Ecotoxicology and Environmental Safety 178 (2019) 146–158

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

A proteomic and biochemical investigation on the effects of sulfadiazine in *Arabidopsis thaliana*

Nisha Sharma[a], Giorgio Arrigoni[b,c], Leonard Barnabas Ebinezer[a,*], Anna Rita Trentin[a], Cinzia Franchin[b,c], Sabrina Giaretta[a], Paolo Carletti[a], Sören Thiele-Bruhn[d], Rossella Ghisi[a], Antonio Masi[a]

[a] DAFNAE, University of Padova, Viale Università 16, 30520 Legnaro, PD, Italy
[b] Department of Biomedical Sciences, University of Padova, Via U. Bassi 58/B, Padova, Italy
[c] Proteomics Center, University of Padova and Azienda Ospedaliera di Padova, Italy
[d] Soil Science, Trier University, Behringstraße 21, D-54286, Trier, Germany

ARTICLE INFO

Keywords:
Abiotic stress
Antibiotics
Environmental pollution
Peroxidase
Proteomics
Sulfonamides

ABSTRACT

Animal manure or bio-solids used as fertilizers are the main routes of antibiotic exposure in the agricultural land, which can have immense detrimental effects on plants. Sulfadiazine (SDZ), belonging to the class of sulfonamides, is one of the most detected antibiotics in the agricultural soil. In this study, the effect of SDZ on the growth, changes in antioxidant metabolite content and enzyme activities related to oxidative stress were analysed. Moreover, the proteome alterations in Arabidopsis thaliana roots in response to SDZ was examined by means of a combined iTRAQ-LC-MS/MS quantitative proteomics approach. A dose-dependent decrease in leaf biomass and root length was evidenced in response to SDZ. Increased malondialdehyde content at higher concentration (2 μM) of SDZ indicated increased lipid peroxidation and suggest the induction of oxidative stress. Glutathione levels were significantly higher compared to control, whereas there was no increase in ascorbate content or the enzyme activities of glutathione metabolism, even at higher concentrations. In total, 48 differentially abundant proteins related to stress/stimuli response followed by transcription and translation, metabolism, transport and other functions were identified. Several proteins related to oxidative, dehydration, salinity and heavy metal stresses were represented. Upregulation of peroxidases was validated with total peroxidase activity. Pathway analysis provided an indication of increased phenylpropanoid biosynthesis. Probable molecular mechanisms altered in response to SDZ are highlighted.

1. Introduction

Antibiotics are drugs used against infections and inflammations. Since penicillin was first discovered in 1928 (Fleming, 1944, 1946), antibiotics have been increasingly used worldwide for therapeutic purposes in both human and veterinary medicine leading to the dispersion of these substances in the environment through contaminated excreta. Wastewaters, landfills and industrial and hospital effluents are the major sources of contamination of water resources with such drugs (Karthikeyan and Meyer, 2006; Manzetti and Ghisi, 2014). Soils are mainly contaminated through the application of livestock slurry, manure and sewage sludge as a fertilizer to the soil (Boxall et al., 2002; Sarmah et al., 2006; Carter et al., 2014; Tasho et al., 2016; Thiele-Bruhn, 2003).

Among the antibiotics, sulfadiazines (SDZ) are a group of synthetic antibacterial agents that contain the sulfonamide group (R_1-SO_2NH-R_2) (Huschek et al., 2008). They are used in human medicine and are one of the most sold classes of veterinary antimicrobial compounds in EU countries for their low cost and broad-spectrum antibacterial and anticoccidian activity (De Liguoro et al., 2007). Because of field application of contaminated manure and slurry, extractable concentrations of sulfonamides up to 0.4 mg kg^{-1} in soil have been measured (Karcı and

Abbreviations: ABA, Abscisic acid; ASC, Ascorbate; CDNB, 1-chloro-2, 4-dinitrobenzene; EDTA, Ethylene Diamine Tetra Acetic Acid; ER, Endoplasmic Reticulum; EU, European Union; GGT, Gamma Glutamyl Transferase; GO, Gene Ontology; GSH, Glutathione; GST, Glutathione-S-transferase; iTRAQ, Isobaric Tags for Relative and Absolute Quantification; LC-MS/MS, Liquid chromatography–Tandem mass spectrometry; MDA, Malondialdehyde; PO4, Phosphate; POD, Peroxidase; SBD-F, Ammonium 7-fluoro 2,1,3-benzooxadiazole-4-sulphonate; SDZ, Sulfadiazine; TBA, Thiobarbituric acid; TCA, Trichloroacetic acid

* Corresponding author. Viale dell'Università 16, 30520 Legnaro, PD, Italy.
E-mail address: leonardbarnabas.ebinezer@unipd.it (L.B. Ebinezer).

https://doi.org/10.1016/j.ecoenv.2019.04.008
Received 14 January 2019; Received in revised form 26 March 2019; Accepted 3 April 2019

Balcıoğlu, 2009). The extractability of these compounds from soil decreases with time owing to immobilizing processes, which can involve physical-chemical interactions with soil components (Förster et al., 2009; Wegst-Uhrich et al., 2014; Wehrhan et al., 2010) as well as reactions mediated by oxidoreductase enzymes, such as peroxidases and laccases (Bialk et al., 2005; Schwarz et al.,2010, 2015). However, the total contents of sulfadiazine remain high in contaminated soil for months and years (Rosendahl et al., 2011; Schmidt et al., 2008).

It has been proved that plants are able to incorporate various drugs (Boxall et al., 2006; Carter et al., 2014; Carvalho et al., 2014), thus contributing to the entry of these compounds into the food-chain and spreading antibiotic resistance (Jechalke et al., 2013). Hence, information on the distribution of antibiotics in manure-applied and wastewater-irrigated soils and their uptake and accumulation by plants/crops has been accumulating over the recent years (Kang et al., 2013; Pan et al., 2014; Rosendahl et al., 2011; Schmidt et al., 2008). Antibiotics can directly affect plant physiological processes such as photosynthesis, respiration and root functionality (Carvalho et al., 2014; Li et al., 2011; Michelini et al., 2012, 2013). Regarding plant growth, sulfonamides have been shown as either promoting or inhibitory substances (hormesis) depending on the plant and the concentrations used (Migliore et al., 2010; Michelini et al., 2012; Pan and Chu, 2016). The comprehensive information on the global changes in transcriptome, proteome and metabolome in response to antibiotics and related drugs is vital to understand the stress responses induced and the tolerance and detoxification mechanisms in plants. Proteomics, being a powerful tool that yields comprehensive information, it has been extensively applied to understand the effects of emerging environmental pollutants and abiotic stress in plants (Mirzajani et al., 2014; Wang et al., 2019). However, not much is known about the effects of antibiotics on the proteome of plants. Hitherto, to the best of our knowledge, there is no information pertaining to the changes in root proteome of *Arabidopsis* and its correlation with the physiological and biochemical effects in response to SDZ. Hence, this study investigates the effects of SDZ on growth, changes in the anti-oxidant metabolites and enzyme activities and the alterations in the proteome occurring in *Arabidopsis thaliana* roots in response to SDZ.

2. Materials and methods

2.1. Arabidopsis seed sterilization and growth

Arabidopsis Col-O seeds were surface sterilized by 70% ethanol for 2 min followed by 5% hypochlorite solution for 15 min in microfuge tubes. The seeds were rinsed with sterile water 5 times to ensure that all bleach residues were removed. Then they were grown for 3 weeks, in sterilized half-strength Murashige and Skoog medium at 6% sucrose and 1.4% agarose with SDZ antibiotics at the concentrations 0 µM (Control), 0.5 µM, 1 µM and 2 µM. The exposure concentration was chosen in resemblance to the concentrations of sulfonamide measured in soil (Karcı and Balcıoğlu, 2009). Also in a preliminary experiment, SDZ concentration at 5 µM was tested but resulted in the death of all treated seedlings. Based on Rosendahl et al. (2011), the easily extractable concentration of SDZ and its main metabolites decreases rapidly in the environment, with DT_{50} of around 2–3 weeks. Based on this, 21 days were chosen as exposure time in this experiment. Seeds were incubated in vertically oriented Petri dishes in a growth chamber under short day condition (8.5/15.5 h of light/dark cycle) at a temperature of 22/18 °C, at 50% relative humidity and with a light intensity of 120 µE m^{-2} s^{-1}. Root samples were harvested and stored for further analysis.

2.2. MDA content determination

The level of lipid peroxidation in roots was determined by the quantification of malondialdehyde (MDA) based on the spectrophotometric method reported by Heath and Packer (1968) with some modifications. The root tissue sample from five replicates was ground in liquid nitrogen and homogenized with extraction buffer (TCA 0.1% (w/v) in H_2O) in 1:10 ratio. The supernatant was collected after centrifugation at 10000*g* for 10 min. The reaction mix was prepared with sample extract and Thiobarbituric acid (TBA) solution (TCA 20% (w/v) in H_2O, TBA 0.65% (w/v)). The samples were incubated for 15 min at 95 °C, cooled down on the ice and centrifuged at 10000 g for 10 min. The absorbance was read at λ 532 nm, which was subtracted with the unspecific absorbance measured at λ 600 nm. MDA concentration was calculated using the extinction coefficient (157 mM^{-1} cm^{-1}).

2.3. Extraction for determination of GSH and ascorbate content

The root samples from at least five replicates were harvested, weighed and ground to a fine powder with liquid nitrogen. Extraction buffer was 0.1 M HCl and 1 mmol/L Na_2EDTA in ratio of 1:4 (w/v). After homogenisation, samples were centrifuged at 4 °C at 10000 g for 10 min and the supernatant was collected and immediately used for ascorbate determination and for thiol derivatisation. The ascorbate content was determined spectrophotometrically by measuring the absorbance at λ 265 nm, according to the method of Hewitt and Dickes (1961) that provides total ascorbate, reduced ascorbate and dehydroascorbic acid (Trentin et al., 2015).

Thiol derivatisation followed by isocratic separation and determination by HPLC was carried out as described by Masi et al. (2002) with some minor modifications. 50 µl of thiols extract was derivatized with the ammonium 7-fluoro 2,1,3-benzooxadiazole-4-sulfonate (SBD-F) fluorophore. The derivatized samples were further separated by reversed phase HPLC using a Luna 3 µ C_{18} (2) 150 × 4.60 mm column (Phenomenex), in isocratic conditions with 97% of 75 mM ammonium formate, pH 2.9 and 3% methanol, at a flow rate of 0.3 ml/min at RT.

2.4. Protein extraction and enzyme activity analysis

Proteins were extracted from 150 mg of root samples ground in liquid nitrogen and thereafter the extraction buffer (40 mM Tris-HCl pH 8,3% Triton, 1 mM PMSF, 1 mM Benzamidine, 1 M NaCl) was added in 1:5 (mg/µl) ratio and homogenized thoroughly with the root powder. The mixture was incubated for 1 h in agitation, followed by centrifugation at 10,000 g for 12 min at 4 °C. The protein concentration in the supernatant was quantified by Bradford's method (Bradford, 1976).

The GGT activity was assayed spectrophotometrically according to Huseby and Strömme (1974), where the release of *p*-nitroanilide by the GGT from γ-glutamyl-*p*-nitroanilide (GPNA) substrate was measured. The reaction mix was prepared with protein extract, solution A (4.6 mM GPNA in 100 mM NaH_2PO_4 pH 8.0) and solution B (575 mM gly-gly in 100 mM NaH_2PO_4 pH 8.0) directly in the cuvette. Therefore, the absorbance at λ 407 nm was measured every 5 min for 1 h.

For GST enzyme assays, total protein extracts were prepared as described above. The collected root samples were made fine powder and were homogenized in 1:10 ratio of extraction buffer containing 1% of Polyvinylpolypyrrolidone. The collected extract was then mixed with reaction mixture of PO_4 (200 mM), 1-chloro-2, 4-dinitrobenzene (CDNB) 100 mM, Glutathione (GSH) (100 mM), Ethylenediaminetetraacetic acid (EDTA) 100 mM, was then incubated at 30 °C for 5 min. The absorbance was measured at λ 340 nm (Giaretta et al., 2017).

The activity of syringaldazine POD was determined by measuring the increase in absorbance at λ 530 mm of the reaction mixture containing leaf extracts with 100 mM Na-K phosphate buffer, pH 6.0, 2.5 mM H_2O_2 and 2 mM syringaldazine (Ranieri et al., 2000).

Data from all the technical replicates of biochemical assays were tested for statistical significance (at $p < 0.05$) using ANOVA followed by Post Hoc analysis with Van der Waerden (Normal score test) and

Friedman's test using R studio.

2.5. Protein Extraction for iTRAQ labelling and MS analysis

The method described in Tolin et al. (2013) was followed to obtain total root proteins. After quantification, 50 µg of samples were loaded in a 12% homemade gel. The electrophoretic run was stopped when the protein extracts entered the running gel, single bands were excised, cut in small pieces, washed with 50 mM triethylammonium bicarbonate (TEAB), and dried under vacuum. Protein reduction, alkylation and trypsin digestion were carried out as described in Resmini et al. (2017). Peptides were extracted from the gel with 3 changes (50 µL each) of 50% acetonitrile in water and samples dried under vacuum.

2.6. iTRAQ labelling

The method described by Tolin et al. (2013) was followed with few modifications. Labelling was done with an iTRAQ® Reagents 4-plex Kit (AB Sciex, MA, USA). The iTRAQ experiment was performed on protein samples derived from roots collected from 1 µM SDZ concentration and control. Peptides from control and treated samples were re-suspended in an iTRAQ-compatible buffer (TEAB 0.5 M, SDS 0.1%) to a final concentration of 2 µg/µL and labelled with the iTRAQ tags according to manufacturer's instructions. Before mixing the samples, LC-MS/MS analysis was performed on each sample to assess labelling efficiency. All peptides were correctly iTRAQ-modified at the N-terminus and at each lysine residue. Samples were finally pooled and dried under vacuum.

2.7. Strong cation exchange fractionation

Strong cation exchange chromatography (SCX) was performed on a SCX cartridge (AB Sciex). Labelled sample was dissolved in 500 µL of buffer A (10 mM KH_2PO_4, 25% acetonitrile, pH 2.9) and loaded onto the cartridge using a syringe pump with a 50 µL/min flow rate, according to Trentin et al. (2015). The cartridge was washed with 500 µL of buffer A and peptides were eluted in a stepwise manner with 500 µL of KCl in buffer A at the following concentrations: 25, 50, 100, 200, and 350 mM. Samples were desalted using C_{18} cartridges (Sep-Pack, C_{18}, Waters, Milford, MA, USA) according to the manufacturer's instructions and dried under vacuum.

2.8. LC-MS/MS analysis and database search

Each sample was suspended in H_2O/0.1% formic acid and LC-MS/MS analysis was performed with an LTQ-Orbitrap XL mass spectrometer (Thermo Fisher Scientific) coupled online with a nano-HPLC Ultimate 3000 (Dionex-Thermo Fisher Scientific). Chromatographic and instrumental conditions were as described in De Rosa et al. (2015).

Raw files were analysed using Proteome Discoverer 1.4 (Thermo Fisher Scientific). The software was connected to a Mascot Search Engine server, version 2.2.4 (Matrix Science, London, UK). Spectra were searched against an *A. thaliana* database (downloaded from UniProt, version October 2015). Trypsin was selected as enzyme with 1 missed cleavage allowed. Peptide and fragment tolerances were 20 ppm and 0.6 Da, respectively. Methylthiocysteine, 4-plex iTRAQ (N-term and Lys) were set as fixed modifications, while methionine oxidation was selected as variable modification. False discovery rates (FDR) were calculated by the software with the algorithm Percolator and data were filtered to keep only proteins identified with at least two unique peptides with high confidence (FDR 1%). The quantification was performed normalizing the results on the median value of all measured iTRAQ reporter ratios. The ratios of treated-to-control values were averaged and subjected to a two-tailed Z-test ($p \leq 0.05$). A ratio of treated to control ≥ 1.5 or ≤ 0.67 was set as the threshold for increased and decreased abundance, respectively.

2.9. Bioinformatic analyses

All identified proteins were functionally categorized based on UniProt (https://www.uniprot.org/). Pathway analysis using KEGG Mapper - Search & Colour Pathway (Kanehisa et al., 2017) was performed using UniProt accessions against *A. thaliana* database. The amino acid sequences of the identified proteins obtained from UniProt were subjected to multiple bioinformatic servers DeepLoc-1.0 (http://www.cbs.dtu.dk-/services/DeepLoc/) and SignalP (http://www.cbs.dtu.dk/services/SignalP/) to predict sub-cellular localization and proteins secreted by classical (with signal peptide). SecretomeP (http://www.cbs.dtu.dk/services/SecretomeP-2.0/) was used to predict proteins secreted by non-classical pathways (without signal peptide). Integrated Interactome System (IIS) platform 3 (Carazzolle et al., 2014) was used to build the protein interactomes with only differentially regulated proteins limiting to only first neighbours' nodes. Cytoscape 3.5.1 software (Shannon et al., 2003) was used to visualise and analyse the interactome data output from IIS as reported in Roomi et al. (2018).

3. Results and discussion

3.1. Effects on seedling growth, leaf biomass and root length

There was no significant difference in the seed germination percentage with respect to the SDZ concentrations studied. The effect of different concentrations of SDZ (0.5, 1 and 2 µM) on *Arabidopsis* growth was evaluated after three weeks of treatment. Morphologically, there was a reduction in the overall growth and a considerable decrease in the number of leaves and root growth in a dose-dependent manner (Fig. 1A). A significant decrease in lateral root growth was also noticed. Root growth and length were notably reduced in plants treated with 1 µM and 2 µM SDZ already after 10 days of treatment (Fig. 1B). There was a noticeable reduction in overall growth with poorly developed roots during the treatment at the highest SDZ dose (2 µM).

No hormetic effect was observed in the concentration range analysed. Consistent with our observation, sulfonamide class of antibiotics have been reported to reduce the root and stem growth, lower the number of leaves and biomass production in several crops and non-crop plants (Migliore et al., 1995, 1997, 2010). In addition to observing that SDZ was mainly stored inside the roots of willow (*Salix fragilis* L.) and maize (*Zea mays* L.), it was reported to decrease stem length, development and also result in death in *Zea mays* at higher concentrations (Michelini et al., 2012).

3.2. Changes in malondialdehyde content

In order to ascertain if the SDZ treatment induced oxidative stress, changes in MDA content in response to different SDZ concentrations were measured. MDA is one of the final products of oxidative modification of lipids, resulting in damage of membrane integrity and hence considered as a biochemical marker for oxidative stress (Hodges et al., 1999). There was a gradual increase in MDA content with response to SDZ concentration. At 2 µM concentration, the MDA content was significantly higher compared to 0.5 µM and control indicating lipid peroxidation due to oxidative stress after 21 days of treatment (Table 1). Increased MDA content was also reported in wheat seedlings treated with SDZ (Xu et al., 2017).

3.3. Changes in ascorbate and glutathione levels

ROS scavenging system in plants consists of enzymatic and non-enzymatic antioxidant components (Scandalios, 2005). Ascorbate and glutathione are non-enzymatic antioxidants that are an integral part of this system, crucial for the survival of the plant (Mittler et al., 2004; Foyer and Noctor, 2005). Although ascorbate and glutathione function invariably in a compensatory and synergistic manner, there is evidence

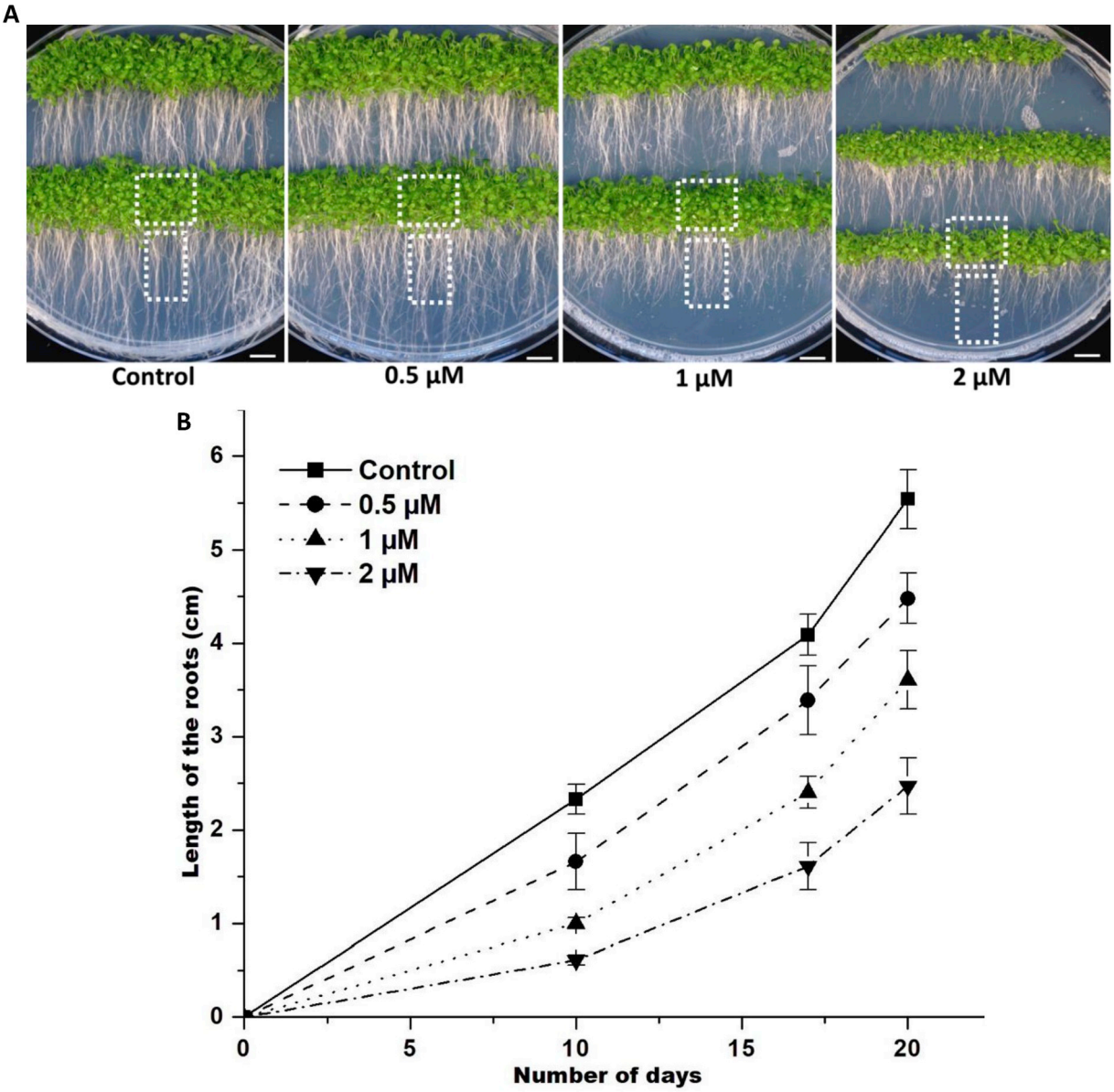

Fig. 1. A Effects of SDZ on *Arabidopsis thaliana* growth, leaf biomass and root length after 3 weeks of treatment. Boxes are of same dimensions highlighting the dose-dependent decrease in leaf biomass, root length and lateral root growth. Fig. 1B Effect of different concentrations of SDZ (0.5, 1, 2 µM) on root length in comparison to control over a period of 3 weeks. Error bars represent standard error (n = 5).

that suggests that they are differentially influenced by environmental stimuli and their interdependence and independence in peroxide metabolism have also been elaborated (Foyer and Noctor, 2011). Here we quantified both ascorbate and dehydroascorbate, but we found that ascorbate was 99% reduced. This supports the notion that sulfadiazine is not altering the ascorbate redox state, at least in our experimental conditions at the concentrations and exposure times we have used. SDZ did not induce significant changes to the ascorbate levels but the GSH content was consistently higher than the control at all concentrations (Table 1). These results indicate that the GSH could be one of the major anti-oxidant metabolites in *Arabidopsis* roots responding to oxidative stress induced by SDZ.

Table 1
Changes in the different biochemical parameters and enzyme activities in *Arabidopsis* roots in response to different concentrations of SDZ in growth medium after 21 days of treatment.

Treatment	MDA (%)	Total ASC ($mol^{*}g^{-1}$ FW)	GSH ($mol^{*}g^{-1}$ FW)	Total Protein (mg $prot^{*}g^{-1}$ FW)	GGT ($U^{*}mg\ prot^{-1}$)	GST ($U^{*}mg\ prot^{-1}$)	POD (µmol $quinone^{*}min^{-1}mg$ prot)
Control	100 ± 25.92^{ab}	0.66 ± 0.22^{a}	50.6 ± 9.10^{b}	3.21 ± 0.54^{b}	0.013 ± 0.003^{a}	22.59 ± 3.07^{a}	0.69 ± 0.15^{b}
0.5 µM	89.67 ± 22.43^{b}	0.51 ± 0.09^{a}	72.10 ± 13.64^{a}	3.63 ± 0.50^{ab}	0.012 ± 0.003^{a}	21.00 ± 5.36^{a}	1.25 ± 0.85^{ab}
1 µM	106.79 ± 20.52^{ab}	0.63 ± 0.22^{a}	74.69 ± 12.25^{a}	3.77 ± 0.53^{ab}	0.012 ± 0.001^{a}	24.84 ± 7.06^{a}	1.10 ± 0.15^{a}
2 µM	125.54 ± 13.12^{a}	0.55 ± 0.16^{a}	78.41 ± 10.52^{a}	4.05 ± 0.46^{a}	0.013 ± 0.003^{a}	23.61 ± 7.72^{a}	0.96 ± 0.24^{ab}

Values after ± indicate standard deviation and different letters indicate statistically significant difference at $p < 0.05$ according to Van der Waerden (Normal score test) and Friedman's tests.

Table 2
Summary of the list of proteins identified.

S.No.	Accession[a]	Protein description[a]	Mascot Score[b]	No. Of unique peptides/ PSMs[b]	% Coverage[b]	Experimental kDa/*pI*[b]	Fold change[b]	Subcellular localization[c]	CS/NCS[d]	Functional category[a]
	P24102	Peroxidase 22 (PER 22)	614.43	3/29	19.77	38.1/6.0	2.522	EX	CS	Stress and stimuli response
	Q9LHB9	Peroxidase 32 (PER 32)	1131.76	8/58	42.90	38.8/6.67	2.395	EX	CS	
	Q9SUT2	Peroxidase 39 (PER 39)	30.87	3/5	10.43	35.6/6.98	1.767	EX	CS	
	Q9SMU8	Peroxidase 34 (PER 34)	144.76	3/9	17.0	38.8/7.64	1.731	EX	CS	
	F4JFY4	L-ascorbate peroxidase S (sAPX)	48.34	2/2	8.38	37.4/9.06	2.128	PL	NCS	
	P42763	Dehydrin ERD14 (ERD14)	159.57	1/6	11.35	20.8/5.48	4.20	NU	–	
	P31168	Dehydrin COR47 (COR47)	69.37	3/7	18.49	29.9/4.77	1.495	NU	–	
	Q9ZVF3	MLP-like protein 328 (MLP328)	1907.37	7/100	60.93	17.5/5.73	1.846	CY	–	
	Q9ZVF2	MLP-like protein 329 (MLP329)	1061.50	5/43	60.93	17.6/5.55	1.596	CY	–	
	Q9FIX1	AIG2-like protein (AIG2LB)	85.18	4/5	20.35	20.0/5.10	1.508	CY	–	
	Q9SIE7	PLAT domain-containing protein 2 (PLAT2)	326.33	4/19	26.78	20.10/5.31	1.569	ER	CS	
	F4JHJ0	HSP20-like chaperone (HSP20)	275.91	5/13	25	25.3/4.56	1.848	NU	–	
	P31265	Translationally-controlled tumour protein homolog (TCTP)	311.67	7/19	31.55	18.9/4.64	2.051	CY	NCS	
	O64517	Metacaspase-4 (AMC4)	240.16	4/7	9.57	45.5/4.82	1.480	CY	–	
	Q8L4N1	Universal stress protein (USP)	45.37	2/3	8.46	28.1/6.25	1.874	CY	–	
	Q9LXC9	Soluble inorganic pyrophosphatase 6 (PPA6)	181.31	4/5	15.0	33.4/6.01	1.998	PL	NCS	
	Q9LHG9	Nascent polypeptide-associated complex subunit alpha-like protein 1 (NAC)	404.70	6/13	40.89	22.0/4.48	1.494	NU	–	
	Q9FGT8	Temperature-induced lipocalin-1 (TIL)	94.40	2/3	9.68	21.4/6.35	1.453	CY	–	
	Q8RUD6	Rhodanese-like domain-containing protein 19, mitochondrial (HAC)	33.35	2/2	17.16	19.0/6.79	1.494	MI	–	
	F4IHK9	Glycine-rich RNA-binding protein 7 (GRP7)	133.59	2/8	23.27	15.5/5.60	1.845	NU	–	
	F4JVC0	Glycine-rich RNA-binding protein 8 (GRP8)	233.43	2/10	35.87	10.2/4.79	2.366	CY	NCS	
	Q9FKA5	PLD-regulated protein 1 (PLDrp1)	387.04	8/16	35.70	43.5/4.78	2.375	NU	–	
	Q9SYT0	Annexin D1 (ANN1)	1491.69	22/70	68.45	36.20/5.38	0.64	CY	–	
	Q9ZVA4	Curculin-like (Mannose-binding) lectin family protein (EP1GP)	1060.40	18/36	41.50	49.0/7.65	0.65	EX	CS	
	O80950	Jacalin-related lectin 22 (JAL22)	479.92	10/22	28.82	50.4/5.30	0.54	CY	–	
	O82762	F17H15.1/F17H15.1 (KHdp)	113.41	2/3	4.11	64.6/5.49	3.448	NU	–	Transcription and translation
	Q9XI36	Methyl-CpG-binding domain-containing protein (MBD)	45.81	2/2	7.81	42.3/4.67	1.784	NU	–	
	Q9LYK9	40S ribosomal protein S26-3 (RPS26C)	79.96	2/5	18.46	14.6/11.09	1.598	CY	–	
	O23515	60S ribosomal protein L15-1 (RPL15A)	139.01	5/7	27.45	24.2/11.44	1.585	CY	–	
	Q9LZH9	60S ribosomal protein L7a-2 (RPL7AB)	469.74	2/31	43.75	29.0/10.15	1.582	CY	–	
	Q9S9P1	40S ribosomal protein S12-1 (RPS12A)	85.27	2/4	13.19	15.4/5.55	1.510	CY	NCS	
	A8MS83	60S ribosomal protein L23a-2 (RPL23AB)	217.17	5/10	35.81	16.7/10.17	1.503	CY	NCS	
	P49692	60S ribosomal protein L7a-1 (RPL7AA)	492.22	3/30	47.86	29.1/10.13	1.452	CY	–	
	O49499	Caffeoyl-CoA O-methyltransferase 1 (CCOAOMT1)	412.52	7/15	30.89	29.1/5.29	1.631	CY	–	Metabolism
	Q0WP12	Thiocyanate methyltransferase 1, Isoform 2 (HOL1)	528.25	7/18	38.33	25.3/4.82	1.671	CY	–	
	O82179	Glycine cleavage system H protein 2 (GDH2)	136.49	2/4	12.82	17.1/5.11	1.809	MI	–	
	Q42599	NADH dehydrogenase [ubiquinone] iron-sulfur protein 8-A (NADHD)	54.68	3/3	14.86	25.5/5.41	1.655	MI	NCS	
	Q8GUN2	Adenylylsulfatase (HINT1)	58.09	2/3	21.77	16.0/7.20	2.319	MI	NCS	
	Q8GW53	Phosphorylase superfamily protein (PSP)	158.57	4/5	14.50	36.9/8.75	0.66	EX	CS	
	O65398	Lactoyl-glutathione lyase (GLX1)	641.22	10/28	36.04	31.9/5.27	0.62	CY	–	
	O82089	Copper transport protein (CCH)	249.09	3/9	32.23	13.0/4.93	2.628	CY	–	Transport
	B3H438	MD-2-related lipid recognition domain-containing protein (MRO11.13)	149.35	3/6	13.04	17.8/4.56	1.542	EX	CS	
	Q8VYM2	Inorganic phosphate transporter 1-1 (PHT1-1)	84.40	4/7	7.63	57.6/9.01	0.64	LY/VA	TM	

(*continued on next page*)

Table 2 (*continued*)

S.No.	Accession[a]	Protein description[a]	Mascot Score[b]	No. Of unique peptides/ PSMs[b]	% Coverage[b]	Experimental kDa/*pI*[b]	Fold change[b]	Subcellular localization[c]	CS/NCS[d]	Functional category[a]
	F4K8S2	12S seed storage protein CRU1 (CRA1)	108.89	3/3	9.12	31.6/8.81	4.335	CY	NCS	Other
	F4IRX7	SOUL heme-binding-like protein (SOUL-1)	106.48	4/4	24.44	24.9/8.81	1.496	MI	–	
	Q93YR3	FAM10 family protein (FAM10)	237.33	5/7	15.87	46.6/4.93	3.080	CY	–	
	Q9SJ81	Fasciclin-like arabinogalactan protein 7 (FLA7)	167.15	2/5	10.63	26.8/6.54	1.854	CM	CS	
	F4J9K9	Neurofilament protein-related protein (NPLP)	108.68	3/4	6.09	73.4/4.35	1.478	NU	–	

[a] - Accession, proteins description and functional categorization are based on UniProt; Corresponding gene names from UniProt were used as abbreviations; b - Information obtained from iTRAQ-LC-MS/MS and Mascot search; c - Subcellular localization (DeepLoc 1.0): EX - Extracellular, PL - Plastid, NU - Nucleus, CY - Cytoplasm, ER - Endoplasmic Reticulum, MI - Mitochondria, LY/VA - Lysosome/Vacuole, CM - Cell membrane; d - Prediction of classical (CS) and non-classical secretory (NCS) pathway (SignalP and SecretomeP).

3.4. Changes in the total protein content and enzyme activities of GST and GGT

An increasing trend in the total protein content in response to SDZ treatment was observed (Table 1). Increased as well as decreased total protein content in crops under abiotic stress have been reported (Hendawey and Kamel, 2015; Jahanbakhsh et al., 2017). Hence, it is reasonable to presume that the change in total protein content depends on the plant species, its inherent varietal tolerance to a specific stress, type of stress and the duration of stress. Nevertheless, it could be correlated in general with the proteome level changes that occur in response to stress, whereby the majority of proteins were upregulated several folds in comparison to untreated control plants. Total protein content is usually considered as a proxy for plant growth. However, protein content accounts for the protein concentration in a tissue, which might be at least partially unrelated to higher biomass. In this study, root growth was reduced in response to SDZ treatment compared to control, while the root protein content increased. Higher protein content not necessarily relates to structural proteins needed for plant growth, it might account for higher levels of proteins related to stress responses, as evidenced in the proteome analysis.

Considering the increase in GSH content, the activities of GST - an enzyme involved in xenobiotic detoxification using GSH as co-substrate (Dixon and Edwards, 2010) and GGT - an enzyme related to GSH metabolism (Masi et al., 2015) were determined. Enzyme activities of both GST and GGT expressed on a protein basis did not show any significant differences between the control and the SDZ treated roots (Table 1). This could possibly indicate that there were other alternate routes of detoxifying the ROS induced by SDZ or that these crucial enzymes were already present in abundance to regulate and maintain the ROS homeostasis. Hence, there could be only subtle changes in these enzymes under such conditions, which might not have been reflected in the total enzyme activity assay.

3.5. Proteome level alterations in Arabidopsis roots in response to SDZ

A quantitative iTRAQ experiment was carried out to understand the alterations that occur at the protein level in *Arabidopsis* roots in response to SDZ. In total, 48 proteins were found to be differentially abundant, of which 42 were upregulated and 6 were downregulated (Table 2). List of all the proteins identified with peptides used for identification and other relevant parameters used for quantification and statistical testing is provided as supplementary material (Table S1). Based on the functional categorization, the differentially regulated proteins represented stress and stimuli response, transcription and translation, metabolism, transport and other functions (Fig. S1). Subcellular localization prediction indicated that the majority of the proteins were localized in the cytoplasm followed by the nucleus and extracellular proteins (Fig. S2). It also indicated that several proteins were intracellularly transported or secreted by non-classical secretory pathway (Table 2).

3.5.1. Proteins related to stress and stimuli response

3.5.1.1. Proteins related to multiple abiotic stress. Two major latex protein (MPL)-like proteins 328 and 329 (MLP328 and MLP329) found to be upregulated in our study were reported to be differentially regulated in response to plant hormones (Yang et al., 2015) and various other abiotic stresses (Stanley Kim et al., 2005; Chen and Dai, 2010; Zhang et al., 2018). Similarly, AIG2-like protein (AIG2LB) reported to be upregulated in response to water deficit and salt stress (Reymond et al., 2000; Qiu et al., 2017). Further, AIG2-like protein also functions as a gamma-glutamylcyclotransferase, which is involved in protecting *Arabidopsis* plants from heavy metal toxicity by ensuring sufficient GSH turnover by recycling glutamate to maintain GSH homeostasis during stress (Paulose et al., 2013).

A PLAT domain-containing protein 2 (**P**olycystin, **L**ipoxygenase,

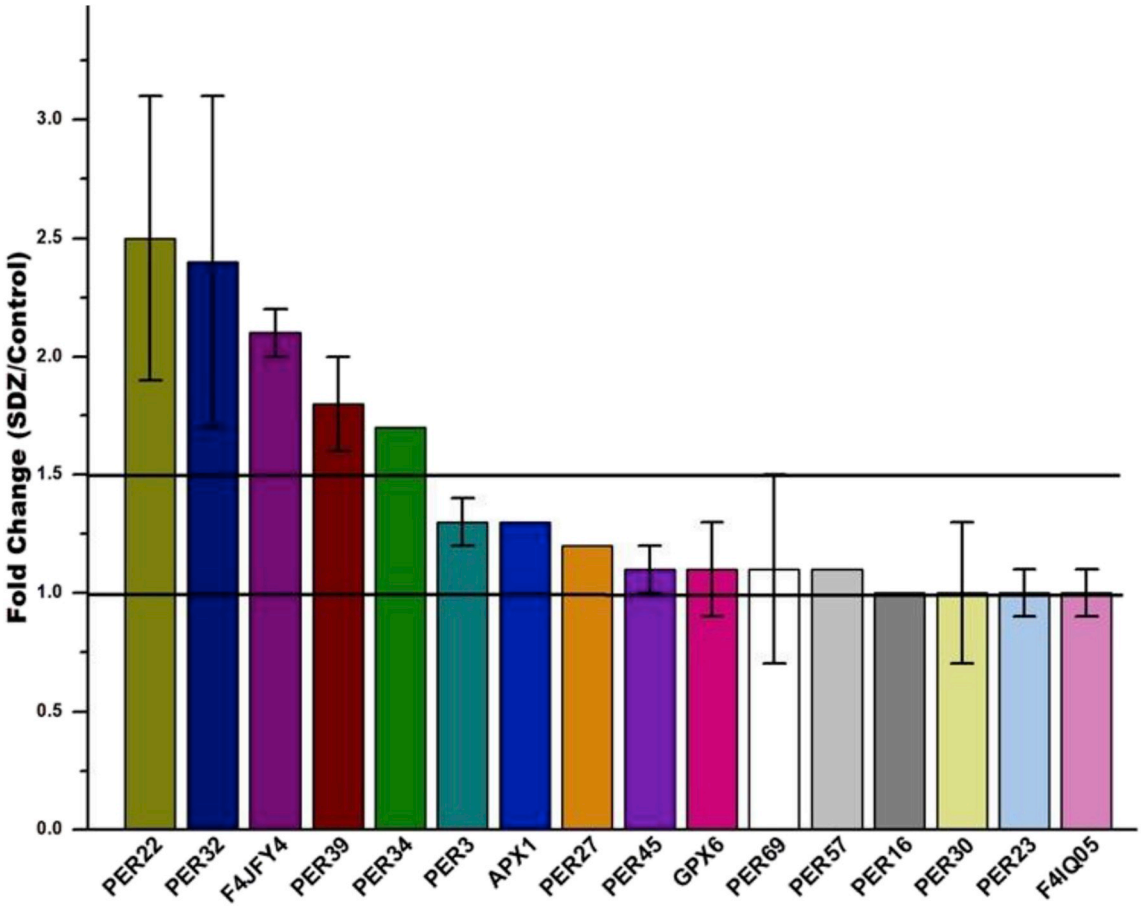

Fig. 2. Fold change variation for peroxidase enzymes in SDZ versus control roots. Error bars indicate standard deviation.

Alpha-toxin and **T**riacylglycerol lipase) (PLAT2) was found to be upregulated 1.6 folds compared to control and it has been reported that overexpression of PLAT domain-containing proteins conferred tolerance to abiotic stress (cold, drought and salt) (Hyun et al., 2014). An HSP20-like chaperone (HSP20), which was upregulated (1.8 folds) primarily functions by avoiding protein denaturation, maintaining native conformation and reorganizing denatured proteins and hence, is induced largely in response to heat, cold, salinity, oxidative and osmotic stresses (Park and Seo et al., 2015). The translationally controlled tumour protein homolog (TCTP) upregulated in this study was reported to be differentially expressed and regulated in abiotic stress conditions such as water limitation, cold and salinity (Lee and Lee, 2003; Vincent et al., 2007).

Universal Stress Protein (USP) was found to be upregulated and belongs to a class of stress-responsive proteins shown to be differentially regulated in salt, drought, cold, heat, and oxidative stress (Kerk et al., 2003; Ndimba et al., 2005; Persson et al., 2007). Inorganic pyrophosphatases (PPA6) are involved in germination, development and stress adaptive responses. Transgenic overexpression of PPA6 has been shown to enhance tolerance to abiotic stress (Gutiérrez-Luna et al., 2018). Similarly, a Nascent polypeptide-associated complex (NAC) protein and a Phospholipase D (PLD) - regulated protein 1 (PLDrp1) were upregulated, both of which were strongly induced in dehydration and salt stress (Karan and Subudhi, 2012; Ufer et al., 2017).

A rhodanese-like domain-containing protein, also known as High Arsenic Content 1 (HAC) protein, was upregulated. In addition, to being responsive to arsenate, it facilitates efflux of toxic arsenic from roots thereby preventing its accumulation and transport (Chao et al., 2014).

Glycine-rich RNA binding proteins (GRP7 and GRP8) upregulated in our study have been shown to be induced during oxidative stress (Schmidt et al., 2010) and these GRPs confer stress tolerance in *Arabidopsis* under dehydration and high salt stress conditions (Kim et al., 2008). Higher expression of transcripts and upregulation of dehydrins, specifically ERD14 and COR47, upregulated in this study, were reported in response to low temperature, salinity and in response to abscisic acid (ABA) (Nylander et al., 2001).

It is interesting that Jacalin-related lectin protein (JAL22) associated with response to various abiotic stresses was downregulated in our study. Similarly, Abebe et al. (2005) reported its downregulation in response to drought, dehydration and ABA.

3.5.1.2. Proteins related to ROS scavenging. Increased ROS generation has been reported in response to various abiotic stresses including antibiotics (Thounaojam et al., 2012; Xu et al., 2017). Increased MDA content in our study suggests that there was lipid peroxidation induced by oxidative stress in response to SDZ (Table 1). The proteome analysis indicated that several proteins related to oxidative stress with direct ROS-scavenging roles were upregulated in response to SDZ in comparison to the untreated control.

In total, there were four type III peroxidases (PODs) (PER 22, PER 32, PER 34 and PER 39) and one stromal ascorbate peroxidase (sAPX) found to be upregulated, while Annexin D1 (ANN1) with POD activity was downregulated in response to SDZ (Table 2). PODs are one of the major classes of antioxidant enzymes that directly catalyse the oxidation of certain electron donors concomitant with the disintegration of H_20_2. At the transcriptome level, several type III POD genes were highly expressed in *Arabidopsis* roots due to oxidative stress in response to salinity (Jiang and Deyholos, 2006). APX, a class I heme-peroxidases is one of the key enzymes in the ascorbate-glutathione cycle, one of the crucial hydrogen peroxide-detoxification systems in plant chloroplasts.

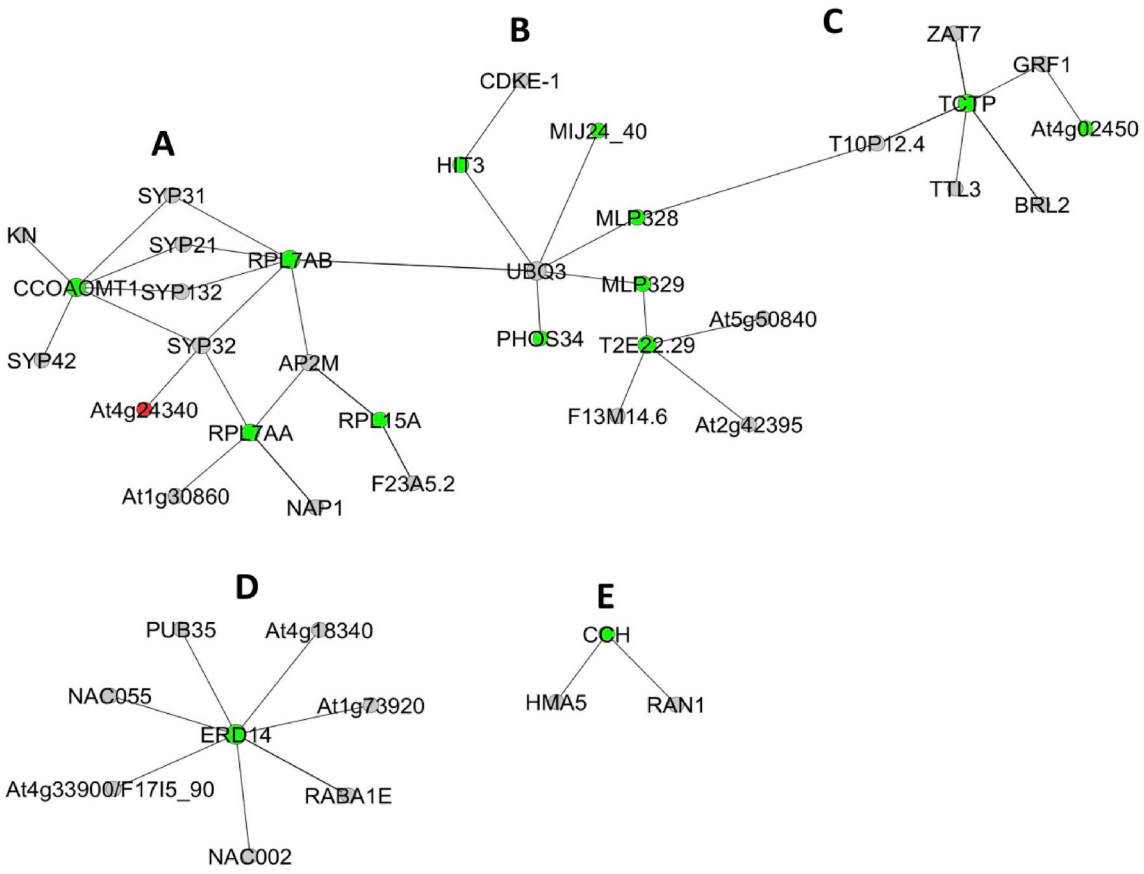

Fig. 3. Interactome analysis of the identified proteins. Colours indicate up regulated (Green), down regulated (Red) and experimentally determined interacting partners (Grey) from the database. Gene names are displayed. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Hence, is also considered as a marker for oxidative stress (Asada, 1992).

The sAPX identified in our study was upregulated 2 folds compared to the control (Table 2), supporting our notion of SDZ induced oxidative stress. Caverzan et al. (2012) reported differential regulation of specific isoforms of APXs in response to various environmental stress/stimuli.

To obtain an overall profile, fold change values of all the PODs identified in this study were compared (Fig. 2). In total, sixteen PODs were identified. Five PODs (PER 22, PER 32, sAPX, PER 39, PER 34) were found to be upregulated more than 1.5 folds compared to control. With a less stringent threshold to determine the fold change (> 1.2), three other PODs (PER 3, APX 1, PER 27) were found to be upregulated, while other PODs were unaltered and interestingly, not downregulated (Fig. 2). Thus, it is evident that the expression of several isoforms of PODs are stimulated in response to SDZ treatment in *Arabidopsis* roots, suggesting that PODs could be the major antioxidant enzymes functioning toward mitigating SDZ induced stress.

In addition to PODs, dehydrins ERD14, COR47 and Temperature induced lipocalin 1 (TIL) were found to be upregulated. Dehydrins are multifunctional proteins and their role in direct ROS scavenging and oxidative stress tolerance have been reported (Heyen et al., 2002; Jaffe et al., 2008).

3.5.1.3. Proteins with cytoprotective function. Abiotic stresses such as drought, high salinity, high temperature and cold ultimately lead to reduced free water available in the cell and result in dehydration. Dehydrins, including ERD14 and COR47 (upregulated 4.2 and 1.5 folds, respectively), are primarily induced in dehydration stress conditions and hence, are considered as molecular markers for identifying drought/dehydration stress and tolerance in plants (Graether and Boddington, 2014). Like other Late Embryogenesis Abundant (LEA) proteins, dehydrins are also accumulated in cells to maintain the cell volume and prevent cell collapse in response to dehydration (Hanin et al., 2011).

12S seed storage protein CRU1 (CRA1) belongs to the cupin superfamily of proteins. Although it is predominantly considered as a nutrient reservoir, upregulation in response to abiotic stress has also been reported (Wang et al., 2014). Considering that CRA1 had the highest fold change (4.3), we speculate that this protein could have similar roles to that of LEA proteins like dehydrins in preventing cell collapse.

3.5.1.4. Proteins related to PCD. TCTP is a multifunction protein that regulates several cellular processes. This protein, upregulated in this study, has been reported to prevent or inhibit the progression of programmed cell death (PCD) in *Arabidopsis* plants treated with PCD activators (Hoepflinger et al., 2013). Coherently, a metacaspase 4 (AMC4) was found to be upregulated and Curculin-like (Mannose-binding) lectin family protein (EP1GP) was downregulated in response to SDZ. Both these proteins are involved in regulating PCD (Hwang and Hwang, 2011; Watanabe and Lam, 2011). Regulation of these proteins suggests that PCD mechanisms are being modulated in response to stress conditions induced by SDZ.

3.5.2. Proteins related to metabolism

Caffeoyl-CoA O-methyltransferase 1 (CCOAOMT1), a protein related to phenylpropanoid pathway was upregulated. It catalyses monolignol formation, thus involved in lignification. It is also a crucial enzyme in various other phenylpropanoid metabolite biosynthesis including scopoletin and suberin, which were directly associated with abiotic stress response (Döll et al., 2018; Franke et al., 2012; Koeppe

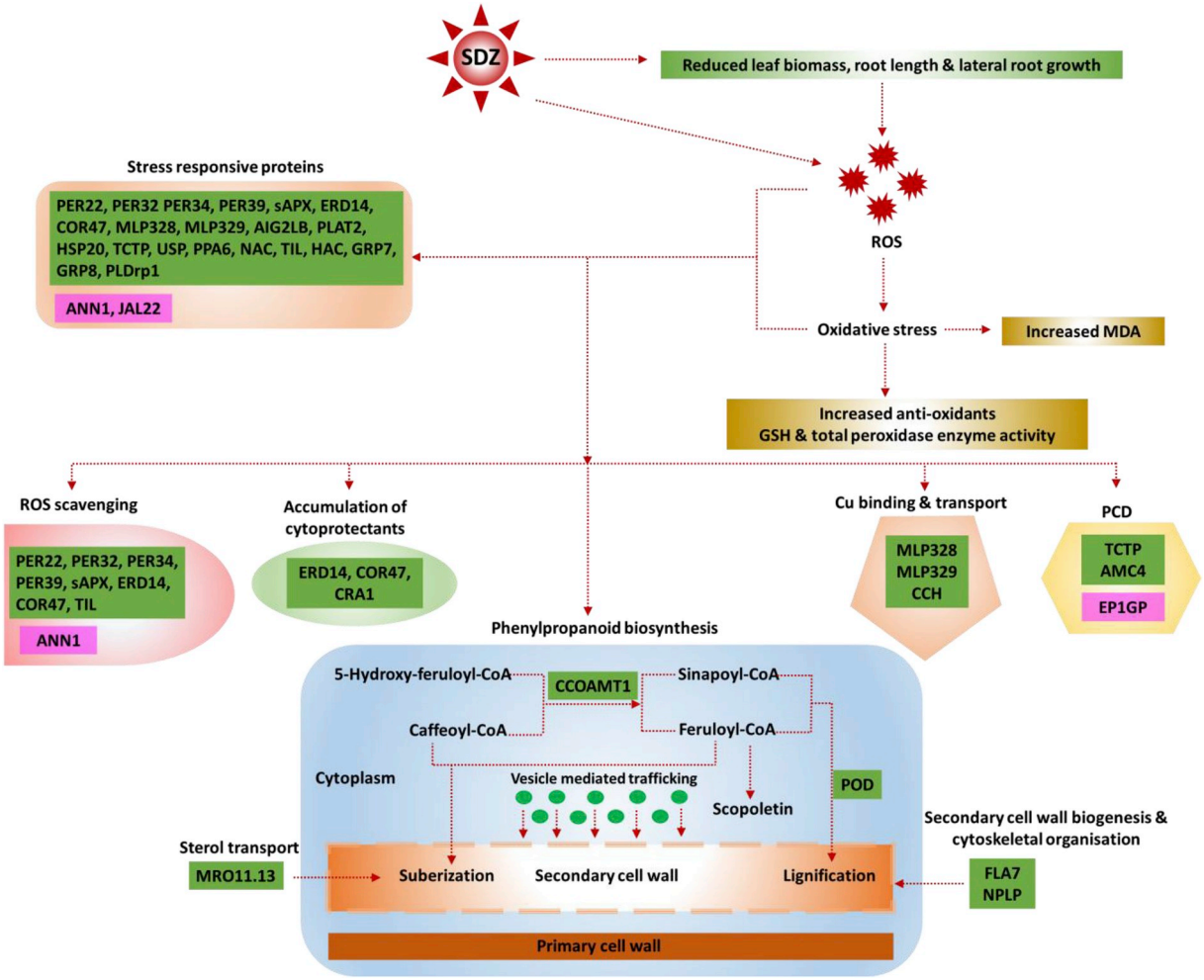

Fig. 4. Conceptual overview highlighting the probable molecular mechanisms modulated in response to SDZ in *Arabidopsis* roots.

et al., 1970).

Elevated levels of glucosinolate could be correlated with the accumulation of osmoprotective compounds (del Carmen Martínez-Ballesta et al., 2013). Thiocyanate methyltransferase 1 (HOL1), a key enzyme in the glucosinolate metabolism was upregulated in our study.

A glycine cleavage system H protein 2 (GDH2) involved in glyoxylate metabolism and glycine degradation and a NADH dehydrogenase [ubiquinone] iron-sulfur protein 8-A (NADHD) associated with the mitochondrial respiratory chain complex I assembly were upregulated. In addition, adenylylsulfatase (HINT1), which belongs to HIS triad family protein associated with purine ribonucleotide metabolism and sulfur metabolism was also upregulated. While a Phosphorylase superfamily protein (PSP) with nucleoside phosphorylase domain related to nucleoside metabolic process and lactoyl-glutathione lyase (GLX1) related to methylglyoxal degradation were downregulated.

In addition to ROS scavenging, type III PODs identified in this study could also be involved in several physiological, cellular and metabolic processes including auxin catabolism, lignification, and suberization in response to stress (Degenhardt and Gimmler, 2000).

3.5.3. Proteins related to transport

Copper transport protein (CCH), upregulated in our study, is involved in copper (Cu) homeostasis and transport. It was reported that this protein was upregulated in response to salt stress and cadmium exposure (Sarry et al., 2006; Jiang et al., 2007). Xu et al. (2017) examined the individual and combined effects of SDZ and copper on wheat seedlings and suggested that amending Cu at specific concentrations in soil could alleviate SDZ induced stress. Interestingly, MDA content was lower in plants treated with SDZ and with Cu at lower concentrations, indicating that Cu could have reduced the oxidative stress induced by SDZ. The mechanism underpinning the Cu mediated alleviation of SDZ induced stress is yet to be understood. Nonetheless, upregulation of this Cu transport protein in our study could be an indication supporting this observation.

An MD-2-related lipid recognition domain-containing protein (MRO11.13) associated with intercellular sterol transport was upregulated suggesting a probable increase in sterol transport. The significance of sterols in drought tolerance and regulation of ROS have been established in *Arabidopsis* (Posé et al., 2009). Inorganic phosphate transporter 1-1 (PHT1-1) is a transmembrane protein, which acts as a high-affinity transporter for inorganic phosphate and was shown that the overexpression of this symporter conferred arsenate sensitivity as it increased arsenate uptake (LeBlanc et al., 2013). Downregulation of this protein in our study suggests that altered preference in the transmembrane transporters could be one of the many mechanisms employed by plants to avoid or limit the import of hazardous compounds including SDZ.

3.5.4. Proteins related to transcription and translation

The protein F17H15.1/F17H15.1 (KHdp) upregulated 3.4 folds in this study consists of a KH domain with putative RNA binding function and has been reported to be an important upstream regulator of stress-responsive gene expression in *Arabidopsis* (Guan et al., 2013). A Methyl-CpG-binding domain (MBD) containing protein, found to be

upregulated in our study, was reported as an important factor that regulates DNA methylation, which is associated with transcriptional level silencing (Lang et al., 2015).

Structural constituents of ribosomes (RPS26C, RPL15A, RPL7AB, RPS12A, RPL23AB, RPL7AA) involved in translation were upregulated indicating a thrust in the protein synthesis, which can be corroborated to the observed increase in the total protein content (Table 1) and the upregulation of several proteins (42) (Table 2).

3.5.5. Proteins with other functions

A SOUL heme-binding-like protein (SOUL-1), which was upregulated is regarded as a cytosolic tetrapyrrole-carrier protein associated with free heme homeostasis (Lee et al., 2012). Upregulation of CRA1 - the 12S seed storage protein in response to SDZ in *Arabidopsis* roots is intriguing as these proteins are generally regarded as a nutrient reservoir for germinating seeds and its possible role as a cytoprotectant is discussed above. FAM10 family protein (FAM10) related to chaperone cofactor-dependent protein refolding, Fasciclin-like arabinogalactan protein 7 (FLA7) and Neurofilament like protein (NPLP) related secondary cell wall biogenesis and cytoskeleton structure, respectively were also upregulated.

3.6. Pathway analysis of the identified proteins

Among the identified proteins, CCOAOMT1 and POD superfamily were mapped to the phenylpropanoid biosynthetic pathway (Fig. S3), indicating that this pathway could be induced in response to SDZ. CCOAOMT1 is a crucial enzyme in monolignol biosynthesis catalysing the formation of feruloyl- CoA (substrate for scopoletin and suberin biosynthesis) and sinapoyl-CoA (substrate for syringyl lignin biosynthesis). Scopoletin accumulates in roots in response to osmotic stress with proposed function in ROS scavenging and forming a protective barrier (Döll et al., 2018); while increased deposition of suberin on roots with protective roles has been reported in drought, osmotic and heavy metal stress (Franke et al., 2012). PODs are involved in oxidative polymerization of monolignols to lignin and a positive association of lignification in response to various abiotic stress has been reported (Le Gall et al., 2015). Lignification and suberization are one of the primary responses of the plant, especially in roots, in response to drought, salinity and heavy metal stress (Franke et al., 2012; Le Gall et al., 2015). In accordance with the increased lignification observed in the roots of barley plants exposed to sulfonamide antibiotics (Michelini et al., 2013), it is probable that lignification and suberization of root cells could also be involved in the responses to SDZ in *Arabidopsis*.

3.7. Interactome of the identified proteins

Of the identified proteins, 26 proteins were found to be interacting with other proteins. Interestingly, several proteins were found to be interacting with Syntaxins associated with SNARE complex involved in vesicle fusion and docking - mediated intracellular transport (Fig. 3A).

This, in turn, redirects to the sub-cellular localization data where several proteins were predicted to be extracellularly secreted and intracellularly transported via the non-classical secretory pathway (Table 2). In addition, CCOAMT1 catalysing the formation of monolignols interacting with the SNARE complex proteins suggests that the monolignols could also be trafficked through vesicles to the secondary cell wall, wherein it is oxidatively polymerised to lignin by peroxidases.

Similarly, it has been indicated that constituents for the suberin biosynthesis are also transported through vesicles (Barros et al., 2015; Vishwanath et al., 2015). Multiple upregulated proteins were found to interact with a polyubiquitin protein (UBQ3) (Fig. 3B) suggesting that there could be ubiquitination-mediated degradation of these proteins to maintain protein homeostasis. TCTP1 - a multifunctional protein, which regulates several crucial cellular processes was found to be interacting with proteins related to auxin signalling and homeostasis, cell redox homeostasis, proteins involved in regulation of hormonal induction in response to stress and other proteins with direct/indirect roles in abiotic stress (Fig. 3C). Dehydrin ERD 14 was found to be interacting with crucial enzymes related to carbohydrate and lipid metabolism, a peroxisomal membrane protein and proteins involved in phosphorylation, jasmonic acid and ABA signalling pathways (Fig. 3D). Dehydrin - a multifunctional protein is established to interact with proteins and enzymes and maintain its native confirmation especially during dehydration and other abiotic stresses. Further, proteins involved in Cu binding and transport were also found to be interacting (Fig. 3E). In the interactome, several proteins related to ABA, auxin, jasmonic acid and brassinosteroid signalling pathways with a known interaction with SDZ-induced proteins evidenced in the present work were found. This suggests that there could be multiple phytohormonal signalling cascades operating with probable cross-talks at various levels.

3.8. Changes in peroxidase enzyme activity

To substantiate the involvement of PODs in response to SDZ and validate the proteomic results, the total POD enzyme activity was determined. There was an overall increase in the POD activity in all the concentrations of SDZ with the highest at 0.5 μM (Table 1), supporting the observed upregulation of PODs (PER 22, PER 32, PER 34 and PER 39) (Table 2; Fig. 2). Increased anti-oxidant enzyme activities were observed in plants exposed to antibiotics (Xie et al., 2011; Nie et al., 2013) and increased POD activity was reported in wheat seedlings treated with SDZ (Xu et al., 2017). It is ascertained that PODs are involved in the responses to SDZ treatment either in ROS-detoxification or in other responses such as lignification and suberization. Bialk et al. (2005) provided direct evidence of PODs-mediated covalent cross-coupling of the sulfonamide sulfamethazine with phenolic substances. This leads to the notion if such sequestration mechanisms mediated by PODs would exist in plants to limit uptake, bioavailability and biological activity of SDZ.

4. Conceptual overview and concluding remarks

Results from this study might be depicted as a comprehensive conceptual snapshot (Fig. 4). There was a significant reduction in leaf biomass, root length and lateral root growth. Congruently, Xu et al. (2017) have reported that SDZ adversely affects the leaf biomass, root formation and length. As a consequence of impaired root growth and function, water uptake and balance would be disturbed, mimicking water deficit-like conditions. This could have resulted in excessive ROS generation as evidenced by increased MDA content, indicating oxidative stress and in turn ROS-induced lipid peroxidation. This was further substantiated by upregulation of several proteins related to oxidative stress and increased anti-oxidants (GSH and PODs). ROS could be one of the primary signalling molecules inducing a cascade of stress responses resulting in upregulation of several stress-related proteins. Their crucial role as stress signalling molecules has been well established (Choudhury et al., 2017).

Comprehensively, in response to SDZ, several proteins related to drought, salinity and heavy metal stress were upregulated, many of which had direct ROS-scavenging roles, notably PODs and dehydrins. Upregulation of dehydrins (ERD14, COR47) and CRA1 - the seed storage protein with a putative cytoprotective function was also observed. There was also an indication of phenylpropanoid biosynthesis. The final outcome of CCOAMT1 and POD is lignification, suberization and accumulation of stress-responsive metabolites such as scopoletin. Along with peroxide detoxification and oxidative cross-linking of monolignols, a possible role of POD in cross-coupling SDZ to phenolic compounds as a means of transformation to limit bioavailability and mobility has also been hypothesised. The interactome analysis also provided an indication of the existing proposed model of lignin monomers and suberin constituents transported through vesicles to the

secondary cell wall. Further, there was an upregulation of proteins involved in sterol transport, secondary cell wall architecture and cytoskeletal organization indicating that there could be rearrangements in the cell wall, possibly by lignification and suberization in response to SDZ. Proteins related to Cu binding and transport and proteins associated with PCD were also upregulated. We contemplate that these could be a few of the mechanisms among the intricate, intertwined and multifaceted responses induced by SDZ in *Arabidopsis* roots.

SDZ at higher concentrations reduced the overall growth, leaf biomass and root length. Biochemical analysis suggested that there is oxidative stress in response to SDZ. Taking into considerations the possible cytotoxic effects of SDZ, the exact mechanism triggering the oxidative stress upon treatment needs to be determined. Increased GSH levels and upregulation of several PODs validated with total POD enzyme activity suggest that these could be the major anti-oxidants responding to SDZ treatment. Proteome analysis indicated that the major proportion of the upregulated proteins were multifunctional stress-responsive proteins. It is possible that SDZ treatment triggers multiple stress responsive pathways in *Arabidopsis* roots.

5. Declarations of interest

None.

Acknowledgements

This work was supported by the grant of the University of Padova, Italy (DOR1621018). The authors wish to thank the Cassa di Risparmio di Padova e Rovigo (Cariparo) Holding for funding the acquisition of the LTQ-Orbitrap XL mass spectrometer. Nisha Sharma was supported by a Cariparo Fellowship for Foreign students. Leonard Barnabas Ebinezer was supported by the grant of the University of Padova (BIRD188182).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoenv.2019.04.008.

References

Abebe, T., Skadsen, R.W., Kaeppler, H.F., 2005. A proximal upstream sequence controls tissue-specific expression of Lem 2, a salicylate-inducible barley lectin-like gene. Planta 221 (2), 170–183. https://doi.org/10.1007/s00425-004-1429-9.

Asada, K., 1992. Ascorbate peroxidase-a hydrogen peroxide-scavenging enzyme in plants. Physiol. Plantarum 85 (2), 235–241. https://doi.org/10.1111/j.1399-3054.1992.tb04728.x.

Barros, J., Serk, H., Granlund, I., Pesquet, E., 2015. The cell biology of lignification in higher plants. Ann. Bot. 115 (7), 1053–1074. https://doi.org/10.1093/aob/mcv046.

Bialk, H.M., Simpson, A.J., Pedersen, J.A., 2005. Cross-coupling of sulfonamide antimicrobial agents with model humic constituents. Environ. Sci. Technol. 39 (12), 4463–4473. https://doi.org/10.1021/es0500916.

Boxall, A.B., Blackwell, P., Cavallo, R., Kay, P., Tolls, J., 2002. The sorption and transport of a sulphonamide antibiotic in soil systems. Toxicol. Lett. 131 (1–2), 19–28. https://doi.org/10.1016/S0378-4274(02)00063-2.

Boxall, A.B., Johnson, P., Smith, E.J., Sinclair, C.J., Stutt, E., Levy, L.S., 2006. Uptake of veterinary medicines from soils into plants. J. Agric. Food Chem. 54 (6), 2288–2297. https://doi.org/10.1021/jf053041t.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72 (1–2), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

Carazzolle, M.F., de Carvalho, L.M., Slepicka, H.H., Vidal, R.O., Pereira, G.A.G., Kobarg, J., et al., 2014. IIS - integrated Interactome System: a web-based platform for the annotation, analysis and visualization of protein-metabolitegene-drug interactions by integrating a variety of data sources and tools. PLoS One 9, e100385. https://doi.org/10.1371/journal.pone.0100385.

Carter, L.J., Harris, E., Williams, M., Ryan, J.J., Kookana, R.S., Boxall, A.B., 2014. Fate and uptake of pharmaceuticals in soil–plant systems. J. Agric. Food Chem. 62 (4), 816–825. https://doi.org/10.1021/jf404282y.

Carvalho, P.N., Basto, M.C.P., Almeida, C.M.R., Brix, H., 2014. A review of plant–pharmaceutical interactions: from uptake and effects in crop plants to phytoremediation in constructed wetlands. Environ. Sci. Pollut. Control Ser. 21 (20), 11729–11763. https://doi.org/10.1007/s11356-014-2550-3.

Caverzan, A., Passaia, G., Rosa, S.B., Ribeiro, C.W., Lazzarotto, F., Margis-Pinheiro, M., 2012. Plant responses to stresses: role of ascorbate peroxidase in the antioxidant protection. Genet. Mol. Biol. 35 (4), 1011–1019. https://doi.org/10.1590/S1415-47572012000600016.

Chao, D.Y., Chen, Y., Chen, J., Shi, S., Chen, Z., Wang, C., Salt, D.E., 2014. Genome-wide association mapping identifies a new arsenate reductase enzyme critical for limiting arsenic accumulation in plants. PLoS Biol. 12 (12), e1002009. https://doi.org/10.1371/journal.pbio.1002009.

Chen, J.-Y., Dai, X.-F., 2010. Cloning and characterization of the *Gossypium hirsutum* major latex protein gene and functional analysis in *Arabidopsis thaliana*. Planta 231 (4), 861–873. https://doi: 10.1007/s00425-009-1092-2.

Choudhury, F.K., Rivero, R.M., Blumwald, E., Mittler, R., 2017. Reactive oxygen species, abiotic stress and stress combination. Plant J. 90 (5), 856–867. https://doi: 10.1111/tpj.13299.

De Liguoro, M., Poltronieri, C., Capolongo, F., Montesissa, C., 2007. Use of sulfadimethoxine in intensive calf farming: evaluation of transfer to stable manure and soil. Chemosphere 68 (4), 671–676. https://doi.org/10.1016/j.chemosphere.2007.02.009.

De Rosa, E., Checchetto, V., Franchin, C., Bergantino, E., Berto, P., Szabò, I., Costantini, P., 2015. [NiFe]-hydrogenase is essential for cyanobacterium *Synechocystis* sp. PCC 6803 aerobic growth in the dark. Sci. Rep. 5, 12424. https://doi.org/10.1038/srep12424.

Degenhardt, B., Gimmler, H., 2000. Cell wall adaptations to multiple environmental stresses in maize roots. J. Exp. Bot. 51 (344), 595–603. https://doi.org/10.1093/jexbot/51.344.595.

del Carmen Martínez-Ballesta, M., Moreno, D.A., Carvajal, M., 2013. The physiological importance of glucosinolates on plant response to abiotic stress in Brassica. Int. J. Mol. Sci. 14 (6), 11607–11625. https://doi.org/10.3390/ijms140611607.

Dixon, D.P., Skipsey, M., Edwards, R., 2010. Roles for glutathione transferases in plant secondary metabolism. Phytochemistry 71 (4), 338–350. https://doi.org/10.1016/j.phytochem.2009.12.012.

Döll, S., Kuhlmann, M., Rutten, T., Mette, M.F., Scharfenberg, S., Petridis, A., Mock, H.P., 2018. Accumulation of the coumarin scopolin under abiotic stress conditions is mediated by the *Arabidopsis thaliana* THO/TREX complex. Plant J. 93 (3), 431–444. https://doi.org/10.1111/tpj.13797.

Fleming, A., 1944. The discovery of penicillin. Br. Med. Bull. 2 (1), 4–5.

Fleming, A., 1946. Penicillin. Its Practical Application. Butterworth And Co. Publishers Ltd., London.

Förster, M., Laabs, V., Lamshoft, M., Groeneweg, J., Zuhlke, S., Spiteller, M., Amelung, W., 2009. Sequestration of manure-applied sulfadiazine residues in soils. Environ. Sci. Technol. 43 (6), 1824–1830. https://doi.org/10.1021/es8026538.

Foyer, C.H., Noctor, G., 2005. Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. Plant Cell Environ. 28 (8), 1056–1071. https://doi.org/10.1111/j.1365-3040.2005.01327.x.

Foyer, C.H., Noctor, G., 2011. Ascorbate and glutathione: the heart of the redox hub. Plant Physiol. 155 (1), 2–18. https://doi.org/10.1104/pp.110.167569.

Franke, R.B., Dombrink, I., Schreiber, L., 2012. Suberin goes genomics: use of a short living plant to investigate a long lasting polymer. Front. Plant Sci. 3, 4. https://doi.org/10.3389/fpls.2012.00004.

Giaretta, S., Prasad, D., Forieri, I., Vamerali, T., Trentin, A.R., Wirtz, M.,, et al., 2017. Apoplastic gamma-glutamyl transferase activity encoded by GGT1 and GGT2 is important for vegetative and generative development. Plant Physiol. Biochem. 115, 44–56. https://doi.org/10.1016/j.plaphy.2017.03.007.

Graether, S.P., Boddington, K.F., 2014. Disorder and function: a review of the dehydrin protein family. Front. Plant Sci. 5, 576. https://doi.org/10.3389/fpls.2014.00576.

Guan, Q., Wen, C., Zeng, H., Zhu, J., 2013. A KH domain-containing putative RNA-binding protein is critical for heat stress-responsive gene regulation and thermotolerance in Arabidopsis. Mol. Plant 6 (2), 386–395. https://doi.org/10.1093/mp/sss119.

Gutiérrez-Luna, F.M., Hernández-Domínguez, E.E., Valencia-Turcotte, L.G., Rodriguez-Sotres, R., 2018. Pyrophosphate and pyrophosphatases in plants, their involvement in stress responses and their possible relationship to secondary metabolism. Plant Sci. 267, 11–19. https://doi.org/10.1016/j.plantsci.2017.10.016.

Hanin, M., Brini, F., Ebel, C., Toda, Y., Takeda, S., Masmoudi, K., 2011. Plant dehydrins and stress tolerance: versatile proteins for complex mechanisms. Plant Signal. Behav. 6 (10), 1503–1509. https://doi.org/10.4161/psb.6.10.17088.

Heath, R.L., Packer, L., 1968. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125 (1), 189–198. https://doi.org/10.1016/0003-9861(68)90654-1.

Hendawey, M.H., Kamel, H.A., 2015. Distribution and bioaccumulation of radiocarbon (14C) into biochemical components of wheat in relation to antioxidant system under saline stress conditions. J. Am. Sci. 11 (10), 8–21. https://doi:10.7537/marsjas111015.02.

Hewitt, E.J., Dickes, G.J., 1961. Spectrophotometric measurements on ascorbic acid and their use for the estimation of ascorbic acid and dehydroascorbic acid in plant tissues. Biochem. J. 78 (2), 384.

Heyen, B.J., Alsheikh, M.K., Smith, E.A., Torvik, C.F., Seals, D.F., Randall, S.K., 2002. The calcium-binding activity of a vacuole-associated, dehydrin-like protein is regulated by phosphorylation. Plant Physiol. 130 (2), 675–687. https://doi.org/10.1104/pp.002550.

Hodges, D.M., DeLong, J.M., Forney, C.F., Prange, R.K., 1999. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta 207 (4), 604–611. https://doi.org/10.1007/s004250050524.

Hoepflinger, M.C., Reitsamer, J., Geretschlaeger, A.M., Mehlmer, N., Tenhaken, R., 2013. The effect of translationally controlled tumour protein (TCTP) on programmed cell

death in plants. BMC Plant Biol. 13, 135. https://doi.org/10.1186/1471-2229-13-135.
Huschek, G., Hollmann, D., Kurowski, N., Kaupenjohann, M., Vereecken, H., 2008. Re-evaluation of the conformational structure of sulfadiazine species using NMR and ab initio DFT studies and its implication on sorption and degradation. Chemosphere 72 (10), 1448–1454. https://doi.org/10.1016/j.chemosphere.2008.05.038.
Huseby, N.E., Stromme, J.H., 1974. Practical points regarding routine determination of γ-glutamyl transferase (γ-GT) in serum with a kinetic method at 37 C. Scand. J. Clin. Lab. Investig. 34 (4), 357–363. https://doi.org/10.3109/00365517409049892.
Hwang, I.S., Hwang, B.K., 2011. The pepper mannose-binding lectin gene CaMBL1 is required to regulate cell death and defense responses to microbial pathogens. Plant Physiol. 155 (1), 447–463. https://doi.org/10.1104/pp.110.164848.
Hyun, T.K., van der Graaff, E., Albacete, A., Eom, S.H., Großkinsky, D.K., Böhm, H., Roitsch, T., 2014. The Arabidopsis PLAT domain protein 1 is critically involved in abiotic stress tolerance. PLoS One 9 (11), e112946. https://doi.org/10.1371/journal.pone.0112946.
Jaffe, R., Charron, T., Puley, G., Dick, A., Strauss, B.H., 2008. Microvascular obstruction and the no-reflow phenomenon after percutaneous coronary intervention. Circulation 117 (24), 3152–3156. https://doi.org/10.1161/circulationaha.107.742312.
Jahanbakhsh, G.S., Chilan, H., Razavi, K., 2017. Effects of water deficit on the physiological response, total protein, and gene expression of RAB17 in wheat (*Triticum aestivum*). J. Plant Process Func. 5 (18), 35–42.
Jechalke, S., Kopmann, C., Rosendahl, I., Groeneweg, J., Weichelt, V., Krögerrecklenfort, E., Heuer, H., 2013. Increased abundance and transferability of resistance genes after field application of manure from sulfadiazine-treated pigs. Appl. Environ. Microbiol. 79 (5), 1704–1711. https://doi.org/10.1128/AEM.03172-12.
Jiang, Y.Q., Deyholos, M.K., 2006. Comprehensive transcriptional profiling of NaCl-stressed *Arabidopsis* roots reveals novel classes of responsive genes. BMC Plant Biol. 6, 25. https://doi.org/10.1186/1471-2229-6-25.
Jiang, Y., Yang, B., Harris, N.S., Deyholos, M.K., 2007. Comparative proteomic analysis of NaCl stress-responsive proteins in Arabidopsis roots. J. Exp. Bot. 58 (13), 3591–3607. https://doi.org/10.1093/jxb/erm207.
Kanehisa, M., Furumichi, M., Tanabe, M., Sato, Y., Morishima, K., 2017. KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Res. 45, D353–D361. https://doi.org/10.1093/nar/gkw1092.
Kang, D.H., Gupta, S., Rosen, C., Fritz, V., Singh, A., Chander, Y., et al., 2013. Antibiotic uptake by vegetable crops from manure-applied soils. J. Agric. Food Chem. 61 (42), 9992–10001. https://doi.org/10.1021/jf404045m.
Karan, R., Subudhi, P.K., 2012. Overexpression nascent polypeptide associated gene complex (*SaβNAC*) of Spartine alternifora improves tolerance to salinity and drought in transgenic Arabidopsis. Biochem. Biophys. Res. Commun. 424 (4), 747–752. https://doi.org/10.1016/j.bbrc.2012.07.023.
Karcı, A., Balcıoğlu, I.A., 2009. Investigation of the tetracycline, sulfonamide, and fluoroquinolone antimicrobial compounds in animal manure and agricultural soils in Turkey. Sci. Total Environ. 407 (16), 4652–4664. https://doi.org/10.1016/j.scitotenv.2009.04.047.
Karthikeyan, K.G., Meyer, M.T., 2006. Occurrence of antibiotics in wastewater treatment facilities in Wisconsin, USA. Sci. Total Environ. 361 (1–3), 196–207. https://doi.org/10.1016/j.scitotenv.2005.06.030.
Kerk, D., Bulgrien, J., Smith, D.W., Gribskov, M., 2003. *Arabidopsis* proteins containing similarity to the universal stress protein domain of bacteria. Plant Physiol. 131 (3), 1209–1219. https://doi.org/10.1104/pp.102.016006.
Kim, J.S., Jung, H.J., Lee, H.J., Kim, K.A., Goh, C.H., Woo, Y., Kang, H., 2008. Glycine-rich RNA-binding protein 7 affects abiotic stress responses by regulating stomata opening and closing in *Arabidopsis thaliana*. Plant J. 55 (3), 455–466. https://doi.org/10.1111/j.1365-313X.2008.03518.x.
Koeppe, D.E., Rohrbaugh, L.M., Rice, E.L., Wender, S.H., 1970. The effect of age and chilling temperature on the concentration of scopolin and caffeoylquinic acids in tobacco. Physiol. Plantarum 23 (2), 258–266. https://doi.org/10.1111/j.1399-3054.1970.tb06415.x.
Lang, Z., Lei, M., Wang, X., Tang, K., Miki, D., Zhang, H., Yan, J., 2015. The methyl-CpG-binding protein MBD7 facilitates active DNA demethylation to limit DNA hyper-methylation and transcriptional gene silencing. Mol. Cell 57 (6), 971–983. https://doi.org/10.1016/j.molcel.2015.01.009.
Le Gall, H., Philippe, F., Domon, J.M., Gillet, F., Pelloux, J., Rayon, C., 2015. Cell wall metabolism in response to abiotic stress. Plants 4 (1), 112–166. https://doi.org/10.3390/plants4010112.
LeBlanc, M.S., McKinney, E.C., Meagher, R.B., Smith, A.P., 2013. Hijacking membrane transporters for arsenic phytoextraction. J. Biotechnol. 163 (1), 1–9. https://doi.org/10.1016/j.jbiotec.2012.10.013.
Lee, J.Y., Lee, D.H., 2003. Use of serial analysis of gene expression technology to reveal changes in gene expression in *Arabidopsis* pollen undergoing cold stress. Plant Physiol. 132 (2), 517–529. https://doi.org/10.1104/pp.103.020511.
Lee, H.J., Mochizuki, N., Masuda, T., Buckhout, T.J., 2012. Disrupting the bimolecular binding of the haem-binding protein 5 (AtHBP5) to haem oxygenase 1 (HY1) leads to oxidative stress in Arabidopsis. J. Exp. Bot. 63 (16), 5967–5978. https://doi.org/10.1093/jxb/ers242.
Li, Y., Han, D., Sommerfeld, M., Hu, Q., 2011. Photosynthetic carbon partitioning and lipid production in the oleaginous microalga Pseudochlorococcum sp. (Chlorophyceae) under nitrogen-limited conditions. Bioresour. Technol. 102 (1), 123–129. https://doi.org/10.1016/j.biortech.2010.06.036.
Manzetti, S., Ghisi, R., 2014. The environmental release and fate of antibiotics. Mar. Pollut. Bull. 79 (1–2), 7–15. https://doi.org/10.1016/j.marpolbul.2014.01.005.
Masi, A., Ghisi, R., Ferretti, M., 2002. Measuring low-molecular-weight thiols by detecting the fluorescence of their SBD-derivatives: application to studies of diurnal and UV-B induced changes in *Zea mays* L. J. Plant Physiol. 159 (5), 499. https://doi.org/10.1078/0176-1617-00655.
Masi, A., Trentin, A.R., Agrawal, G.K., Rakwal, R., 2015. Gamma-glutamyl cycle in plants: a bridge connecting the environment to the plant cell? Front. Plant Sci. 6, 252. https://doi.org/10.3389/fpls.2015.00252.
Michelini, L., Reichel, R., Werner, W., Ghisi, R., Thiele-Bruhn, S., 2012. Sulfadiazine uptake and effects on *Salix fragilis* L. and *Zea mays* L. plants. Water, Air, Soil Pollut. 223 (8), 5243–5257. https://doi.org/10.1007/s11270-012-1275-5.
Michelini, L., La Rocca, N., Rascio, N., Ghisi, R., 2013. Structural and functional alterations induced by two sulfonamide antibiotics on barley plants. Plant Physiol. Biochem. 67, 55–62. https://doi.org/10.1016/j.plaphy.2013.02.027.
Migliore, L., Brambilla, G., Cozzolino, S., Gaudio, L., 1995. Effect on plants of sulphadimethoxine used in intensive farming (Panicum miliaceum, Pisum sativum and Zea mays). Agric. Ecosyst. Environ. 52 (2–3), 103–110. https://doi.org/10.1016/0167-8809(94)00549-T.
Migliore, L., Civitareale, C., Brambilla, G., Di Delupis, G.D., 1997. Toxicity of several important agricultural antibiotics to Artemia. Water Res. 31 (7), 1801–1806. https://doi.org/10.1016/S0043-1354(96)00412-5.
Migliore, L., Rotini, A., Cerioli, N.L., Cozzolino, S., Fiori, M., 2010. Phytotoxic antibiotic sulfadimethoxine elicits a complex hormetic response in the weed *Lythrum salicaria* L. Dose Res. 8, 414–427. https://doi.org/10.2203/dose-response.09-033.
Mirzajani, F., Askari, H., Hamzelou, S., Schober, Y., Römpp, A., Ghassempour, A., Spengler, B., 2014. Proteomics study of silver nanoparticles toxicity on *Oryza sativa* L. Ecotoxicol. Environ. Saf. 108, 335–339. https://doi.org/10.1016/j.ecoenv.2014.07.013.
Mittler, R., Vanderauwera, S., Gollery, M., Van Breusegem, F., 2004. Reactive oxygen gene network of plants. Trends Plant Sci. 9 (10), 490–498. https://doi.org/10.1016/j.tplants.2004.08.009.
Ndimba, B.K., Chivasa, S., Simon, W.J., Slabas, A.R., 2005. Identification of Arabidopsis salt and osmotic stress responsive proteins using two-dimensional difference gel electrophoresis and mass spectrometry. Proteomics 5 (16), 4185–4196. https://doi.org/10.1002/pmic.200401282.
Nie, X.P., Liu, B.Y., Yu, H.J., Liu, W.Q., Yang, Y.F., 2013. Toxic effects of erythromycin, ciprofloxacin and sulfamethoxazole exposure to the antioxidant system in *Pseudokirchneriella subcapitata*. Environ. Pollut. 172, 23–32. https://doi.org/10.1016/j.envpol.2012.08.013.
Nylander, M., Svensson, J., Palva, E.T., Welin, B.V., 2001. Stress-induced accumulation and tissue-specific localization of dehydrins in *Arabidopsis thaliana*. Plant Mol. Biol. 45 (3), 263–279. https://doi: 10.1023/A:1006469128280.
Pan, M., Chu, L.M., 2016. Adsorption and degradation of five selected antibiotics in agricultural soil. Sci. Total Environ. 545, 48–56. https://doi.org/10.1016/j.scitotenv.2015.12.040.
Pan, M., Wong, C.K., Chu, L.M., 2014. Distribution of antibiotics in wastewater-irrigated soils and their accumulation in vegetable crops in the Pearl River Delta, Southern China. J. Agric. Food Chem. 62 (46), 11062–11069. https://doi.org/10.1021/jf503850v.
Park, C.J., Seo, Y.S., 2015. Heat shock proteins: a review of the molecular chaperones for plant immunity. Plant Pathol. J. 31 (4), 323. https://doi.org/10.5423/PPJ.RW.08.2015.0150.
Paulose, B., Chhikara, S., Coomey, J., Jung, H.I., Vatamaniuk, O., Dhankher, O.P., 2013. A γ-glutamyl cyclotransferase protects Arabidopsis plants from heavy metal toxicity by recycling glutamate to maintain glutathione homeostasis. Plant Cell tpc-113. https://doi.org/10.1105/tpc.113.111815.
Persson, Ö., Valadi, Å., Nyström, T., Farewell, A., 2007. Metabolic control of the Escherichia coli universal stress protein response through fructose-6-phosphate. Mol. Microbiol. 65 (4), 968–978. https://doi.org/10.1111/j.1365-2958.2007.05838.x.
Posé, D., Castanedo, I., Borsani, O., Nieto, B., Rosado, A., Taconnat, L., Botella, M.A., 2009. Identification of the Arabidopsis dry 2/sqe1-5 mutant reveals a central role for sterols in drought tolerance and regulation of reactive oxygen species. Plant J. 59 (1), 63–76. https://doi.org/10.1111/j.1365-313X.2009.03849.x.
Qiu, N., Liu, Q., Li, J., Zhang, Y., Wang, F., Gao, J., 2017. Physiological and transcriptomic responses of Chinese cabbage (Brassica rapa L. ssp. pekinensis) to salt stress. Int. J. Mol. Sci. 18 (9), 1953. https://doi.org/10.3390/ijms18091953.
Ranieri, A., Petacco, F., Castagna, A., Soldatini, G.F., 2000. Redox state and peroxidase system in sunflower plants exposed to ozone. Plant Sci. 159 (1), 159–167. https://doi.org/10.1016/S0168-9452(00)00352-6.
Resmini, G., Rizzo, S., Franchin, C., Zanin, R., Penzo, C., Pegoraro, S., Manfioletti, G., 2017. HMGA1 regulates the Plasminogen activation system in the secretome of breast cancer cells. Sci. Rep. 7 (1), 11768. https://doi.org/10.1038/s41598-017-11409-4.
Reymond, P., Weber, H., Damond, M., Farmer, E.E., 2000. Differential gene expression in response to mechanical wounding and insect feeding in Arabidopsis. Plant Cell 12 (5), 707–719. https://doi.org/10.1105/tpc.12.5.707.
Roomi, S., Masi, A., Conselvan, G.B., Trevisan, S., Quaggiotti, S., Pivato, M., et al., 2018. Protein profiling of *Arabidopsis* roots treated with humic substances: insights into the metabolic and interactome networks. Front. Plant Sci. 9 (1812). https://doi.org/10.3389/fpls.2018.01812.
Rosendahl, I., Siemens, J., Groeneweg, J., Linzbach, E., Laabs, V., Herrmann, C., Amelung, W., 2011. Dissipation and sequestration of the veterinary antibiotic sulfadiazine and its metabolites under field conditions. Environ. Sci. Technol. 45 (12), 5216–5222. https://doi.org/10.1021/es200326t.
Sarmah, A.K., Meyer, M.T., Boxall, A.B., 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere 65 (5), 725–759. https://doi.org/10.1016/j.chemosphere.2006.03.026.
Sarry, J.E., Kuhn, L., Ducruix, C., Lafaye, A., Junot, C., Hugouvieux, V.,, et al., 2006. The early responses of Arabidopsis thaliana cells to cadmium exposure explored by protein and metabolite profiling analyses. Proteomics 6 (7), 2180–2198. https://doi.org/

10.1002/pmic.200500543.
Scandalios, J.G., 2005. Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defenses. Braz. J. Med. Biol. Res. 38 (7), 995–1014. https://doi.org/10.1590/S0100-79X2005000700003.
Schmidt, B., Ebert, J., Lamshöft, M.A.R.C., Thiede, B., Schumacher-Buffel, R., Ji, R., Schäffer, A., 2008. Fate in soil of 14C-sulfadiazine residues contained in the manure of young pigs treated with a veterinary antibiotic. J. Environ. Sci. Health 43 (1), 8–20. https://doi.org/10.1080/03601230701734824.
Schmidt, F., Marnef, A., Cheung, M.K., Wilson, I., Hancock, J., Staiger, D., Ladomery, M., 2010. A proteomic analysis of oligo (dT)-bound mRNP containing oxidative stress-induced Arabidopsis thaliana RNA-binding proteins ATGRP7 and ATGRP8. Mol. Biol. Rep. 37 (2), 839–845. https://doi.org/10.1007/s11033-009-9636-x.
Schwarz, J., Aust, M.O., Thiele-Bruhn, S., 2010. Metabolites from fungal laccase-catalysed transformation of sulphonamides. Chemosphere 81 (11), 1469–1476. https://doi.org/10.1016/j.chemosphere.2010.08.053.
Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., et al., 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 13, 2498–2504. https://doi.org/10.1101/gr.1239303.
Stanley Kim, H., Yu, Y., Snesrud, E.C., Moy, L.P., Linford, L.D., Haas, B.J., Quackenbush, J., 2005. Transcriptional divergence of the duplicated oxidative stress-responsive genes in the Arabidopsis genome. Plant J. 41 (2), 212–220. https://doi.org/10.1111/j.1365-313X.2004.02295.x.
Tasho, R.P., Cho, J.Y., 2016. Veterinary antibiotics in animal waste, its distribution in soil and uptake by plants: a review. Sci. Total Environ. 563, 366–376. https://doi.org/10.1016/j.scitotenv.2016.04.140.
Thounaojam, T.C., Panda, P., Mazumdar, P., Kumar, D., Sharma, G.D., Sahoo, L., Sanjib, P., 2012. Excess copper induced oxidative stress and response of antioxidants in rice. Plant *Physiol. Biochem.* 53, 33–39. https://doi.org/10.1016/j.plaphy.2012.01.006.
Thiele-Bruhn, S., 2003. Pharmaceutical antibiotic compounds in soils. J. Plant Nutr. Soil Sci. 166 (2), 145–167. https://doi.org/10.1002/jpln.200390023.
Tolin, S., Arrigoni, G., Trentin, A.R., Veljovic-Jovanovic, S., Pivato, M., Zechman, B., Masi, A., 2013. Biochemical and quantitative proteomics investigations in Arabidopsis ggt1 mutant leaves reveal a role for the gamma-glutamyl cycle in plant's adaptation to environment. Proteomics 13 (12–13), 2031–2045. https://doi.org/10.1002/pmic.201200479.
Trentin, A.R., Pivato, M., Mehdi, S.M., Barnabas, L.E., Giaretta, S., Fabrega-Prats, M., Masi, A., 2015. Proteome readjustments in the apoplastic space of Arabidopsis thaliana ggt1 mutant leaves exposed to UV-B radiation. Front. Plant Sci. 6, 128. https://doi: 10.3389/fpls.2015.00128.
Ufer, G., Gertzmann, A., Gasulla, F., Röhrig, H., Bartels, D., 2017. Identification and characterization of the phosphatidic acid-binding A. thaliana phosphoprotein PLD rp1 that is regulated by PLD α1 in a stress-dependent manner. Plant J. 92 (2), 276–290. https://doi.org/10.1111/tpj.13651.
Vincent, D., Ergül, A., Bohlman, M.C., Tattersall, E.A., Tillett, R.L., Wheatley, M.D., Schooley, D.A., 2007. Proteomic analysis reveals differences between Vitis vinifera L. cv. Chardonnay and cv. Cabernet Sauvignon and their responses to water deficit and salinity. J. Exp. Bot. 58 (7), 1873–1892. https://doi.org/10.1093/jxb/erm012.
Vishwanath, S.J., Delude, C., Domergue, F., Rowland, O., 2015. Suberin: biosynthesis, regulation, and polymer assembly of a protective extracellular barrier. Plant Cell Rep. 34 (4), 573–586. https://doi.org/10.1007/s00299-014-1727-z.
Wang, X., Zhang, H., Gao, Y., Sun, G., Zhang, W., Qiu, L., 2014. A comprehensive analysis of the Cupin gene family in soybean (Glycine max). PLoS One 9 (10), e110092. https://doi.org/10.1371/journal.pone.0110092.
Wang, Y., Peng, X., Salvato, F., Wang, Y., Yan, X., Zhou, Z., Lin, J., 2019. Salt-adaptive strategies in oil seed crop *Ricinus communis* early seedlings (cotyledon vs. true leaf) revealed from proteomics analysis. Ecotoxicol. Environ. Saf. 171, 12–25. https://doi.org/10.1016/j.ecoenv.2018.12.046.
Watanabe, N., Lam, E., 2011. *Arabidopsis* metacaspase 2d is a positive mediator of cell death induced during biotic and abiotic stresses. Plant J. 66 (6), 969–982. https://doi.org/10.1111/j.1365-313X.2011.04554.x.
Wegst-Uhrich, S.R., Navarro, D.A., Zimmerman, L., Aga, D.S., 2014. Assessing antibiotic sorption in soil: a literature review and new case studies on sulfonamides and macrolides. Chem. Cent. J. 8 (1), 5. https://doi.org/10.1186/1752-153X-8-5.
Wehrhan, A., Streck, T., Groeneweg, J., Vereecken, H., Kasteel, R., 2010. Long-term sorption and desorption of sulfadiazine in soil: experiments and modeling. J. Environ. Qual. 39 (2), 654–666. https://doi.org/10.2134/jeq2009.0001.
Xie, X., Zhou, Q., Lin, D., Guo, J., Bao, Y., 2011. Toxic effect of tetracycline exposure on growth, antioxidative and genetic indices of wheat (Triticum aestivum L.). Environ. Sci. Pollut. Control Ser. 18 (4), 566–575. https://doi.org/10.1007/s11356-010-0398-8.
Xu, Y., Yu, W., Ma, Q., Zhou, H., Jiang, C., 2017. Toxicity of sulfadiazine and copper and their interaction to wheat (*Triticum aestivum* L.) seedlings. Ecotoxicol. Environ. Saf. 142, 250–256. https://doi: 10.1016/j.ecoenv.2017.04.007.
Yang, C.L., Liang, S., Wang, H.Y., Han, L.B., Wang, F.X., Cheng, H.Q., Xia, G.X., 2015. Cotton major latex protein 28 functions as a positive regulator of the ethylene responsive factor 6 in defense against *Verticillium dahliae*. Mol. Plant 8 (3), 399–411. https://doi.org/10.1016/j.molp.2014.11.023.
Zhang, N., Li, R., Shen, W., Jiao, S., Zhang, J., Xu, W., 2018. Genome-wide evolutionary characterization and expression analyses of major latex protein (MLP) family genes in *Vitis vinifera*. Mol. Genet. Genom. 1–15. https://doi.org/10.1007/s00438-018-1440-7.