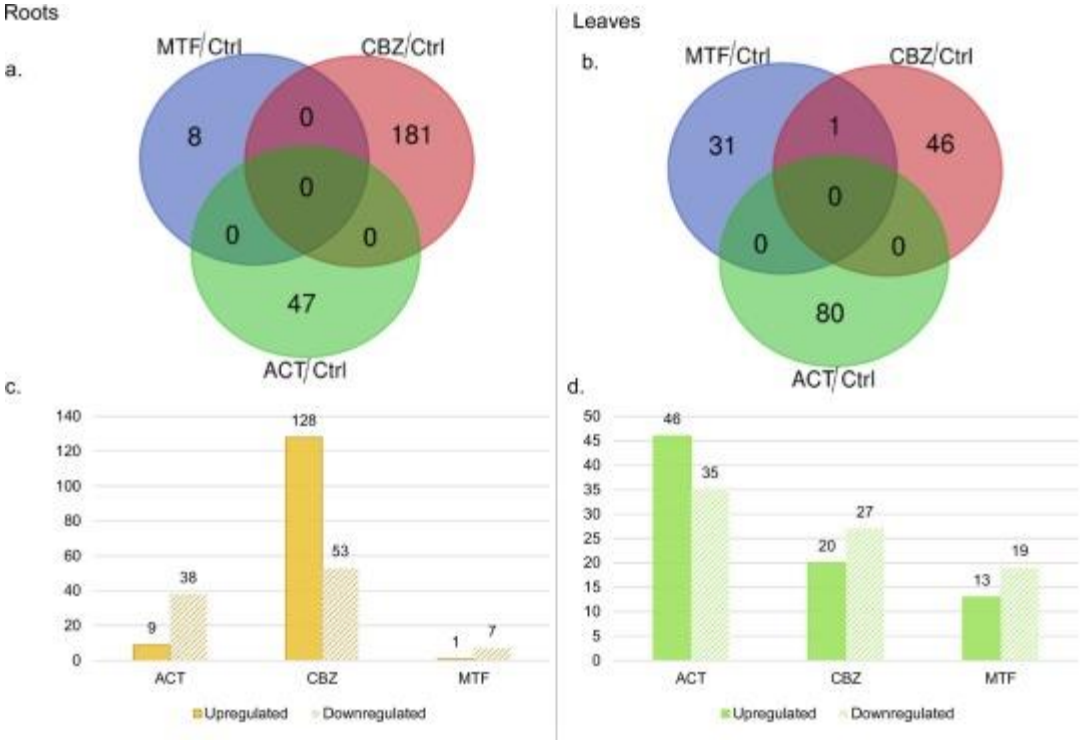
In all contaminations, it is possible to state that differences between tissue responses were observed. Roots revealed (in general) less differences by comparison to control. Nevertheless, in both tissues, HSPs, IDH and other protein species related with stress response showed to have higher levels of accumulation in contaminated plants, showing that these contaminants may induce oxidative stress. The comparison between roots and leaves will be furthered discussed. Protein species previously mentioned were grouped according to the different metabolic pathways. According to the data from Table 1, Table 2, Table 3, several aquaporins and protein species related with cell respiration were differentially accumulated in both tissues. However in some cases, the pattern observed in abundance is different, e.g. aquaporins were found to be more abundant in roots of control group by comparison to all the other groups and similar roles may be inferred. In the case of ACT and CBZ contamination, lower abundance was observed for aquaporins PIP1-1 (ACT and CBZ) and PIP1-2 (CBZ). However, in leaves, different abundances (higher abundance in contaminated groups) were observed. Also, detoxification protein species related with cytochrome P450 showed higher abundance in roots under CBZ, while in leaves this behaviour was detected in ACT and MTF contaminations as well. Therefore, roots and leaves show different responses to the abiotic stress caused by these three pharmaceuticals.

3.2. Protein profile characterization

The proteomic characterization and identification of specific protein species in plant samples allows a better understanding of the metabolic processes according to applied contaminants. The list of identified protein species is presented in Supplementary data (S2). In our study the number of identified protein species varied among tissues and among conditions. In detail, a total of 1240 and 919 protein species were identified for

roots and leaves, respectively. Of these, 639 protein species for roots and 237 protein species for leaves were found to have differential accumulation. To perform a comparative analysis, the three contaminants were compared to the control taking into account the normalized abundance values.

These comparisons were established in order to characterize the contamination influence on the proteome. Fig. 2a, b shows that some protein species were expressed only in certain conditions in both tissues, and it is possible to observe that for each contaminant studied (MTF, ACT, CBZ), a different number of protein species were obtained. This suggests that different contaminations affect tissues possibly in different pathways. Regarding the influence of the contaminant on lettuce tissues, Fig. 2c, d shows the number of protein species with higher and lower normalized abundance when compared to control plants. Overall, Fig. 2 shows that CBZ was the pharmaceutical that most influenced protein species abundance in roots, while in leaves, ACT had the higher number of more abundant protein species in comparison to controls.

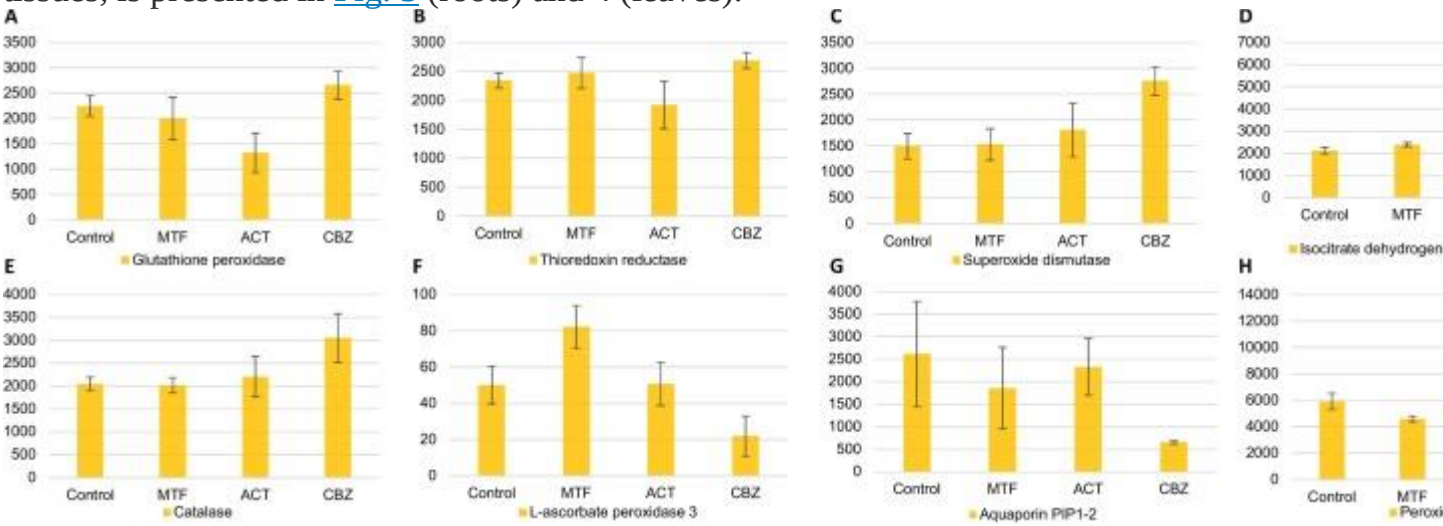


1. Download : Download high-res image (262KB)
2. Download : Download full-size image

Fig. 2. Venn diagram (top) for roots (a) and leaves (b) of differentially significant proteins species for each contamination VS compared to Control, obtained

from Bioinformatic and Evolutionary Genomics tool (http://bioinformatics.psb.ugent.be/webtools/Venn/). Bar figure (down) with number of proteins with higher and lower abundance (down) in roots (c) and leaves (d) contrasting each contamination with the control condition.

Considering the main objective of our study, some particular protein species related with stress response were selected. The mean abundance between conditions, in both tissues, is presented in Fig. 3 (roots) and 4 (leaves).



1. Download : Download high-res image (340KB)
2. Download : Download full-size image

Fig. 3. Differential abundance (y-value) of some proteins species (potential stress-related) identified as proteins of interest, in roots of different conditions. Legend: A – Glutathione peroxidase, B – Thioredoxin reductase, C – Superoxide dismutase, D - Isocitrate dehydrogenase, E – Catalase, F – L-ascorbate peroxidase 3, G – Aquaporin PIP1-2, H – Peroxidase.

Principal Component Analysis (PCA) is represented for both roots and leaves in Fig. 5 including all the experimental conditions and different patterns were observed for the two tissues. In roots, CBZ group showed a clear differentiation regarding the other contaminations and the control, which may be also observed in the case of the abundance of different protein species (Fig. 2c, d). MTF contamination was the one that showed more similarity to the controls as may be observed in the PCA, indicating that MTF does not influence as much the protein species abundance when compared to controls as the other two contaminants. In contrast, for leaves, the PCA clearly indicates a clear differentiation between protein species abundances in control and contaminated plants.

In order to provide an overview of protein species abundance in several condition,s Fig. 6a, b shows a hierarchical cluster analysis and heatmap of the protein species with differences in abundance in studied conditions, in both leaves and roots, in direct comparison to control.
In roots, it is possible to observe a clear differentiation between CBZ contamination and the other contaminants. In leaves, this type of differentiation occurs between control and contaminated conditions in general. The difference between contaminants is not as evident in leaves as it is in roots.

3.3. Molecular and biological functions analysis

Fig. 7 shows the different molecular functions of the differentially accumulated protein species between roots and leaves for each condition against control. Differences in molecular functions were detected between tissues and contaminations. In all contaminations, binding and catalytic activity are the two main MF, independently of contamination and tissue. The number of protein species related with catalytic activity remained higher in roots (80%) and leaves (42%) under CBZ contamination as well as in roots (80%) under MTF contamination. Yet, the first one may be associated with high number of differentially accumulated protein species observed in CBZ group, while in case of MTF group the 80% of catalytic activity may be related to the low number of differentially expressed protein species in roots. In ACT group, a distribution between functions was observed, the more frequent being binding (35%), catalytic activity (46%) and transporter activity (12%) in roots, while in leaves the main molecular functions were binding (38%), catalytic activity (26%) and translation regulator activity (34%).
In Fig. 8, the protein species differentially expressed in relation to the control are classified according to the biological processes (BP), both in leaves and in roots. Regarding BP grouping it is important to highlight that some protein species related with response to stimulus were detected in plant under CBZ (30%, in roots) and MTF (20%, in roots). In ACT contamination plants showed similar values of cellular and metabolic processes in both tissues.

4. Discussion

This proteomics-based approach contributes to an overall understanding of the MTF, ACT and CBZ effect in the metabolism of lettuce helping to identify the biological processes that are more affected by the induced stress. Therefore, a special emphasis is placed on stress related protein species in order to ascertain the toxic effects of the different compounds. A summary of the main changes induced in lettuce cells is present in Fig. 9, according to tissue and contaminant. As stress related protein species are the main focus of our study, protein species related with ROS scavenging and cell oxidative stress were preferentially selected according to abundance in different contaminations. Literature regarding lettuce proteomic studies was already conducted by several authors [27,[48], [49], [50], [51]], some of the works will be considered in this discussion in order to provide a broad analysis of the protein species related with other types of abiotic stress. Moreover, a previous study showed important differences in antioxidative stress response of lettuce under CBZ contamination [52].

4.1. Effects on roots

4.1.1. Stress response protein species

In the roots of plants exposed to MTF contamination, several protein species revealed higher abundance compared to control, in a pattern that was also observed for the CBZ contamination. One example is ascorbate peroxidase, an enzyme that is involved in the ascorbate-glutathione cycle and has a crucial role under oxidative stress conditions. Focusing on the proteins of interest mentioned in Fig. 3, only L-ascorbate peroxidase (APX) showed higher abundance by comparison to control, whereas the other proteins of interest (Fig. 3) remained at similar levels. This may indicate an influence on maintaining the ascorbate pools when roots are subjected to MTF contamination, as an antioxidative response. This result may influence the activity of IDH and consequently be the reason for its lower abundance in roots under MTF contamination.

Regarding ACT contamination, BZIP domain-containing protein was more abundant when compared to control. Some authors addressed its relation with responses to abiotic stress [53,54]. In our study it showed an increase in the abundance of this protein when plants were subjected to the exposure to the three pharmaceuticals. ACT was the contamination that most affected the abundance of this protein. For instance,

the abundance on MTF leaves was 2 times lower than those of leaves contaminated with ACT.

Glutamate decarboxylase (GAD) was detected in higher abundance in ACT roots and revealed a downregulation over control roots in the case of CBZ contamination. Some authors suggest that the degradation of GABA will contribute to the scavenging of ROS, preventing oxidative stress damage in cells [55]. In our study, the higher abundance of GAD will likely increase GABA contents as response to stress caused by MTF exposure. GAD is modulated by Ca^{2+}-calmodulin (CaM) complex, therefore calcium pools will influence GAD abundance or activity. Compared to other contaminants, ACT was the one that most influenced the abundance of this protein. CBZ showed a reduction of GAD production rate in cells leading to a lower abundance of GAD. This result may influence the pools of NADH and succinate available for the Krebs cycle. Indeed, succinate dependent proteins also decreased their abundance when subjected to CBZ contamination, in turn a possible consequence of alterations in GAD abundance.

Peroxidases and superoxide dismutase increased their abundance in CBZ roots when compared to control conditions. These are two of the most important protein species related to the enzymatic response to oxidative stress, controlling ROS imbalance. The increase of ROS in contaminated cells, are usually the main cause for this increase in abundance.

An overall analysis of differentially expressed stress protein species in roots, for the three contaminants highlights that the major effects are due to CBZ contamination. In terms of proteins of interest (Fig. 3) roots under CBZ showed a clear trend to increase the abundance of SOD, CAT, glutathione peroxidase (GPX) and other peroxidases, while MTF revealed more effects on APX abundance. The high contents of ROS are known to be related with SOD, CAT, POX, thioredoxin reductase (TrxR) and thioredoxin peroxidase (TrxP) so the higher abundance of these protein species in this contamination may be related to the activation of some ROS scavenging enzymes. Indeed significant differences in lettuce roots under CBZ were observed in H_2O_2 contents as well as in scavenging enzymes in previous conducted experiments [52]. However, in case of APX, a decrease in abundance was observed. It is thus clear that these contaminants act differently on plant metabolism as they induced a response involving different stress protein species.

4.1.2. Heat shock proteins

HSP 70 protein showed higher abundance in the ACT contaminated roots. This protein species is frequently associated to ROS and stress responses [56].HSPs are indeed induced by a wide range of stresses such as drought, temperature or osmotic stress, and even heavy metals or other chemicals stresses [57].
In roots under CBZ contamination, the abundance of HSPs remains higher than those of controls. Two different HSP were identified: HPS70 and HSP90 (HATPase_c domain-containing protein). In fact, these two HPS revealed higher abundance in all contaminant exposure, being CBZ the contaminant where the abundance was higher. Some authors studied the effects of phytohormones and heavy metals in HSP90 revealing that this group of proteins may respond to this type of stress [58]. Therefore, it is possible to assume that CBZ may have induced the abundance of HSPs, similarly to other contaminants.

4.1.3. Aquaporins

Aquaporins are integral membrane proteins responsible for the formation of water-selective channels across the membrane. However, some of these protein species may act as H_2O_2 transporters and be affected by different types of abiotic stresses.
The higher abundance of these protein species in roots of control plants indicates a good regulation of water transport through the membrane in comparison to roots exposed to contaminants. These protein species are considered to be crucial for the water permeability of the plasma membrane and for the root system morphology [59]. Aquaporins were found to be more abundant in roots of control group by comparison to all the other groups and similar roles may be inferred. In the case of ACT and CBZ contamination, a decrease was observed for Aquaporins PIP1-1 (ACT and CBZ) and PIP1-2 (CBZ), the latter shown in Fig. 3. These results may be related with possible protein denaturation in contaminated roots. In general, all aquaporins identified in our study were less abundant in contaminated roots suggesting there is interference disruption of the different pharmaceuticals on water transport process. This outcome will induce negative effects on water transport into cells and possibly affect or denature important proteins, mainly related with osmotic state, composition and integrity of cell walls or lignin biosynthesis catalysed by peroxidases.

4.1.4. Detoxification proteins

In roots under CBZ contamination, cytochrome P450 81Q32 and cytochrome P450 82C4 showed significant increase in abundance in comparison to control. Proteins from cytochrome P450 complex are frequently associated with xenobiotics detoxification or transport [60]. Therefore, CBZ influenced the abundance of cytochrome P450 complex in roots.
ABC transporters were identified in higher abundance on ACT contaminated roots. These protein species were identified as transporters involved in detoxification processes and are required for plant development and response to abiotic stresses [61]. Also, ABC transporter C family member 5 participated in ATPase-coupled xenobiotic transmembrane transporter activity as molecular function and xenobiotic detoxification by transmembrane export across the plasma membrane and response to stress as biological processes. Conversely, the other contaminants had accumulation patterns for this protein species similar to those found on ACT roots, revealing higher abundances values than those of control roots. Thus, the abundance of ABC transporters may be related to xenobiotic transport into the vacuole.

4.1.5. Secondary metabolism related proteins

Cinnamyl alcohol dehydrogenase was more abundant in CBZ roots when compared to control. This protein species is involved in the phenylpropanoids metabolism. Usually, phenylpropanoids are associated with stress conditions as reported [62,63], suggesting that CBZ may interfere with the plant's secondary metabolism.
4-coumarate-CoA ligase protein was also more abundant in CBZ roots, revealing lower abundance on control and other contaminated roots. So, a closer relation between CBZ and secondary metabolism may be established. This protein species characterized by phenylpropanoid and auxin biosynthetic biological processes will induce a different response from roots under CBZ contamination. Moreover, phenylalanine ammonia-lyase (PAL) showed higher abundance in roots under CBZ contamination over control. The activity of PAL is known to increase according to environmental factors such as oxidative stress [63]. Consequently, abiotic stress caused by CBZ may induce changes on phenylpropanoids biosynthesis. Additionally,

the lettuce variety of our experiments is a red leaf variety which may present higher antioxidative response involving secondary metabolism compounds.

4.1.6. Cell respiration proteins

Different protein species from the mitochondrial electron transport such as cytochrome *c* biological process were identified as differentially expressed. Cytochrome b-c1 complex subunit Rieske-2, mitochondrial (complex III) was identified in higher abundance in ACT and in lower abundance in CBZ (Supplementary material 2). These results may suggest that CBZ may interfere with redox reaction on ubiquinone, since it is frequently addressed as a marker for oxidative stress. This may result in some negative impact on electron transport chain, affecting mitochondrial metabolism. However, results regarding protein species from cytochrome complexes are different according to the b-type, as in the case of NADH-cytochrome *b*5 reductase 1 (complex III as well), the lower abundances were found for CBZ group, following the same trend as described above and also for the ACT group.

The cytochrome *c* domain-containing protein called complex IV, also known as cytochrome *c* oxidase, is involved in the latter stages of the electron transport chain. In our study, this protein species showed lower abundance in roots contaminated with ACT in comparison to controls. Cytochrome-c is a peripheral protein involved in the transfer of electrons between complexes III and IV in the mitochondrial electron transport chain [64]. ACT was the contaminant that most affected the abundance of cytochrome *c* domain-containing protein followed by CBZ. However, CBZ revealed similar values to control in this specific protein. Recently, Racca et al. [65] studied the effect of reducing the levels of the mitochondrial electron carrier cytochrome *c* in *Arabidopsis thaliana* treated with gibberellins studying also the interplay with cytochrome *c*. The results showed that plants with cytochrome *c* deficiency have delayed growth and development and tend to accumulate starch and glucose. This outcome may influence pentose phosphate pathway and consequently the Krebs cycle. Alterations in starch and glucose metabolism were also reported by Qin et al., [48] which described this functional pathway as included in the group of enriched pathways. Roots contaminated with ACT did not reveal damages or changes in development in our

experiment. Indeed, Complex I, II, III and IV are often mentioned as being inhibited by several compounds [64]. As plants have an alternative pathway for oxygen reduction, involving alternative oxidases, changes in this complex may not be directly related to root cell damage.

Glucose-6-phosphate 1-dehydrogenase (G6PDH) plays a role in redox balance and oxidative stress producing cytosolic NADPH [66]. It was found that G6PDH was more abundant in roots under CBZ contamination. The increase in G6PDH abundance may be related with a response to oxidative stress acting on control of excess ROS. Several authors described already similar patterns in other cultures such as rice under salt stress [67], soybeans subject to drought stress [68] and sugarcane under salt, drought, low temperature and heavy metal stresses [69]. In all of them, a correlation between cell redox balance and ROS metabolism was highlighted. Moreover, Liu et al. [68], reported a role of G6PDH in maintaining GSH and AsA pools levels regulating H_2O_2 contents in cells.

IDH [NADP] and Iso dh domain-containing protein had lower abundance in MTF and ACT respectively, by comparison to controls. Such results may indicate an abundance decrease of these protein species in contaminated roots, possibly showing that Krebs cycle and consequently the primary metabolism of the contaminated roots show significant changes in this metabolic pathway as a whole. This lower abundance was also detected in CBZ in both protein species but to a lesser extent. Some authors reported the inhibition of some metabolites from the first phases of Krebs cycle, in roots exposed to stress conditions [70]. For instance Lehmann et al. [71] showed a decrease in α-ketoglutarate, which may be a consequence of low activity of IDH, in *Arabidopsis* roots under menadione stress. This enzyme is also less abundant in MTF and ACT contamination and is correlated with the cellular ratio NADH/NADPH, which in turn affects the regulation of other metabolites such as GSH/GSSG pools and ascorbate pathway [72,73]. Thus, a possible negative effect of these contaminations may be the regulation of other metabolites responsible for the response to stress and redox homeostasis.

Under CBZ contamination lettuce roots IDH showed to be more abundant in comparison to controls. This trend was also reported by Leterrier et al. [74] in *Arabidopsis thaliana* subjected to salinity stress. Therefore, alterations in the Krebs cycle caused by CBZ may also induce some changes on oxidative

phosphorylation, glycolysis and secondary metabolism processes directly dependent of Krebs cycle sub products. This trend was only detected in CBZ contaminated roots, indicating a different effect on cell respiration processes compared to ACT and MTF. Another Krebs cycle enzyme, Succinate-CoA ligase, was also detected in lower abundance in ACT roots. Maršálová et al. [75] conducted a proteomic analysis to study the influence of salinity stress in glycophytes and halophyte plants and showed that Krebs cycle enzymes, including succinate-CoA ligase, enhanced their abundance in control plants, reinforcing that under stress conditions succinate-CoA ligase is less abundant. A similar trend was also shown in MTF and CBZ contaminations, albeit to a lower extent, indicating that the three contaminants have a similar effects on the Krebs pathway, as suggested by those authors.
Despite the presence of contaminants, roots are able to maintain a good regulation of the Krebs cycle, ensuring that the primary metabolism is not completely affected. This is probably what is supporting a certain tolerance of the plant to these pharmaceuticals.

4.1.7. Cell wall proteins

UDP-glucose 6-dehydrogenase (UGHD) is described as a key enzyme involved in the biosynthesis of the plant cell wall. In this experiment, it showed a lower abundance in ACT contaminated roots. This result may indicate an impact of the pharmaceutical on cell wall biosynthesis or even on glycosylation of proteins and lipids as suggested by Kleczkowski et al. [76].
The previously mentioned decrease of several aquaporins abundance in contaminated roots, may also have some impact on cell wall processes due to the imbalance on water transport into the cells causing changes on cell wall integrity.

Interestingly, α-galactosidase and β-galactosidase changed their abundance in roots under CBZ, showing higher values of normalized abundance in comparison to controls. In the case of α -galactosidase higher abundance in CBZ roots, other authors suggested its higher abundance when plants were subjected to abiotic stress in different tissues [77]. Similar results were reported by Gutsch et al. [78] in *Medicago sativa* L. subjected to heavy metal stress. Differences between contaminants (CBZ, MTF and ACT) were also detected in α-galactosidase abundance since CBZ remain at

least 40 times higher than the abundances presented in roots under ACT and MTF. CBZ contamination may induce changes in cell wall proteins promoting structural degradation and deficient root development. Indeed, Fig. 1 shows a strong differentiation for root length under CBZ contamination (d) when compared to control plants (a).

In the roots of MTF plants, Ras-related protein Rab11C was identified as more abundant in comparison to controls. This protein species is considered one of the most complex protein species between Rab families. In plants, Rab11 proteins were detected in Golgi bodies (pea) and endosomes (tomato) [79]. Also, Rab11 homologs have been implicated in the secretion of cell wall enzymes, in ripening tomato [80].

4.1.8. Nutrients assimilation and nitrogen metabolism proteins

Enzymes such as glutamine synthase and ferredoxin-nitrite reductase were more abundant in control roots in comparison to CBZ. In general, MTF and ACT also affected the abundance of these protein species, however CBZ was the contamination that most affected the abundance levels (lower mean). The lower abundance of glutamine synthase found may indicate a poorer regulation in contaminated roots. It is however important to state that the presence of proteins is not always necessarily linked to higher activities. Other studies have reported a decrease in glutamine synthase activity when rice was subjected to cadmium contamination [81].

Ferredoxins have been reported to be less abundant in plants when environmental stress occurs [82]. In our case, the same trend was observed, with lower abundance of ferredoxin-nitrite reductase in CBZ roots. Lodeyro et al. [82] observed that in wild type tobacco plants under stress conditions ferredoxin levels decreased and NADPH accumulated in cells leading to changes in ROS. A possible interference on ammonium assimilation may be correlated with the presence of pharmaceuticals. Consequently, for longer periods of exposure to pharmaceuticals, NH_4^+ and NO_2^- pools may suffer alterations leading to changes on plant growth and development.

4.1.9. Other proteins

ATPase_AAA proteins are responsible for protein homeostasis as defined by Yedidi et al. [83] and an increase in its abundance was observed in roots under CBZ

contamination. The higher abundance of these proteins in rice cells under salinity stress, was described by Shen et al. [84]. In accordance, our results suggest a possible interference of CBZ with protein homeostasis which induced the increase of ATPase_AAA proteins. CBZ was the contamination with which the abundance of this protein species increased the most, suggesting a clear influence on protein homeostasis.

Some DEAD-box proteins showed high differential abundance in roots of CBZ condition, with higher abundance when compared to control. These proteins were described by Tujeda et al. [85] as playing a role in stabilizing growth in plants under stress conditions throughout the regulation of some stress-induced pathways.

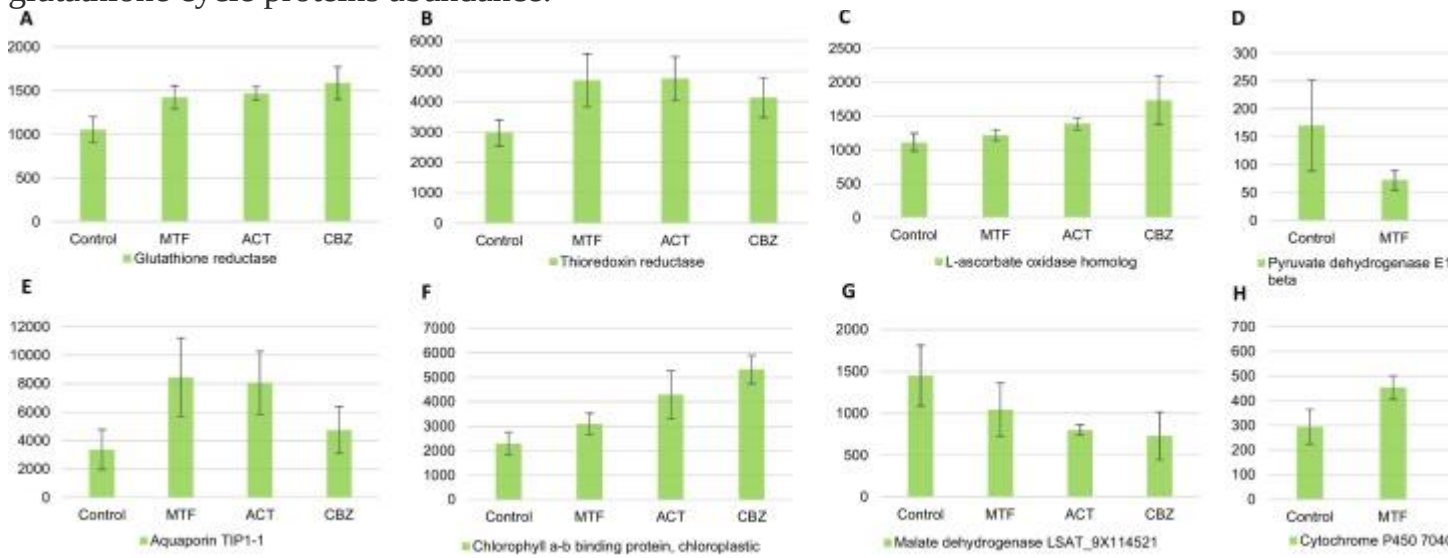
4.2. Effects on leaves

4.2.1. Stress response proteins

Different stress response protein species were identified as more abundant in contaminated leaves. It was noticed that the abundance of sulfurtransferase in leaves contaminated with ACT and CBZ was higher when compared to control leaves, suggesting a biochemical response to this type of abiotic stress. Previous studies suggest that sulfurtransferase is often involved with detoxification of ROS, cyanide and heavy metals [86,87].

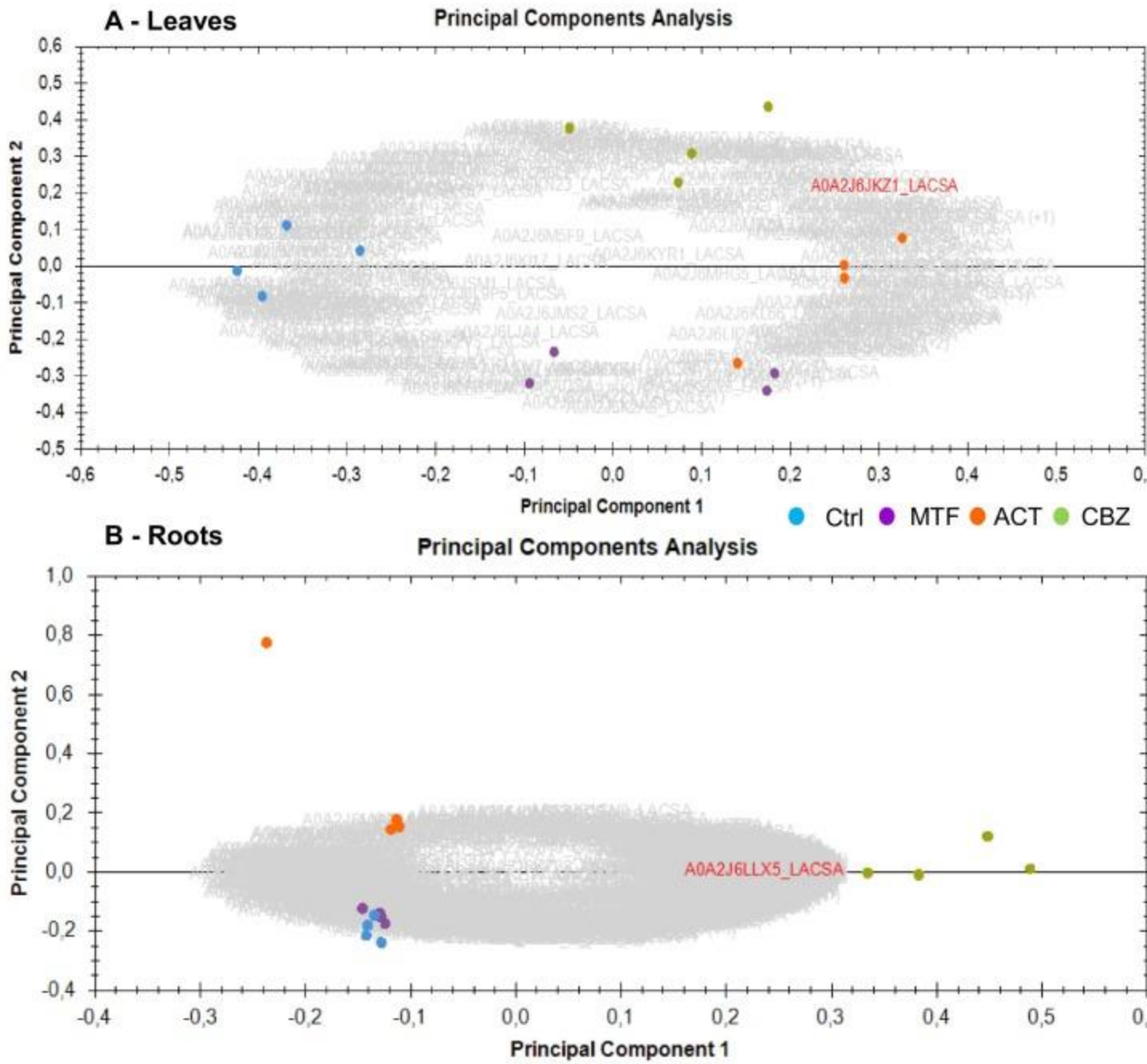
Additionally, thioredoxin reductase increased its abundance on leaves contaminated with ACT, CBZ and MTF (Fig. 4). Thioredoxins are present in different organelles (cytosol, mitochondria and chloroplast) using different substrates and playing a role in defence mechanisms against oxidative stress [88]. This protein species may be affected by the presence of ROS in cells, which in turn may be related the higher abundance of thioredoxin, often linked to role in ROS scavenging [89]. Indeed GOs identified for this protein species were removal of superoxide radicals as well as cell redox homeostasis. Additionally, this protein species will act on NADPH pools, using it as a substrate, available to the glutathione cycle and thus alterations in thioredoxin reductase may induce changes in this cycle. However, the increase of glutathione reductase abundance in contaminated leaves allows us to conclude that the consumption of NADPH triggered by thioredoxin reductase did not interfere on NADPH pools available for GR activity. Indeed, NADPH-Thioredoxin and

glutathione systems are known to play overlapping roles in physiological development, however the degree of interplay is yet not fully described [90]. Therefore NADPH-dependent thioredoxins may not reflect a direct decrease of glutathione cycle proteins abundance.



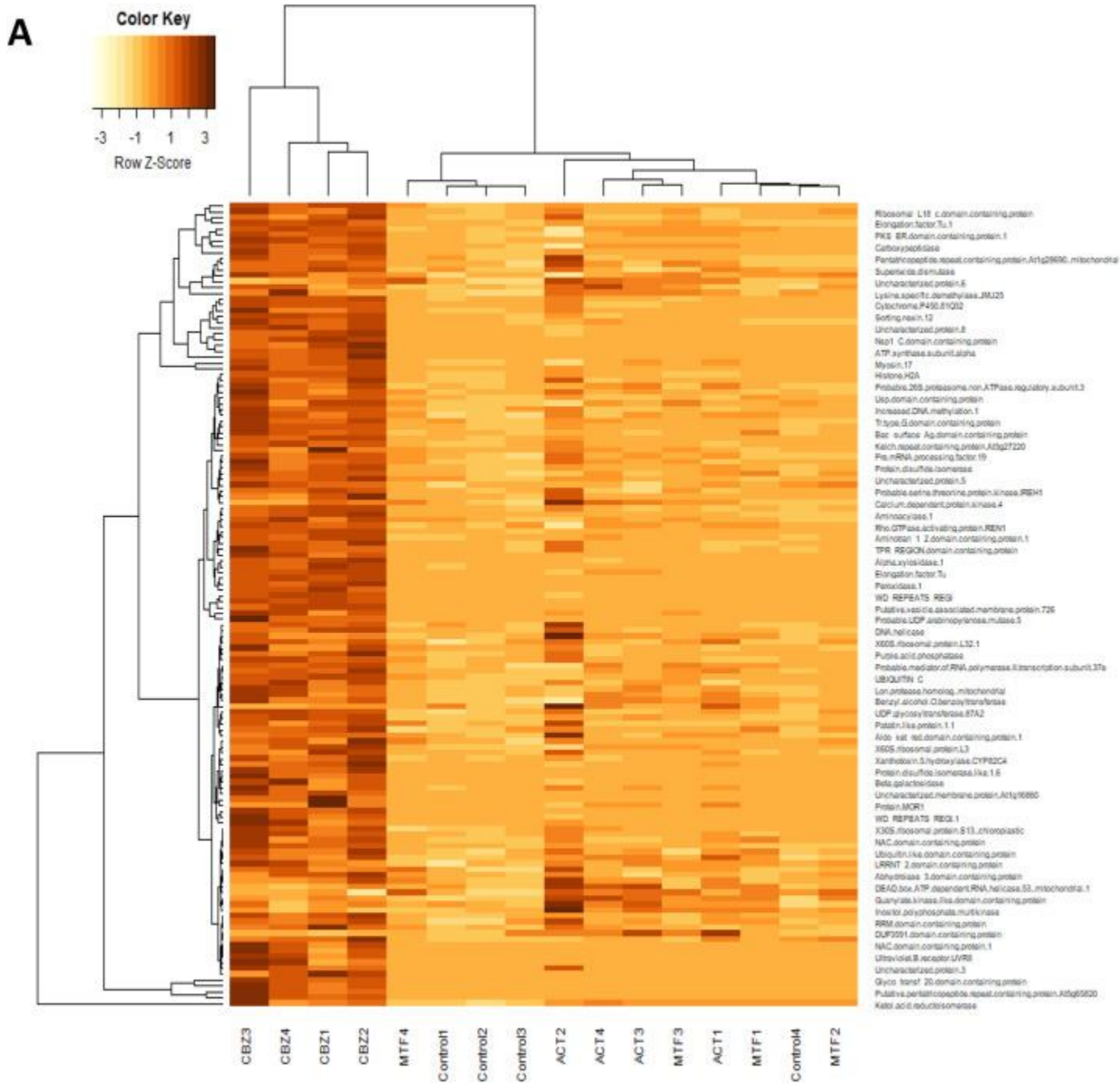
1. Download : Download high-res image (325KB)
2. Download : Download full-size image

Fig. 4. Differential abundance (y-value) of some proteins species (potential stress-related) identified as proteins of interest, in leaves under different conditions. Legend: A – Glutathione reductase abundance, B- Thioredoxin reductase, C – L-ascorbate oxidase, D- Pyruvate dehydrogenase E1 componente, E- Aquaporin TIP1-1, F – Chlorophyll a-b binding protein, G – Malate dehyrogenase, H – Cytochrome P450.

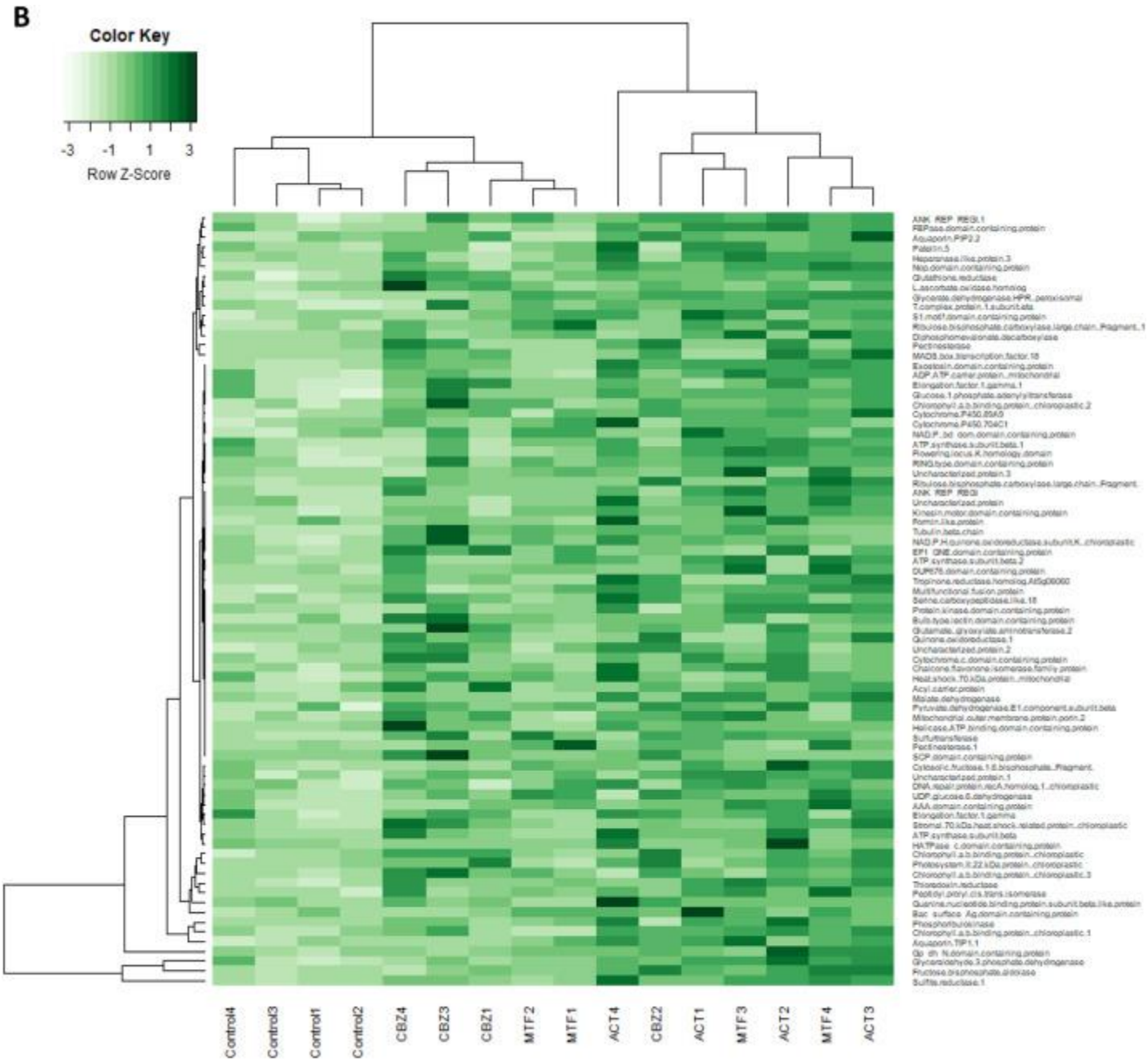


1. Download : Download high-res image (808KB)
2. Download : Download full-size image

Fig. 5. Principal Component Analysis carried out with Progenesis QIP for the lettuce leaves (A) and roots (B) for Ctrl, MTF, ACT and CBZ conditions, with the filters: ANOVA P-value < 0.05, at least 1 unique peptide, at least 2 peptides identified per protein with a fold change higher than 1.5.

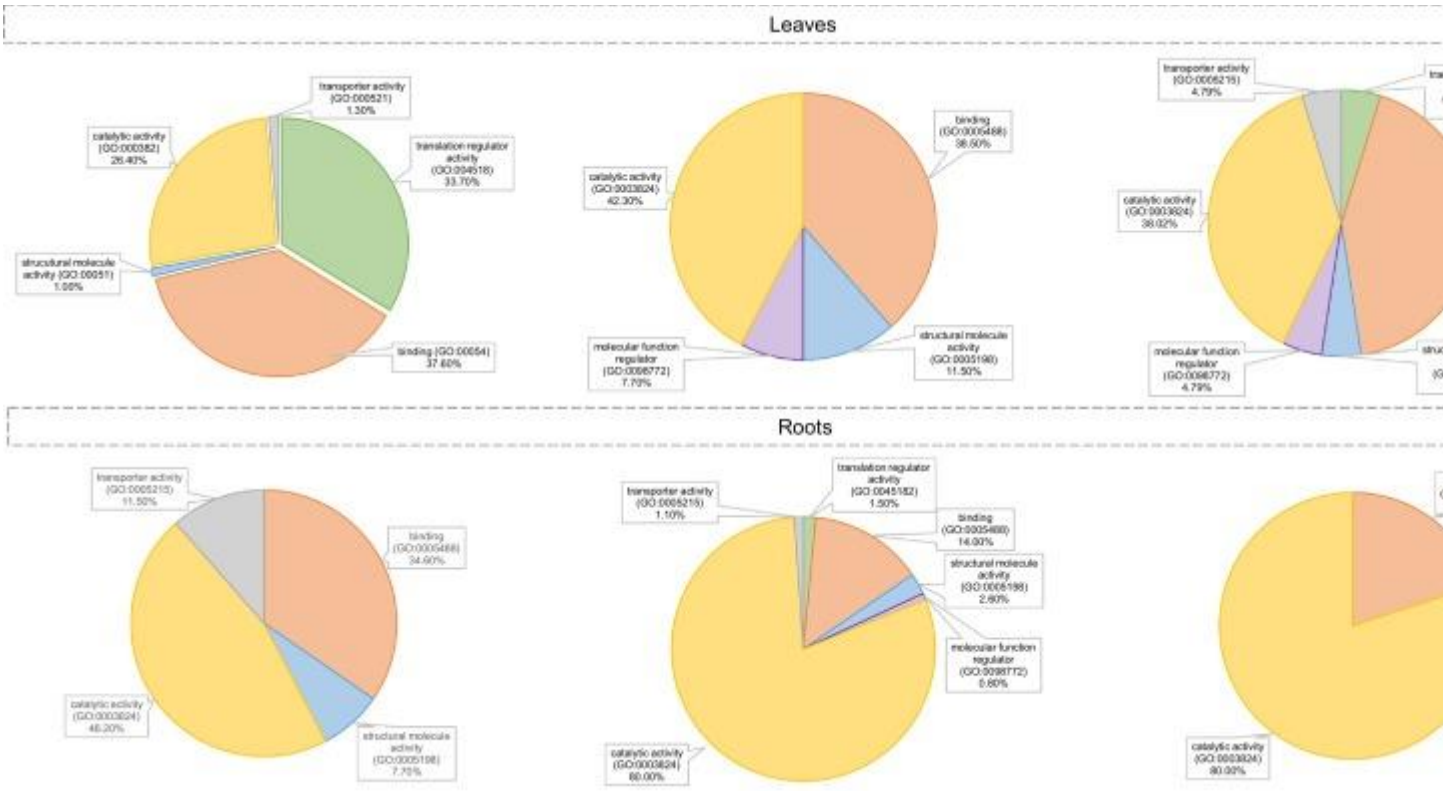


1. Download : Download high-res image (579KB)
2. Download : Download full-size image



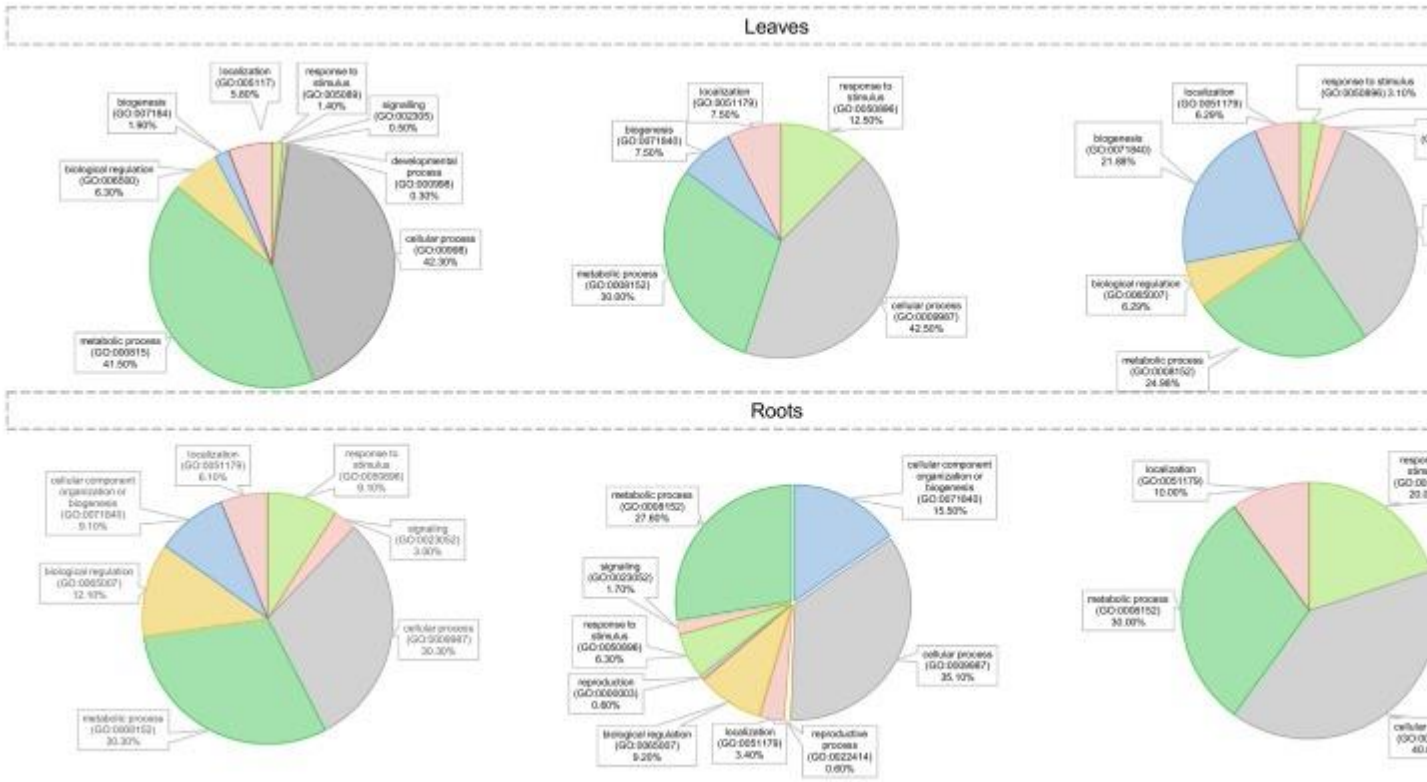
1. Download : Download high-res image (582KB)
2. Download : Download full-size image

Fig. 6. Heatmaps of differentially abundant proteins species across conditions over control. A – roots, B – leaves. Lines represent different proteins and columns are different replicates. For roots (A) dark orange indicates higher abundance, whereas light orange indicates lower abundance. For leaves (B) dark green indicates higher abundance, whereas light green indicates lower abundance. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



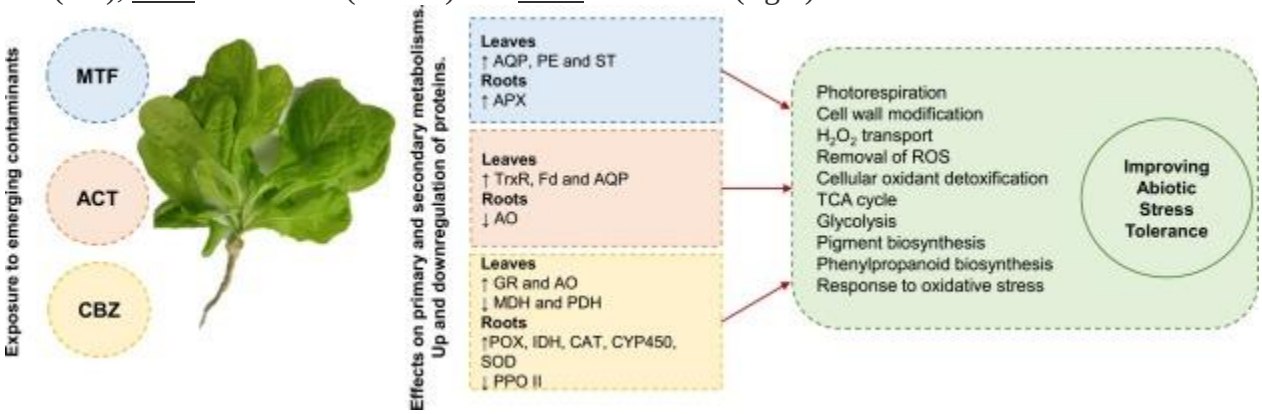
1. Download : Download high-res image (456KB)
2. Download : Download full-size image

Fig. 7. Molecular Functions analysis of three contaminations vs control for Leaves (top) and Roots (down), by performing a search in the PANTHER tool. ACT vs Control (left), CBZ vs Control (middle) and MTF vs Control (right).



1. Download : Download high-res image (525KB)
2. Download : Download full-size image

Fig. 8. Biological processes analysis of three contaminations vs control for Leaves (top) and Roots (down), by performing a search in the PANTHER tool. ACT vs Control (left), CBZ vs Control (middle) and MTF vs Control (right).



1. Download : Download high-res image (325KB)
2. Download : Download full-size image

Fig. 9. Proteins of interest and main biological processes associated to the overall response of lettuce to pharmaceuticals (MTF, ACT and CBZ). Legend: AQP – Aquaporin, PE – Pectinesterase, ST – Sulfotransferase, APX – Ascorbate Peroxidase, TrxR – Thioredoxin Reductase, Fd – Ferredoxin (or Sulphite Reductase), AO – Ascorbate oxidase, GR – Glutathione Reductase, MDH – Malate Dehydrogenase, PDH – Pyruvate Dehydrogenase, POX – Peroxidase, IDH – Isocitrate Dehydrogenase, CAT – Catalase, CYP450 – Cytochrome P450, SOD – Superoxide Dismutase, PPO II – Polyphenol oxidase II.

As mentioned before, protein species such as GR and ascorbate L-oxidase were more abundant in contaminated leaves, not only with CBZ but also with ACT contamination. The contribution of these proteins to defence mechanisms has already been described by other authors [91,92]. The activity of these protein species may interfere with ascorbate pools available for the scavenging of ROS by ascorbate peroxidase [93]. The regeneration of the reduced form of ascorbate may occur throughout the ascorbate-glutathione cycle [94]. Indeed, the higher content of GR reflects this mechanism in lettuce leaves under CBZ, ACT and MTF contamination. The increase in normalized abundance of protein species related with sulphur assimilation was observed, which may reinforce an impact in glutathione cycle regulation. In the case of L-ascorbate oxidase the major differences in comparison control were observed in CBZ contamination. This supports the possibility of a CBZ effect on the ascorbate pools in leaves, as a response to stress.

4.2.2. Heat shock proteins

Similarly to roots, two different HSPs (HATPase_c domain-containing protein, Heat shock 70 kDa protein, mitochondrial) were identified as more abundant in leaves under ACT. This type of response in ACT leaves may translate an influence of this contaminant in both tissues. The linking of HSP to ROS and stress response is known and these proteins are considered as potential biomarkers for this stress [95]. The abundance of heat shock proteins was already reported in lettuce under zinc and saline stress [50].

Also the synthesis of HSP in plants under stress will improve the protection of proteins in stress conditions [57]. Stromal 70 kDa heat shock-related (chloropastic) protein was identified as more abundant in CBZ leaves, as well as HSP90. The latter had higher abundance in leaves under CBZ and ACT. Among all the contaminants

studied, MTF was the only one that did not show as many differences in HSPs abundance in leaves. This pattern was also observed in roots suggesting a similar response to MTF exposure in both tissues.

4.2.3. Aquaporins

The higher abundance of Aquaporin TIP1–1 in the MTF leaves over control condition is likely related to a putative higher content of H_2O_2 in MTF exposed leaves. Similarly to the MTF condition, ACT also showed a higher abundance when compared to control leaves, as presented in Fig. 4a. A similar trend was observed in aquaporin PIP2–2 which showed higher abundance in leaves contaminated with ACT when compared to controls. These results were similar to those reported by Lucini and Bernardo [50] in lettuce plants under zinc stress. Once again, the contents of hydrogen peroxide may support a possible explanation for the abundance of this aquaporin in leaves under stress.

In leaves under CBZ contamination, aquaporin PIP2–1 revealed higher normalized abundance. Unlike roots, where aquaporin proteins were less abundant under contamination, the three pharmaceuticals revealed a high impact on different aquaporin abundance in leaves. These results may point out to differences between leaf and root cell metabolism, as leaves reflect a higher level of response to this type of abiotic stress.

4.2.4. Detoxification proteins

Proteins from cytochrome P450 complex (Cytochrome P450 89A9 and Cytochrome P450 704C1) revealed also higher abundance in ACT contaminated leaves, a trend followed also by MTF and CBZ. These protein species frequently show an increased activity under stress conditions [96].

Cytochromes P450 (CYP450) in lettuce leaves follow a similar trend in every contamination which seems to be a response to the presence of different xenobiotics. The direct involvement of CYP450 in the secondary metabolism enables detoxification processes as well as the activation of different scavenging by-products as a response to the induced stress. The increased abundance of CYP450 was identified by other authors carrying out studies of different abiotic stresses. For

instance, the increase of CYPs abundance have been reported in wheat and rice under heavy metal stress and in *Arabidopsis thaliana* under salinity stress [96,97].

4.2.5. Cell respiration proteins

Cytochrome c domain-containing protein (complex IV) revealed a higher abundance in ACT-contaminated leaves, a different trend when compared to roots under ACT contamination as well. This result confirms that different tissues may have differences between metabolic pathways.

FBPase domain-containing protein, cytosolic fructose1,6-bisphosphate (fragment) and fructose-bisphosphate aldolase are three proteins related with glycolysis processes and highly expressed in ACT contaminated leaves. Fructose 1,6-biphosphatase is responsible for the allocation between glycolysis and sucrose synthesis. Fructose-bisphosphate aldolase is involved in glycolysis and gluconeogenesis, in cytosol, and in Calvin cycle at plastids level. Indeed, several protein species related to the glycolysis process are often addressed as moonlighting proteins. Taking into account its abundance in ACT leaves, it is possible to link this protein species with a response to abiotic stress. Lu et al. [98] focus their research in fructose 1,6-bisphosphate aldolase genes in *Arabidopsis thaliana* and showed a positive response not only in terms of plant growth and development, but also in stress response. Similar results were reported by Lv et al. [99] in wheat under different abiotic stresses (drought, cold, heat, ABA, salt). Previous studies indicated that oxidative stress modifies functions of cytosolic aldolase [70].This type of alteration may promote C-flux into the oxidative pentose-phosphate pathway to regenerate NADPH necessary to ROS detoxification processes. Moreover, aldolase was addressed as a moonlighting protein that display redox-dependent changes according to the subcellular localization and biological functions [100,101].

Malate and pyruvate dehydrogenases were also two protein species considered to be of interest to our study and both are involved in the cell respiration process. Under ACT contamination, leaves showed higher abundance of these two protein species over control leaves. The detected high abundances of Krebs cycle proteins may indicate a strategy for defence mechanisms in order to withstand stress conditions. Other authors suggested that enzymes such as malate and pyruvate dehydrogenases may comprise potential targets for redox regulation [102]. Indeed, these two protein

species are closely related since malate dehydrogenase will produce pyruvate as a substrate to pyruvate dehydrogenase. Therefore, the higher abundance of the first may be consequently linked to the increase in abundance of the latter.

On the other hand, aconitase_C domain-containing protein showed significantly lower abundance in leaves under ACT exposure, and also with CBZ and MTF contaminated leaves but to a lower extent. Several authors reported on the importance of plant aconitases in mediation of oxidative stress and cell death. Lehmann et al. [71] carried out a study with *Arabidopsis* roots where a concentration of 60 μM menadione caused a decrease of aconitase activity, and a similar trend was observed by Obata et al. [103] in *Arabidopsis* seedling during oxidative stress. This type of fluctuation on aconitase levels, was also reported by Moeder et al. [104] in *Arabidopsis* and *Nicotiana benthamiana* plants, where reduced levels of aconitase displayed enhanced resistance to oxidative stress. We may infer that a similar situation happened in our experiment, supporting the idea that aconitase plays a role in ROS homeostasis and stress physiology.

On the other hand, homologs malate and pyruvate dehydrogenases protein species revealed distinct behaviour in leaves under CBZ contamination. Pyruvate dehydrogenase E1 component subunit beta and malate dehydrogenase suffered a decrease in abundance when under CBZ contamination. This result does not follow the same trend as observed for other contaminations and tissues, for instance MTF did not affect malate dehydrogenase abundance. ACT revealed different trends in these protein species, as previously mentioned. Thus, between the pharmaceuticals studied, CBZ is the one that affects the most enzymes from Krebs cycle and consequently the energy processes. Kumar and co-workers [105] observed also a decrease in total activities of mitochondrial and chloroplast malate dehydrogenase for rice seedlings under high salinity levels on less tolerant cultivars. So, it is possible to state that this type of fluctuations in normalized abundance may be a consequence of different abiotic stresses.

This may represent an interesting outcome of our study, suggesting that the exposure to different pharmaceuticals will induce a different response and affect cell respiration pathways differently.

4.2.6. Cell wall proteins

UGHD is a key sugar nucleotide involved in the biosynthesis of the plant cell wall. Similarly, pectinesterase which is also related to cell wall modifications. In both cases, it was possible to find an increase in normalized abundance in leaves under MTF contamination. Indeed, Vítámvás et al. [106] reported an increase of UGHD, under drought stress conditions, which may indicate an enhanced synthesis of pectins and hemicelluloses. Le Gall et al. [107] pointed out some correlation between different types of stresses and pectinesterase activity, showing that in some abiotic stresses the regulation of this protein species, or even pectin content itself, increased. Therefore, both proteins may lead to changes on cell walls as a response to the induced stress. The relation between these two protein species and abiotic stress also occurred in leaves under ACT and CBZ contaminations, yet with minor differences to control. Interestingly, the result for UGDH abundance revealed an opposite pattern from the one observed in roots. This points to a dissimilar pattern of response between tissues.

4.2.7. Photosynthesis and Calvin cycle

Photosynthesis was not so affected by the presence of MTF, ACT and CBZ. Direct measures on lettuce leaves under CBZ did not revealed negative alterations [52]. During the experiments no visual effects were observed in terms of green leaf coloration on day 8, as it is possible to observe in Fig. 1. However, some changes were observed in chlorophyll *a-b* binding protein which revealed higher abundance in CBZ leaves in comparison to control. MTF and ACT also influenced the pools of chlorophyll *a-b* binding protein, however this difference was not as high as the one with CBZ contamination.

Ribulose bisphosphate carboxylase large chain (fragment, rbcL) (Table 1) showed a higher abundance in contaminated leaves, particularly concerning the MTF contamination. On the other hand, RuBisCO_large domain-containing protein (Table 2) was identified in lower abundance in contaminated leaves when compared to controls, showing a lower abundance in ACT leaves. The proportion of fold change (8.2) is higher than the max fold change for the first one (3.2). A decrease in RubisCO abundance has been reported in different stress conditions as in soybean subjected to drought stress [108]. Such results are likely related with a shutdown of photosynthetic activity as reported by Vu et al. [109] and by Freitas et al. [110]. However, in our

study, no alterations on photosynthesis were shown during experiments and the abundance of chlorophyll *a-b* binding protein reveals that alterations in RuBisCO did not impact the plant photosynthetic ability.

4.2.8. Nutrients assimilation proteins

The plasma membrane ATPase generates the gradient of electrochemical potentials of H^+ across the plasma membrane. This protein species plays a crucial role in the regulation of intracellular pH and proton export across plasma membrane as well as proton transmembrane transport. ATPase plays a fundamental role in the resistance of plants to abiotic and biotic stress [111]. Results of plasma membrane ATPase may fluctuate according to tissue and types of abiotic stress, however the lower abundance in contaminated leaves in the present study may reflect a pattern for abundance decrease of this protein species as a consequence of the exposure to these pharmaceuticals. Thus, the lower abundance of plasma membrane ATPase may cause a negative impact on solute transport into and across membrane. Shen et al. [84] reported an increase in plasma membrane ATPase activity when rice was exposed to salinity stress. On the other hand, Wakeel et al. [112] reported a decrease on plasma membrane ATPase activity in sugar cane under salt stress.

The maintenance of sulphur levels is crucial for a good regulation of S assimilation pathway. Leaves are often addressed as more active than roots in what concerns S assimilation. Sulphite reductase was accumulated in contaminated leaves, particularly in ACT contamination. According to Brychkova et al. [113], an increase in sulphite content was reported under dark stress in tomato leaves. The presence of sulphite reductase may also result from an accumulation of sulphite in cells. Hänsch et al. [114] indicated that when the cell shows a higher content of sulphite, it may be oxidized by hydrogen peroxide in a non-enzymatic reaction occurring in the peroxisomes. This reaction will promote ROS detoxification in the cells. Therefore, the abundance of sulphite reductase may be display high accumulation of sulphite in cells, as a consequence of a response to oxidative stress. Also, according to our results, the abundance of sulphite may be a precursor of other important metabolites such as glutathione.

5. Conclusions

Several protein groups were differentially accumulated in our study. Herein we have highlighted the main protein species related to the stress response, glycolysis, cell respiration and cell wall processes, among others. In the course of this work we aimed to understand the effect of the different stresses on protein species accumulation patterns and relating such information with stress physiology. If biomarker establishment would be the main focus of our work, a subsequent validation step would be interesting to conduct. However, it is noteworthy to mention that a very strict set of filters was applied in order to confer robustness to our results and outputs. Additionally, specific protein species of significance to ROS scavenging, cell homeostasis and photosynthesis were identified in order to form a complete picture of cell regulation processes.

An overall analysis of differentially expressed stress protein species, for the three contaminants, highlights that the major effects are due to CBZ contamination. However, the three contaminants revealed different patterns regarding stress protein species. It is therefore possible to state that different organic compounds will influence the regulation and possible detoxification pathways in lettuce cells. In general, cytochrome P450 proteins revealed higher abundance under contamination in both tissues showing a clear activation of detoxification pathways. Besides pharmaceuticals contamination also interfered on cell respiration proteins suggesting a correlation between oxidative stress and dysregulation on Krebs cycle together with glycolysis pathways. Unlike stress proteins, where CBZ was the contaminant that most influence the redox state of cells, ACT was the one that most influence respiration metabolism, mainly in leaves. MTF was clearly the contaminant that less affected metabolic pathways, in the set of roots and leaves, revealing the lower number of abundant and differentially expressed proteins. Lettuce plants revealed a higher tolerance to MTF and to be able to cope with CBZ and ACT with a different response to each of them, in a short period of exposure. Longer periods of exposure to these pharmaceuticals, even in lower concentrations, may lead to more damages in cells and possible alterations in biomass parameters affecting productivity standards, and even consumers safety.

Future proteomics-based approach together with metabolomics will improve data regarding emerging contaminants and their interference in metabolic pathways. In addition, the interaction between pharmaceuticals and essential minerals will be an

asset in future research in order to provide a complete analysis not only in terms of minerals uptake but also correlating this with higher and lower abundance of different protein species.

The following are the supplementary data related to this article. Download : **Download Powerpoint document (3MB)**

Fig. S1. Experimental design images and proteomics workflow.

Download : **Download spreadsheet (1MB)**

Raw data of proteomics experiment in leaves and roots.

Funding

Inês Leitão acknowledges funding from the Universidade de Lisboa in the form of a PhD grant. This work was partially supported by the Fundação para a Ciência e Tecnologia (FCT) through the research unit LEAF - Linking Landscape, Environment, Agriculture and Food (UID/AGR/04129/2013). David Miguel Ribeiro acknowledges a PhD fellowship (SFRH/BD/143992/2019) granted by FCT.

Authors' contributions

All authors read and agreed on the final version of the manuscript.

Acknowledgments

Authors thank the Luxembourg Institute of Science and Technology, particularly to Sebastien Planchon for the contribution to this work with laboratory support as well as the final review of the manuscript.

Data availability

Data will be made available on request.

References

[1]

A. Galieni, C. Di Mattia, M. De Gregorio, S. Speca, D. Mastrocola, M. Pisante, F. Stagnari

Effects of nutrient deficiency and abiotic environmental stresses on yield, phenolic compounds and antiradical activity in lettuce (*Lactuca sativa* L.)
Sci. Hortic., 187 (2015), pp. 93-101, 10.1016/j.scienta.2015.02.036
ArticleDownload PDFView Record in ScopusGoogle Scholar
[2]
M. Kolahi, E. Mohajel Kazemi, M. Yazdi, A. Goldson-Barnaby

Oxidative stress induced by cadmium in lettuce (*Lactuca sativa* Linn.): oxidative stress indicators and prediction of their genes
Plant Physiol. Biochem., 146 (2020), pp. 71-89, 10.1016/j.plaphy.2019.10.032
ArticleDownload PDFView Record in ScopusGoogle Scholar
[3]
D. Jiménez-Arias, F.J. García-Machado, S. Morales-Sierra, J.C. Luis, E. Suarez, M. Hernández, F. Valdés, A.A. Borges

Lettuce plants treated with L-pyroglutamic acid increase yield under water deficit stress
Environ. Exp. Bot., 158 (2019), pp. 215-222, 10.1016/j.envexpbot.2018.10.034
ArticleDownload PDFView Record in ScopusGoogle Scholar
[4]
M.S. Kalhor, S. Aliniaeifard, M. Seif, E.J. Asayesh, F. Bernard, B. Hassani, T. Li

Title: enhanced salt tolerance and photosynthetic performance: Implication of γ-amino butyric acid application in salt-exposed lettuce (*Lactuca sativa* L.) plants
Plant Physiol. Biochem., 130 (2018), pp. 157-172, 10.1016/j.plaphy.2018.07.003
ArticleDownload PDFView Record in ScopusGoogle Scholar
[5]
M.S.S. Carvalho, L.F. Andrade-Vieira, F.E. dos Santos, F.F. Correa, M. das Graças Cardoso, L.R. Vilela

Allelopathic potential and phytochemical screening of ethanolic extracts from five species of *Amaranthus* spp. in the plant model *Lactuca sativa*
Sci. Hortic., 245 (2019), pp. 90-98, 10.1016/j.scienta.2018.10.001
ArticleDownload PDFView Record in ScopusGoogle Scholar
[6]
F.E. dos Santos, M.S.S. Carvalho, G.L. Silveira, F.F. Correa, M.G. das Cardoso, L.F. Andrade-Vieira, L.R. Vilela

Phytotoxicity and cytogenotoxicity of hydroalcoholic extracts from *Solanum muricatum* Ait. and *Solanum betaceum* Cav. (Solanaceae) in the plant model *Lactuca sativa*
Environ. Sci. Pollut. Res., 26 (2019), pp. 27558-27568, 10.1007/s11356-017-1015-x
View PDF
View Record in ScopusGoogle Scholar
[7]
J. Charles, B. Sancey, N. Morin-Crini, P.M. Badot, F. Degiorgi, G. Trunfio, G. Crini

Evaluation of the phytotoxicity of polycontaminated industrial effluents using the lettuce plant (*Lactuca sativa*) as a bioindicator
Ecotoxicol. Environ. Saf., 74 (2011), pp. 2057-2064, 10.1016/j.ecoenv.2011.07.025
ArticleDownload PDFView Record in ScopusGoogle Scholar

[8]

L.L. Martins, M.P. Mourato, A.I. Cardoso, A.P. Pinto, A.M. Mota, M.L. Maria, A. de Varennes

Oxidative stress induced by cadmium in *Nicotiana tabacum* L.: effects on growth parameters, oxidative damage and antioxidant responses in different plant parts
Acta Physiol. Plant., 33 (2011), pp. 1375-1383, 10.1007/s11738-010-0671-y
View PDF
View Record in ScopusGoogle Scholar

[9]

A. Sofo, R. Bochicchio, M. Amato, N. Rendina, A. Vitti, M. Nuzzaci, M.M. Altamura, G. Falasca, F. Della Rovere, A. Scopa

Plant architecture, auxin homeostasis and phenol content in Arabidopsis thaliana grown in cadmium- and zinc-enriched media
J. Plant Physiol. (2017), 10.1016/j.jplph.2017.06.008
View PDF
Google Scholar

[10]

B.D. Blair, J.P. Crago, C.J. Hedman, R.D. Klaper

Pharmaceuticals and personal care products found in the Great Lakes above concentrations of environmental concern
Chemosphere., 93 (2013), pp. 2116-2123, 10.1016/j.chemosphere.2013.07.057
ArticleDownload PDFView Record in ScopusGoogle Scholar

[11]

C. Trautwein, J.D. Berset, H. Wolschke, K. Kümmerer

Occurrence of the antidiabetic drug Metformin and its ultimate transformation product Guanylurea in several compartments of the aquatic cycle
Environ. Int., 70 (2014), pp. 203-212, 10.1016/j.envint.2014.05.008
ArticleDownload PDFView Record in ScopusGoogle Scholar

[12]

T. Eggen, C. Lillo

Antidiabetic II drug metformin in plants: uptake and translocation to edible parts of cereals, oily seeds, beans, tomato, squash, carrots, and potatoes
J. Agric. Food Chem., 60 (2012), pp. 6929-6935, 10.1021/jf301267c
View PDF
View Record in ScopusGoogle Scholar

[13]

Y. Li, J.B. Sallach, W. Zhang, S.A. Boyd, H. Li

Insight into the distribution of pharmaceuticals in soil-water-plant systems
Water Res., 152 (2019), pp. 38-46, 10.1016/j.watres.2018.12.039
ArticleDownload PDFGoogle Scholar

[14]

Y.H. Chuang, C.H. Liu, J.B. Sallach, R. Hammerschmidt, W. Zhang, S.A. Boyd, H. Li

Mechanistic study on uptake and transport of pharmaceuticals in lettuce from water
Environ. Int., 131 (2019), p. 104976, 10.1016/j.envint.2019.104976
ArticleDownload PDFView Record in ScopusGoogle Scholar

[15]

X. Wu, L.K. Dodgen, J.L. Conkle, J. Gan

Plant uptake of pharmaceutical and personal care products from recycled water and biosolids: a review
Sci. Total Environ., 536 (2015), pp. 655-666, 10.1016/j.scitotenv.2015.07.129
ArticleDownload PDFView Record in ScopusGoogle Scholar

I.T. Carvalho, L. Santos

Antibiotics in the aquatic environments: a review of the European scenario
Environ. Int., 94 (2016), pp. 736-757, 10.1016/j.envint.2016.06.025
ArticleDownload PDFView Record in ScopusGoogle Scholar

M.S. Fram, K. Belitz

Occurrence and concentrations of pharmaceutical compounds in groundwater used for public drinking-water supply in California
Sci. Total Environ., 409 (2011), pp. 3409-3417, 10.1016/j.scitotenv.2011.05.053
ArticleDownload PDFView Record in ScopusGoogle Scholar

C. Wu, J.D. Witter, A.L. Spongberg, K.P. Czajkowski

Occurrence of selected pharmaceuticals in an agricultural landscape, western Lake Erie basin
Water Res., 43 (2009), pp. 3407-3416, 10.1016/j.watres.2009.05.014
ArticleDownload PDFView Record in ScopusGoogle Scholar

A. Bahlmann, W. Brack, R.J. Schneider, M. Krauss

Carbamazepine and its metabolites in wastewater: analytical pitfalls and occurrence in Germany and Portugal
Water Res., 57 (2014), pp. 104-114, 10.1016/j.watres.2014.03.022
ArticleDownload PDFView Record in ScopusGoogle Scholar

R. Loos, B.M. Gawlik, G. Locoro, E. Rimaviciute, S. Contini, G. Bidoglio

EU-wide survey of polar organic persistent pollutants in European river waters
Environ. Pollut., 157 (2009), pp. 561-568, 10.1016/j.envpol.2008.09.020
ArticleDownload PDFView Record in ScopusGoogle Scholar

M. Mourato, R. Reis, L. Martins

Characterization of plant antioxidative system in response to abiotic stresses: A focus on heavy metal toxicity
Adv. Sel. Plant Physiol. Asp (2012), pp. 23-44, 10.5772/34557
View PDF
View Record in ScopusGoogle Scholar

G. Noctor, C. Lelarge-trouverie, A. Mhamdi

Phytochemistry the metabolomics of oxidative stress
Phytochemistry., 112 (2015), pp. 33-53, 10.1016/j.phytochem.2014.09.002
ArticleDownload PDFView Record in ScopusGoogle Scholar

F.R. Pinto, M.P. Mourato, J.R. Sales, I.N. Moreira, F.R. Pinto, M.P. Mourato, J.R. Sales, I.N. Moreira, L.L. Martins, J.R. Sales

Oxidative stress response in spinach plants induced by cadmium
J. Plant Nutr., 40 (2017), pp. 268-276, 10.1080/01904167.2016.1240186
View PDF
View Record in ScopusGoogle Scholar

K. Kosová, P. Vítámvás, I.T. Prášil, J. Renaut

Plant proteome changes under abiotic stress - contribution of proteomics studies to understanding plant stress response
J. Proteome, 74 (2011), pp. 1301-1322, 10.1016/j.jprot.2011.02.006
ArticleDownload PDFView Record in ScopusGoogle Scholar

S. Chen, A.C. Harmon

Advances in plant proteomics
Proteomics, 6 (2006), pp. 5504-5516, 10.1002/pmic.200600143
View PDF
View Record in ScopusGoogle Scholar

I.A. Abreu, A.P. Farinha, S. Negrão, N. Gonçalves, C. Fonseca, M. Rodrigues, R. Batista, N.J.M. Saibo, M.M. Oliveira

Coping with abiotic stress: proteome changes for crop improvement
J. Proteome, 93 (2013), pp. 145-168, 10.1016/j.jprot.2013.07.014
ArticleDownload PDFView Record in ScopusGoogle Scholar

M. Freitas, A. Campos, J. Azevedo, A. Barreiro, S. Planchon, J. Renaut, V. Vasconcelos

Lettuce (*Lactuca sativa* L.) leaf-proteome profiles after exposure to cylindrospermopsin and a microcystin-LR/cylindrospermopsin mixture: a concentration-dependent response
Phytochemistry, 110 (2015), pp. 91-103, 10.1016/j.phytochem.2014.12.004

ArticleDownload PDFView Record in ScopusGoogle Scholar

A.V. Dordio, M. Belo, D. Martins Teixeira, A.J. Palace Carvalho, C.M.B. Dias, Y. Picó, A.P. Pinto

Evaluation of carbamazepine uptake and metabolization by Typha spp., a plant with potential use in phytotreatment
Bioresour. Technol., 102 (2011), pp. 7827-7834, 10.1016/j.biortech.2011.06.050
ArticleDownload PDFView Record in ScopusGoogle Scholar

G.D. Alkimin, D. Daniel, S. Frankenbach, J. Serôdio, A.M.V.M. Soares, C. Barata, B. Nunes

Evaluation of pharmaceutical toxic effects of non-standard endpoints on the macrophyte species *Lemna minor* and *Lemna gibba*
Sci. Total Environ., 657 (2019), pp. 926-937, 10.1016/j.scitotenv.2018.12.002
ArticleDownload PDFView Record in ScopusGoogle Scholar

B. Bartha, C. Huber

Effects of acetaminophen in *Brassica juncea* L. Czern.: investigation of uptake, translocation, detoxification, and the induced defense pathways
Environ. Sci. Pollut. Res. (2010), pp. 1553-1562, 10.1007/s11356-010-0342-y
View PDF
View Record in ScopusGoogle Scholar

T. Brumbarova, A. Matros, H.P. Mock, P. Bauer

A proteomic study showing differential regulation of stress, redox regulation and peroxidase proteins by iron supply and the transcription factor FER
Plant J., 54 (2008), pp. 321-334, 10.1111/j.1365-313X.2008.03421.x
View PDF
View Record in ScopusGoogle Scholar

J. Zhu, S. Alvarez, E.L. Marsh, M.E. LeNoble, I.J. Cho, M. Sivaguru, S. Chen, H.T. Nguyen, Y. Wu, D.P. Schachtman, R.E. Sharp

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 (2007), pp. 1533-1548, 10.1104/pp.107.107250
View PDF
View Record in ScopusGoogle Scholar

P. Kieffer, J. Dommes, L. Hoffmann, J.F. Hausman, J. Renaut

Quantitative changes in protein expression of cadmium-exposed poplar plants
Proteomics, 8 (2008), pp. 2514-2530, 10.1002/pmic.200701110
View PDF
View Record in ScopusGoogle Scholar

X. Wang, P. Fan, H. Song, X. Chen, X. Li, Y. Li

Comparative proteomic analysis of differentially expressed proteins in shoots of *Salicornia europaea* under different salinity
J. Proteome Res., 8 (2009), pp. 3331-3345, 10.1021/pr801083a
View PDF
View Record in ScopusGoogle Scholar

A.J. Castro, C. Carapito, N. Zorn, C. Magné, E. Leize, A. Van Dorsselaer, C. Clément

Proteomic analysis of grapevine (*Vitis vinifera* L.) tissues subjected to herbicide stress
J. Exp. Bot., 56 (2005), pp. 2783-2795, 10.1093/jxb/eri271
View PDF
View Record in ScopusGoogle Scholar

D.R. Hoagland, D.I. Arnon

The water-culture method for growing plants without soil
Coll. Agric. Univ. Calif. Circular, 3 (1950)
Google Scholar

M. Shenker, D. Harush, J. Ben-Ari, B. Chefetz

Uptake of carbamazepine by cucumber plants - a case study related to irrigation with reclaimed wastewater
Chemosphere., 82 (2011), pp. 905-910, 10.1016/j.chemosphere.2010.10.052
ArticleDownload PDFView Record in ScopusGoogle Scholar

C. Huber, B. Bartha, R. Harpaintner, P. Schröder

Metabolism of acetaminophen (paracetamol) in plants-two independent pathways result in the formation of a glutathione and a glucose conjugate
Environ. Sci. Pollut. Res., 16 (2009), pp. 206-213, 10.1007/s11356-008-0095-z
View PDF
View Record in ScopusGoogle Scholar

J. Kotyza, P. Soudek, Z. Kafka, T. Vaněk

Phytoremediation of pharmaceuticals-preliminary study
Int. J. Phytoremediat., 12 (2010), pp. 306-316, 10.1080/15226510903563900
View PDF
View Record in ScopusGoogle Scholar

T. Eggen, T.N. Asp, K. Grave, V. Hormazabal

Uptake and translocation of metformin, ciprofloxacin and narasin in forage- and crop plants
Chemosphere, 85 (2011), pp. 26-33, 10.1016/j.chemosphere.2011.06.041
ArticleDownload PDFView Record in ScopusGoogle Scholar

D.M. Ribeiro, S. Planchon, C.C. Leclercq, K. Raundrup, S.P. Alves, R.J.B. Bessa, J. Ren aut

The muscular, hepatic and adipose tissues proteomes in muskox (*Ovibos moschatus*): differences between males and females
J. Proteome, 208 (2019), p. 103480, 10.1016/j.jprot.2019.103480
ArticleDownload PDFView Record in ScopusGoogle Scholar

E. Woith, G. Guerriero, J.F. Hausman, J. Renaut, C.C. Leclercq, C. Weise, S. Legay, A. Weng, M.F. Melzig

Plant extracellular vesicles and nanovesicles: focus on secondary metabolites, proteins and lipids with perspectives on their potential and sources
Int. J. Mol. Sci., 22 (2021), pp. 1-20, 10.3390/ijms22073719
View PDF
Google Scholar

J. Figueiredo, A.R. Cavaco, L. Guerra-Guimarães, C. Leclercq, J. Renaut, J. Cunha, J. Eiras-Dias, C. Cordeiro, A.R. Matos, M. Sousa Silva, A. Figueiredo

An apoplastic fluid extraction method for the characterization of grapevine leaves proteome and metabolome from a single sample
Physiol. Plant., 171 (2021), pp. 343-357, 10.1111/ppl.13198
View PDF
View Record in ScopusGoogle Scholar

D.M. Ribeiro, S. Planchon, C.C. Leclercq, M.T.P. Dentinho, R.J.B. Bessa, J. Santos-Silva, K. Paulos, E. Jerónimo, J. Renaut, A.M. Almeida

The effects of improving low dietary protein utilization on the proteome of lamb tissues
J. Proteome, 223 (2020), p. 103798, 10.1016/j.jprot.2020.103798
ArticleDownload PDFView Record in ScopusGoogle Scholar

I.C. Ribeiro, C.C. Leclercq, N. Simões, A. Toureiro, I. Duarte, J.B. Freire, M.M. Chaves, J. Renaut, C. Pinheiro

Identification of chickpea seed proteins resistant to simulated in vitro human digestion
J. Proteome, 169 (2017), pp. 143-152, 10.1016/j.jprot.2017.06.009
ArticleDownload PDFView Record in ScopusGoogle Scholar

E.W. Deutsch, A. Csordas, Z. Sun, A. Jarnuczak, Y. Perez-Riverol, T. Ternent, D.S. Campbell, M. Bernal-Llinares, S. Okuda, S. Kawano, R.L. Moritz, J.J. Carver, M. Wang, Y. Ishihama, N. Bandeira, H. Hermjakob, J.A. Vizcaíno

The ProteomeXchange consortium in 2017: supporting the cultural change in proteomics public data deposition
Nucleic Acids Res., 45 (2017), pp. D1100-D1106, 10.1093/nar/gkw936
View PDF
View Record in ScopusGoogle Scholar

J.A. Vizcaíno, A. Csordas, N. Del-Toro, J.A. Dianes, J. Griss, I. Lavidas, G. Mayer, Y. Perez-Riverol, F. Reisinger, T. Ternent, Q.W. Xu, R. Wang, H. Hermjakob

2016 update of the PRIDE database and its related tools
Nucleic Acids Res., 44 (2016), pp. D447-D456, 10.1093/nar/gkv1145
View PDF
Google Scholar

X. Qin, P. Li, S. Lu, Y. Sun, L. Meng, J. Hao, S. Fan

Phosphoproteomic analysis of lettuce (*Lactuca sativa* L.) reveals starch and sucrose metabolism functions during bolting induced by high temperature
PLoS One, 15 (2020), pp. 1-15, 10.1371/journal.pone.0244198
View PDF
View Record in ScopusGoogle Scholar

J.H. Hao, L.L. Zhang, P.P. Li, Y.C. Sun, J.K. Li, X.X. Qin, L. Wang, Z.Y. Qi, S. Xiao, Y. Y. Han, C.J. Liu, S.X. Fan

Quantitative proteomics analysis of lettuce (*Lactuca sativa* L.) reveals molecular basis-associated auxin and photosynthesis with bolting induced by high temperature
Int. J. Mol. Sci., 19 (2018), pp. 1-23, 10.3390/ijms19102967

View PDF
Google Scholar

L. Lucini, L. Bernardo

Comparison of proteome response to saline and zinc stress in lettuce
Front. Plant Sci., 6 (2015), pp. 1-12, 10.3389/fpls.2015.00240
View PDF
View Record in ScopusGoogle Scholar

M. Di Carli, P. De Rossi, P. Paganin, A. Del Fiore, F. Lecce, C. Capodicasa, L. Bianco, G. Perrotta, A. Mengoni, G. Bacci, L. Daroda, C. Dalmastri, M. Donini, A. Bevivino

Bacterial community and proteome analysis of fresh-cut lettuce as affected by packaging
FEMS Microbiol. Lett., 363 (2015), pp. 1-7, 10.1093/femsle/fnv209
View PDF
Google Scholar

I. Leitão, M.P. Mourato, L. Carvalho, M.C. Oliveira, M.M. Marques, L.L. Martins

Antioxidative Response of Lettuce (*Lactuca sativa*) to Carbamazepine-Induced Stress
(2021)
Google Scholar

L.G.G. Corrêa, D.M. Riaño-Pachón, C. Guerra Schrago, R. Vicentini dos Santos, B. Mueller-Roeber, M. Vincentz

The role of bZIP transcription factors in green plant evolution: adaptive features emerging from four founder genes
PLoS One, 3 (2008), 10.1371/journal.pone.0002944
View PDF
Google Scholar

C.M. Llorca, M. Potschin, U. Zentgraf

bZIPs and WRKYs: two large transcription factor families executing two different functional strategies
Front. Plant Sci., 5 (2014), pp. 1-14, 10.3389/fpls.2014.00169
View PDF
View Record in ScopusGoogle Scholar

N. Bouché, H. Fromm

GABA in plants: just a metabolite?

Trends Plant Sci., 9 (2004), pp. 110-115, 10.1016/j.tplants.2004.01.006
ArticleDownload PDFView Record in ScopusGoogle Scholar

N. Suzuki, R. Mittler

Reactive oxygen species and temperature stresses: a delicate balance between signaling and destruction
Physiol. Plant., 126 (2006), pp. 45-51, 10.1111/j.0031-9317.2005.00582.x
View PDF
View Record in ScopusGoogle Scholar

A.M. Timperio, M.G. Egidi, L. Zolla

Proteomics applied on plant abiotic stresses: role of heat shock proteins (HSP)
J. Proteome, 71 (2008), pp. 391-411, 10.1016/j.jprot.2008.07.005
ArticleDownload PDFView Record in ScopusGoogle Scholar

P. Krishna, G. Gloor

The Hsp90 family of proteins in *Arabidopsis thaliana*
Cell Stress Chaperones, 6 (2001), pp. 238-246, 10.1379/1466-1268(2001)006<0238:thfopi>2.0.co;2
View PDF
View Record in ScopusGoogle Scholar

J. Bellati, C. Champeyroux, S. Hem, V. Rofidal, G. Krouk, C. Maurel, V. Santoni

Novel aquaporin regulatory mechanisms revealed by interactomics
Mol. Cell. Proteomics, 15 (2016), pp. 3473-3487, 10.1074/mcp.M116.060087
ArticleDownload PDFView Record in ScopusGoogle Scholar

J. Xu, X.Y. Wang, W.Z. Guo

The cytochrome P450 superfamily: key players in plant development and defense
J. Integr. Agric., 14 (2015), pp. 1673-1686, 10.1016/S2095-3119(14)60980-1
ArticleDownload PDFView Record in ScopusGoogle Scholar

J. Kang, J. Park, H. Choi, B. Burla, T. Kretzschmar, Y. Lee, E. Martinoia

Plant ABC transporters
Arab. B. (2011), pp. 1-25, 10.1199/tab. 0153
View PDF
Google Scholar

R.A. Dixon, N.L. Paiva

Stress-induced phenylpropanoid metabolism

Plant Cell, 7 (1995), pp. 1085-1097, 10.1105/tpc.7.7.1085
View PDF
View Record in ScopusGoogle Scholar

A. Sharma, B. Shahzad, A. Rehman, R. Bhardwaj, M. Landi, B. Zheng

Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress
Molecules, 24 (2019), pp. 1-22, 10.3390/molecules24132452
View PDF
Google Scholar

L. Taiz, E. Zeiger

Physiology Plants
(5th ed.) (2014)
http://www.sinauer.com/media/wysiwyg/tocs/PlantPhysiology5.pdf
Google Scholar

S. Racca, E. Welchen, D.E. Gras, D. Tarkowská, V. Turečková, V.G. Maurino, D.H. Gon zalez

Interplay between cytochrome c and gibberellins during Arabidopsis vegetative development
Plant J., 94 (2018), pp. 105-121, 10.1111/tpj.13845
View PDF
View Record in ScopusGoogle Scholar

X. Long, B. He, Y. Fang, C. Tang

Identification and characterization of the glucose-6-phosphate dehydrogenase gene family in the para rubber tree, *Hevea brasiliensis*
Front. Plant Sci., 7 (2016), pp. 1-14, 10.3389/fpls.2016.00215
View PDF
Google Scholar

L. Zhang, J. Liu, X. Wang, Y. Bi

Glucose-6-phosphate dehydrogenase acts as a regulator of cell redox balance in rice suspension cells under salt stress
Plant Growth Regul., 69 (2013), pp. 139-148, 10.1007/s10725-012-9757-4
View PDF
View Record in ScopusGoogle Scholar

J. Liu, X. Wang, Y. Hu, W. Hu, Y. Bi

Glucose-6-phosphate dehydrogenase plays a pivotal role in tolerance to drought stress in soybean roots
Plant Cell Rep., 32 (2013), pp. 415-429, 10.1007/s00299-012-1374-1
View PDF
View Record in ScopusGoogle Scholar

Y. Yang, Z. Fu, Y. Su, X. Zhang, G. Li, J. Guo, Y. Que, L. Xu

A cytosolic glucose-6-phosphate dehydrogenase gene, ScG6PDH, plays a positive role in response to various abiotic stresses in sugarcane
Sci. Rep., 4 (2014), pp. 1-10, 10.1038/srep07090
View PDF
Google Scholar

S. Dumont, J. Rivoal

Consequences of oxidative stress on plant glycolytic and respiratory metabolism
Front. Plant Sci., 10 (2019), pp. 1-16, 10.3389/fpls.2019.00166
View PDF
View Record in ScopusGoogle Scholar

M. Lehmann, M. Schwarzländer, T. Obata, S. Sirikantaramas, M. Burow, C.E. Olsen, T. Tohge, M.D. Fricker, B.L. Møller, A.R. Fernie, L.J. Sweetlove, M. Laxa

The metabolic response of Arabidopsis roots to oxidative stress is distinct from that of heterotrophic cells in culture and highlights a complex relationship between the levels of transcripts, metabolites, and flux
Mol. Plant, 2 (2009), pp. 390-406, 10.1093/mp/ssn080
ArticleDownload PDFView Record in ScopusGoogle Scholar

T.N. Popova, M.Â.A. Pinheiro de Carvalho

Citrate and isocitrate in plant metabolism
Biochim. Biophys. Acta Bioenerg., 1364 (1998), pp. 307-325, 10.1016/S0005-2728(98)00008-5
ArticleDownload PDFView Record in ScopusGoogle Scholar

W.L. Araújo, A. Nunes-Nesi, Z. Nikoloski, L.J. Sweetlove, A.R. Fernie

Metabolic control and regulation of the tricarboxylic acid cycle in photosynthetic and heterotrophic plant tissues
Plant Cell Environ., 35 (2012), pp. 1-21, 10.1111/j.1365-3040.2011.02332.x
View PDF
View Record in ScopusGoogle Scholar

M. Leterrier, J.B. Barroso, J.M. Palma, F.J. Corpas

Cytosolic NADP-isocitrate dehydrogenase in Arabidopsis leaves and roots
Biol. Plant., 56 (2012), pp. 705-710, 10.1007/s10535-012-0244-6
View PDF
View Record in ScopusGoogle Scholar

L. Maršálová, P. Vítámvás, R. Hynek, I.T. Prášil, K. Kosová

Proteomic response of *Hordeum vulgare* cv. Tadmor and *Hordeum marinum* to salinity stress: similarities and differences between a glycophyte and a halophyte
Front. Plant Sci., 7 (2016), pp. 1-19, 10.3389/fpls.2016.01154
View PDF
Google Scholar

L.A. Kleczkowski, S. Kunz, M. Wilczynska

Mechanisms of UDP-glucose synthesis in plants
CRC. Crit. Rev. Plant Sci., 29 (2010), pp. 191-203, 10.1080/07352689.2010.483578
View PDF
View Record in ScopusGoogle Scholar

M. Hara, K. Tokunaga, T. Kuboi

Isolation of a drought-responsive alkaline α-galactosidase gene from New Zealand spinach
Plant Biotechnol., 25 (2008), pp. 497-501, 10.5511/plantbiotechnology.25.497
View PDF
View Record in ScopusGoogle Scholar

A. Gutsch, E. Keunen, G. Guerriero, J. Renaut, A. Cuypers, J.F. Hausman, K. Sergeant

Long-term cadmium exposure influences the abundance of proteins that impact the cell wall structure in *Medicago sativa* stems
Plant Biol., 20 (2018), pp. 1023-1035, 10.1111/plb.12865
View PDF
View Record in ScopusGoogle Scholar

B.H.J. De Graaf, A.Y. Cheung, T. Andreyeva, K. Levasseur, M. Kieliszewski, H.M. Wu

Rab11 GTPase-regulated membrane trafficking is crucial for tip-focused pollen tube growth in tobacco
Plant Cell, 17 (2005), pp. 2564-2579, 10.1105/tpc.105.033183
View PDF
View Record in ScopusGoogle Scholar

C. Lu, Z. Zainal, G.A. Tucker, G.W. Lycett

Developmental abnormalities and reduced fruit softening in tomato plants expressing an antisense Rab11 GTPase gene
Plant Cell, 13 (2001), p. 1819, 10.2307/3871321
View PDF
View Record in ScopusGoogle Scholar

H.J. Lee, S.E. Abdula, D.W. Jang, S.H. Park, U.H. Yoon, Y.J. Jung, K.K. Kang, I.S. Nou, Y.G. Cho

Overexpression of the glutamine synthetase gene modulates oxidative stress response in rice after exposure to cadmium stress
Plant Cell Rep., 32 (2013), pp. 1521-1529, 10.1007/s00299-013-1464-8
View PDF
View Record in ScopusGoogle Scholar

A.F. Lodeyro, R.D. Ceccoli, J.J. Pierella Karlusich, N. Carrillo

The importance of flavodoxin for environmental stress tolerance in photosynthetic microorganisms and transgenic plants. Mechanism, evolution and biotechnological potential
FEBS Lett., 586 (2012), pp. 2917-2924, 10.1016/j.febslet.2012.07.026
ArticleDownload PDFView Record in ScopusGoogle Scholar

R.S. Yedidi, P. Wendler, C. Enenkel

AAA-ATPases in protein degradation
Front. Mol. Biosci., 4 (2017), pp. 1-14, 10.3389/fmolb.2017.00042
View PDF
Google Scholar

P. Shen, R. Wang, W. Jing, W. Zhang

Rice phospholipase Dα is involved in salt tolerance by the mediation of H+-ATPase activity and transcription
J. Integr. Plant Biol., 53 (2011), pp. 289-299, 10.1111/j.1744-7909.2010.01021.x
View PDF
View Record in ScopusGoogle Scholar

N. Tuteja, M.B. Singh, M.K. Misra, P.L. Bhalla, R. Tuteja, R.N.C. Mishra, S. Carolina

Molecular mechanisms of DNA damage and repair: progress in plants
Crit. Rev. Biochem. Mol. Biol. (2001), 10.1080/20014091074219
View PDF
Google Scholar

P. Most, J. Papenbrock

Possible roles of plant sulfurtransferases in detoxification of cyanide, reactive oxygen species, selected heavy metals and arsenate
Molecules, 20 (2015), pp. 1410-1423, 10.3390/molecules20011410
View PDF
View Record in ScopusGoogle Scholar

J. Papenbrock, A. Schmidt

Characterization of a sulfurtransferase from *Arabidopsis thaliana*
Eur. J. Biochem., 267 (2000), pp. 145-154, 10.1046/j.1432-1327.2000.00980.x
View PDF
View Record in ScopusGoogle Scholar

L. Michelet, M. Zaffagnini, D. Lemaire

Thioredoxins and related proteins
Chlamydomonas Sourceb (2009), pp. 401-443, 10.1016/B978-0-12-370873-1.00019-8
3-Vol Set, 2nd Edition
ArticleDownload PDFView Record in ScopusGoogle Scholar

J.Y. Cha, D.N. Barman, M.G. Kim, W.Y. Kim

Stress defense mechanisms of NADPH-dependent thioredoxin reductases (NTRs) in plants
Plant Signal. Behav., 10 (2015), pp. 1-3, 10.1080/15592324.2015.1017698
View PDF
View Record in ScopusGoogle Scholar

[90]

G. Noctor, A. Mhamdi, S. Chaouch, Y. Han, J. Neukermans, B. Marquez-Garcia, G. Queval, C.H. Foyer

Glutathione in plants: an integrated overview
Plant Cell Environ., 35 (2012), pp. 454-484, 10.1111/j.1365-3040.2011.02400.x
View PDF
View Record in ScopusGoogle Scholar

[91]

J. Venkatesh, S.W. Park

Role of L-ascorbate in alleviating abiotic stresses in crop plants
Bot. Stud., 55 (2014), pp. 38-55, 10.1186/1999-3110-55-38
View PDF
View Record in ScopusGoogle Scholar

[92]

N.A. Akram, F. Shafiq, M. Ashraf

Ascorbic acid-a potential oxidant scavenger and its role in plant development and abiotic stress tolerance

Front. Plant Sci., 8 (2017), pp. 1-17, 10.3389/fpls.2017.00613
View PDF
Google Scholar
[93]
R. Batth, K. Singh, S. Kumari, A. Mustafiz

Transcript profiling reveals the presence of abiotic stress and developmental stage specific ascorbate oxidase genes in plants
Front. Plant Sci., 8 (2017), pp. 1-15, 10.3389/fpls.2017.00198
View PDF
Google Scholar
[94]
C.H. Foyer, G. Noctor

Ascorbate and Glutathione: The Heart of the Redox Hub 1
155 (2011), pp. 2-18, 10.1104/pp.110.167569
View PDF
View Record in ScopusGoogle Scholar
[95]
H.E. Ireland, S.J. Harding, G.A. Bonwick, M. Jones, C.J. Smith, J.H.H. Williams

Evaluation of heat shock protein 70 as a biomarker of environmental stress in *Fucus serratus* and *Lemna minor*
Biomarkers., 9 (2004), pp. 139-155, 10.1080/13547500410001732610
View PDF
View Record in ScopusGoogle Scholar
[96]
B.A. Pandian, R. Sathishraj, M. Djanaguiraman, P.V.V. Prasad, M. Jugulam

Role of cytochrome P450 enzymes in plant stress response
Antioxidants, 9 (2020), pp. 1-15, 10.3390/antiox9050454
View PDF
Google Scholar
[97]
G. Mao, T. Seebeck, D. Schrenker, O. Yu

CYP709B3, a cytochrome P450 monooxygenase gene involved in salt tolerance in *Arabidopsis thaliana*
BMC Plant Biol., 13 (2013), p. 1, 10.1186/1471-2229-13-169
View PDF
View Record in ScopusGoogle Scholar
[98]
W. Lu, X. Tang, Y. Huo, R. Xu, S. Qi, J. Huang, C. Zheng, C. Ai Wu

Identification and characterization of fructose 1,6-bisphosphate aldolase genes in Arabidopsis reveal a gene family with diverse responses to abiotic stresses
Gene, 503 (2012), pp. 65-74, 10.1016/j.gene.2012.04.042

ArticleDownload PDFGoogle Scholar

[99]

G.Y. Lv, X.G. Guo, L.P. Xie, C.G. Xie, X.H. Zhang, Y. Yang, L. Xiao, Y.Y. Tang, X.L. Pan, A.G. Guo, H. Xu

Molecular characterization, gene evolution, and expression analysis of the fructose-1, 6-bisphosphate Aldolase (FBA) gene family in wheat (*Triticum aestivum* L.)
Front. Plant Sci., 8 (2017), pp. 1-18, 10.3389/fpls.2017.01030
View PDF
Google Scholar

[100]

K. van der Linde, N. Gutsche, H.M. Leffers, C. Lindermayr, B. Müller, S. Holtgrefe, R. Scheibe

Regulation of plant cytosolic aldolase functions by redox-modifications
Plant Physiol. Biochem., 49 (2011), pp. 946-957, 10.1016/j.plaphy.2011.06.009
ArticleDownload PDFView Record in ScopusGoogle Scholar

[101]

B. O'Leary, W.C. Plaxton

Multifaceted functions of post-translational enzyme modifications in the control of plant glycolysis
Curr. Opin. Plant Biol., 55 (2020), pp. 28-37, 10.1016/j.pbi.2020.01.009
ArticleDownload PDFView Record in ScopusGoogle Scholar

[102]

Y. Balmer, W.H. Vensel, C.K. Tanaka, W.J. Hurkman, E. Gelhaye, N. Rouhier, J.P. Jacquot, W. Manieri, P. Schürmann, M. Droux, B.B. Buchanan

Thioredoxin links redox to the regulation of fundamental processes of plant mitochondria
Proc. Natl. Acad. Sci. U. S. A., 101 (2004), pp. 2642-2647, 10.1073/pnas.0308583101
View PDF
View Record in ScopusGoogle Scholar

[103]

T. Obata, A. Matthes, S. Koszior, M. Lehmann, W.L. Araújo, R. Bock, L.J. Sweetlove, A.R. Fernie

Alteration of mitochondrial protein complexes in relation to metabolic regulation under short-term oxidative stress in Arabidopsis seedlings
Phytochemistry, 72 (2011), pp. 1081-1091, 10.1016/j.phytochem.2010.11.003
ArticleDownload PDFView Record in ScopusGoogle Scholar

[104]

W. Moeder, Æ. Olga, D.A. Navarre, Æ.G.B. Martin, D.F. Klessig

Aconitase plays a role in regulating resistance to oxidative stress and cell death in *Arabidopsis* and *Nicotiana benthamiana*
Plant Mol. Biol., 63 (2007), pp. 273-287, 10.1007/s11103-006-9087-x

View PDF
View Record in ScopusGoogle Scholar

[105]

R.G. Kumar, K. Shah, R.S. Dubey

Salinity induced behavioural changes in malate dehydrogenase and glutamate dehydrogenase activities in rice seedlings of differing salt tolerance
Plant Sci., 156 (2000), pp. 23-34, 10.1016/S0168-9452(00)00224-7
ArticleDownload PDFView Record in ScopusGoogle Scholar

[106]

P. Vítámvás, M.O. Urban, Z. Škodácek, K. Kosová, I. Pitelková, J. Vítámvás, J. Renaut, I .T. Prášil

Quantitative analysis of proteome extracted from barley crowns grown under different drought conditions
Front. Plant Sci., 6 (2015), pp. 1-18, 10.3389/fpls.2015.00479
View PDF
Google Scholar

H. Le Gall, F. Philippe, J.M. Domon, F. Gillet, J. Pelloux, C. Rayon

Cell wall metabolism in response to abiotic stress
Plants., 4 (2015), pp. 112-166, 10.3390/plants4010112
View PDF
View Record in ScopusGoogle Scholar

S. Das, M. Sen, C. Saha

Isolation and expression analysis of partial sequences of heavy metal transporters from *Brassica juncea* by coupling high throughput cloning with a molecular fingerprinting technique

Planta., 234 (2011), pp. 139-156, 10.1007/s00425-011-1376-1
View PDF
View Record in ScopusGoogle Scholar

J.C.V. Vu, R.W. Gesch, L.H. Allen, K.J. Boote, G. Bowes

CO2 enrichment delays a rapid, drought-induced decrease in rubisco small subunit transcript abundance
J. Plant Physiol., 155 (1999), pp. 139-142, 10.1016/S0176-1617(99)80156-4
ArticleDownload PDFView Record in ScopusGoogle Scholar

M. Freitas, J. Azevedo, E. Pinto, J. Neves, A. Campos, V. Vasconcelos

Effects of microcystin-LR, cylindrospermopsin and a microcystin-LR/cylindrospermopsin mixture on growth, oxidative stress and mineral content in lettuce plants (*Lactuca sativa* L.)
Ecotoxicol. Environ. Saf., 116 (2015), pp. 59-67, 10.1016/j.ecoenv.2015.02.002
ArticleDownload PDFView Record in ScopusGoogle Scholar

M. Janicka, A. Wdowikowska, G. Kłobus

Assay of plasma membrane H+-ATPase in plant tissues under abiotic stresses
Methods Mol. Biol., 1696 (2018), pp. 205-215, 10.1007/978-1-4939-7411-5_14
View PDF
View Record in ScopusGoogle Scholar

A. Wakeel, S. Hanstein, B. Pitann, S. Schubert

Hydrolytic and pumping activity of H+-ATPase from leaves of sugar beet (*Beta vulgaris* L.) as affected by salt stress
J. Plant Physiol., 167 (2010), pp. 725-731, 10.1016/j.jplph.2009.12.018

G. Brychkova, D. Yarmolinsky, A. Batushansky, V. Grishkevich, I. Khozin-Goldberg, A. Fait, R. Amir, R. Fluhr, M. Sagi

Sulfite oxidase activity is essential for normal sulfur, nitrogen and carbon metabolism in tomato leaves
Plants., 4 (2015), pp. 573-605, 10.3390/plants4030573
View PDF
View Record in ScopusGoogle Scholar

R. Hänsch, C. Lang, H. Rennenberg, R.R. Mendel

Significance of plant sulfite oxidase
Plant Biol., 9 (2007), pp. 589-595, 10.1055/s-2007-965433
View PDF
View Record in ScopusGoogle Scholar