Environmental and Experimental Botany 228 (2024) 105953

Contents lists available at ScienceDirect

Environmental and Experimental Botany

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

Research Paper

Transcriptional responses of durum wheat to chronic chromium exposure reveal candidate proteins involved in metal detoxification and compartmentalization

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ARTICLE INFO

Keywords:
Triticum turgidum L
Trace metals
RNA-seq
Gene expression
Organic acids
Metal chelating

ABSTRACT

Chromium phytotoxicity results in relevant alterations to plant physiology, gene expression, and genomic DNA methylation at a transgenerational level. Herein, transcriptional responses of durum wheat (*Triticum turgidum* L.) to chronic chromium exposure were explored in roots and leaves by RNA-seq approach. Plants grown all the time in a hydroponic system supplemented with 2.5 and 10 μM hexavalent chromium were compared to unstressful control plants, assessing biomass and seed yield analyses after senescence. Then, transcriptomic analysis was performed with these plants kept under 10 μM chromium 50 days after the onset of exposure. The chromium concentrations used were considered the lowest dose sufficient to alter gene expression without impeding plant development, while the sampling time reflected the effects in the pre-harvest phase and long-lasting defense mechanisms. Root and leaf samples from plants kept under 10 μM chromium stress and from unstressful control plants were analyzed, generating 12 RNA-seq libraries. In total, 965 and 810 transcripts were found to be differentially expressed, respectively, in roots and leaves in response to 10 μM chromium stress. In roots, transcriptional changes were noted in the primary and secondary metabolism, redox homeostasis, protein modification, solute transport, nutrient uptake, and external stimuli responses. Meanwhile, the transcriptional changes in leaves were primarily found in the secondary metabolism, hormone-related pathways, chromatin modifications, cell division, protein modification and homeostasis, solute transport, and nutrient uptake. In particular, the metal uptake and translocation pathways were studied with greater emphasis to identify key proteins involved in chromium transport and compartmentalization. Furthermore, several genes involved in the biosynthesis of malate-derived organic acids, trace metal transport/detoxification/chelation, and vacuolar compartmentalization were linked to primary defense responses, and some of them were also associated with two putative gene clusters. Therefore, these genes and gene clusters are suggested as valuable biotechnological targets for future proof-of-concept studies aimed at genetic engineering of durum wheat to improve plant tolerance to chromium exposure.

1. Introduction

Environmental pollution with materials containing trace metals is an eminent dangerous risk for all living organisms, from plants to humans worldwide (Bertucci et al., 2018; Han et al., 2023). Among trace metals present in contaminated soils, chromium is one of the most abundant elements, since it is used in very diverse industrial activities such as leather tanning, dyeing processes, metallurgical materials, chemicals, pigments, printing, and others (Lunk, 2015). The disposal and decomposition of these chrome-containing materials in the environment leads to the gradual accumulation of chromium in the soil and water. Consequently, the uptake and compartmentalization of chromium in

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https://doi.org/10.1016/j.envexpbot.2024.105953
Received 7 July 2024; Received in revised form 21 August 2024; Accepted 24 August 2024

contaminated plant organelles, as well as in agricultural products and plant-derived foods, exposes people to several health risks (Zayed and Terry, 2003; Xu et al., 2022a; Ali et al., 2023; Soni et al., 2023). In green plants, hexavalent chromium [Cr(VI)] is actively uptake by unspecific carriers of other essential ions such as sulfate or iron, whereas trivalent chromium [Cr(III)] is taken up passively by cation exchange (Shanker et al., 2005; Sharma et al., 2020). In general, crops tolerate low levels of chromium contamination probably due to their effective compartmentalization, through chelating mechanisms, and by removal of reactive oxygen species (ROS) accumulating during chromium-induced stress (Viehweger, 2014; Abdullah et al., 2024). However, concentrations above the minimum toxicity threshold cause severe damage to plants (Kundu et al., 2018; Yu et al., 2023). In particular, chromium phytotoxicity disrupts normal cellular activity and inhibits seed germination, root and seedling growth, plant development, reproduction, and seed yield (Panda and Choudhury, 2005; Shanker et al., 2005; Medda and Mondal, 2017; Saud et al., 2022; Colzi et al., 2023).

Previous studies have shown that exposure to chronic or acute chromium phytotoxicity impairs photosynthesis, induces oxidative stress, and alters nutrient and water uptake (Kumar et al., 2019; Patra et al., 2019; Christou et al., 2020; Colzi et al., 2023). Simultaneously, *Arabidopsis thaliana* exposed to 200 μM chromium stress presented the downregulation of several genes associated with redox potential, secondary metabolism, and energy metabolism, while several genes associated with response defense, photosynthesis, sulfur metabolism, indole-3-acetic acid, and carotenoids were up-regulated (Liu et al., 2020). Epigenetic changes associated with a transgenerational priming effect were also demonstrated in *A. thaliana* when submitted to 2.5 and 5 μM chromium stress (Colzi et al., 2023). In *Miscanthus sinensis* under chromium exposure, a total of 83,645 differentially expressed genes were identified and shown as mainly involved in trace metal transport, metal ion chelation, and photosynthesis, reinforcing that glutathione metabolism and ABC transporters pathways play an important role in plant tolerance to chromium stress (Nie et al., 2021b). However, the understanding of the molecular mechanism involved in the defense response and compartmentalization of chromium in durum wheat remains not completely understood.

Durum wheat (*Triticum turgidum* Desf. var. *durum*) is an allotetraploid (AABB genome) cereal with a genome of about 10.45 Gb organized in 14 chromosomes ($2n = 4x = 28$) containing 63,993 coding genes (Morris and Sears, 1967; Blake et al., 1999; Zhang et al., 2018). It originated from hybridization between *Triticum urartu* ($2n = 2x = 14$, AA genome) and *Aegilops speltoides* ($2n = 2x = 14$, BB-related genome) (Zohary and Feldman, 1962). The durum wheat is a crop adapted to high temperatures and semiarid climates, widely cultivated throughout Europe (*e.g.*, Italy and France), North America, North Africa, and Asia, and traditionally used to produce pasta (mainly in Europe and America), bulgur (Asia), couscous (Africa), and some bread (South Italy) (Beres et al., 2020; Xynias et al., 2020). In particular, the main regions with the highest durum wheat cultivation have soil with variable types and levels of trace metals (Paunov et al., 2018; Aprile et al., 2019). In recent years, many studies have reported information about the implications of trace metals in durum wheat, revealing that durum wheat exposed to chromium toxicity showed a high metal accumulation in roots, while a small part was translocated to shoots (Rascio et al., 2023). However, very little is known about the molecular responses of these plants to chromium exposure (Aprile et al., 2018, 2019; Sabella et al., 2022). This knowledge could contribute to improving the understanding of plant defense mechanisms and would provide new biotechnological tools for genetic engineering or durum wheat breeding to improve plant resilience (Basso et al., 2019, 2020; Colzi et al., 2023).

In this study, genome-wide transcriptional changes induced by chronic chromium exposure in roots and leaves of durum wheat were analyzed using an RNA-seq approach. The transcriptional profile of the wheat cultivar widely grown in Europe was obtained 50 days after the start of chromium treatment to identify long-lasting defense mechanisms. At the same time, biomass and seed yield were determined in the senescence phase. Several genes associated with the broad plant defense response toward chromium stress were identified as differentially modulated. In particular, the putative primary molecular mechanism involved in chromium transport, detoxification/chelation, and vacuolar compartmentalization were emphasized and discussed.

2. Materials and methods

2.1. Plant material

For this study, durum wheat cv. Creso (pedigree: Cpb144 × [(Yt54-N10-B)Cp2–63] Tc2) was chosen since it is a variety widely grown in Italy (Vergine et al., 2017), its cultivation is at risk of exposure to chromium (Tóth et al., 2016; Ertani et al., 2017), and previous data report its response to cadmium (Aprile et al., 2018) and lead (Aprile et al., 2019), while data related to chromium toxicity is non-existent. Seeds were surface sterilized with a 1.2 % NaClO solution for 20 minutes, then thoroughly washed and immersed in a 1 mM $CaCl_2$ solution that had been aerated for a full day. Following this, the seeds began the germination process on plastic Petri dishes with vermiculite, maintained in complete darkness at a 10°C temperature (Fig. 1A). After a period spanning 3–4 days, three of these germinated seeds were transferred to plastic containers measuring 10 cm (diameter) x 60 cm (height), filled with perlite. They were moistened using deionized water and then integrated into a hydroponic setup as described by Sabella et al. (2021). In every pot, three young plants were placed, and three separate tubes were set up for each specific treatment, acting as three distinct biological replicates. Regular hydration was maintained for the plants using a hydroponic mixture at fixed timeframes (every 4 hours) lasting 5 minutes, ensuring the perlite remained damp while preventing waterlogging. Plants were grown under 40–60 % relative humidity, 8–22°C temperature day/night of seedling growth to flowering, and photoperiod day/night ranging from 10/14 (seedling growth) to 16/8 (flowering) hours in a Fitotron® growth chamber (Weiss Technik, UK), as detailed by Vergine et al. (2017). The durum wheat plants were arranged following a randomized block design. Each block consisted of an equal number of plants, systematically allocated to different treatment groups to ensure uniformity across varying environmental conditions within the growth chamber. This arrangement was aimed at minimizing the potential confounding effects of spatial variability on the experimental outcomes. The nutrient solution was prepared using reverse osmosis water ($<30\ \mu S/cm^{-1}$) and contained 1.1 mM KNO_3, 3 mM $[Ca(NO_3)_2\ 2\ H_2O]$, 0.2 mM NH_4NO_3, 1.2 mM K_2HPO_4, 0.04 g/L FeEDDHA, 2 mM $MgSO_4$, 70 μM H_3BO_3, 1.2 μM Na_2MoO_4, 1 μM $ZnSO_4$, 1 μM $CuSO_4$, and 10 μM $MnSO_4$, while pH was maintained between 5.5 and 6.0 adjusting it every two days with a portable pH meter and 10 % sulfuric acid solution. The hydroponic solution was continuously aerated, the hoagland solution was changed every five days, and the treated plant set was cultivated by adding 2.5 and 10 μM chromium (potassium dichromate, $K_2Cr_2O_7$, chromium(VI)). Cr(VI) was preferred because it is a stable and most toxic form of chromium (Becquer et al., 2003; Shanker et al., 2005). These two concentrations were selected to study the responses to chromium exposure at a non-symptomatic level at the macroscopic level of plant growth to identify genes and molecular mechanisms modulated at limited concentrations, similar to the previous studies carried out in *A. thaliana* (Colzi et al., 2023; Guarino et al., 2024) and environmental concentrations (Ertani et al., 2017).

2.2. Plant biomass, seed yield, and chromium accumulation

The same plants kept under continuous 2.5 and 10 μM chronic chromium stress and unstressful control conditions were evaluated for root, panicle, and vegetative biomass and seed yield after the senescence phase. Roots, panicles, and shoots were dried at 60°C to a constant weight. Then, the dry weight per plant, number of panicles per plant,

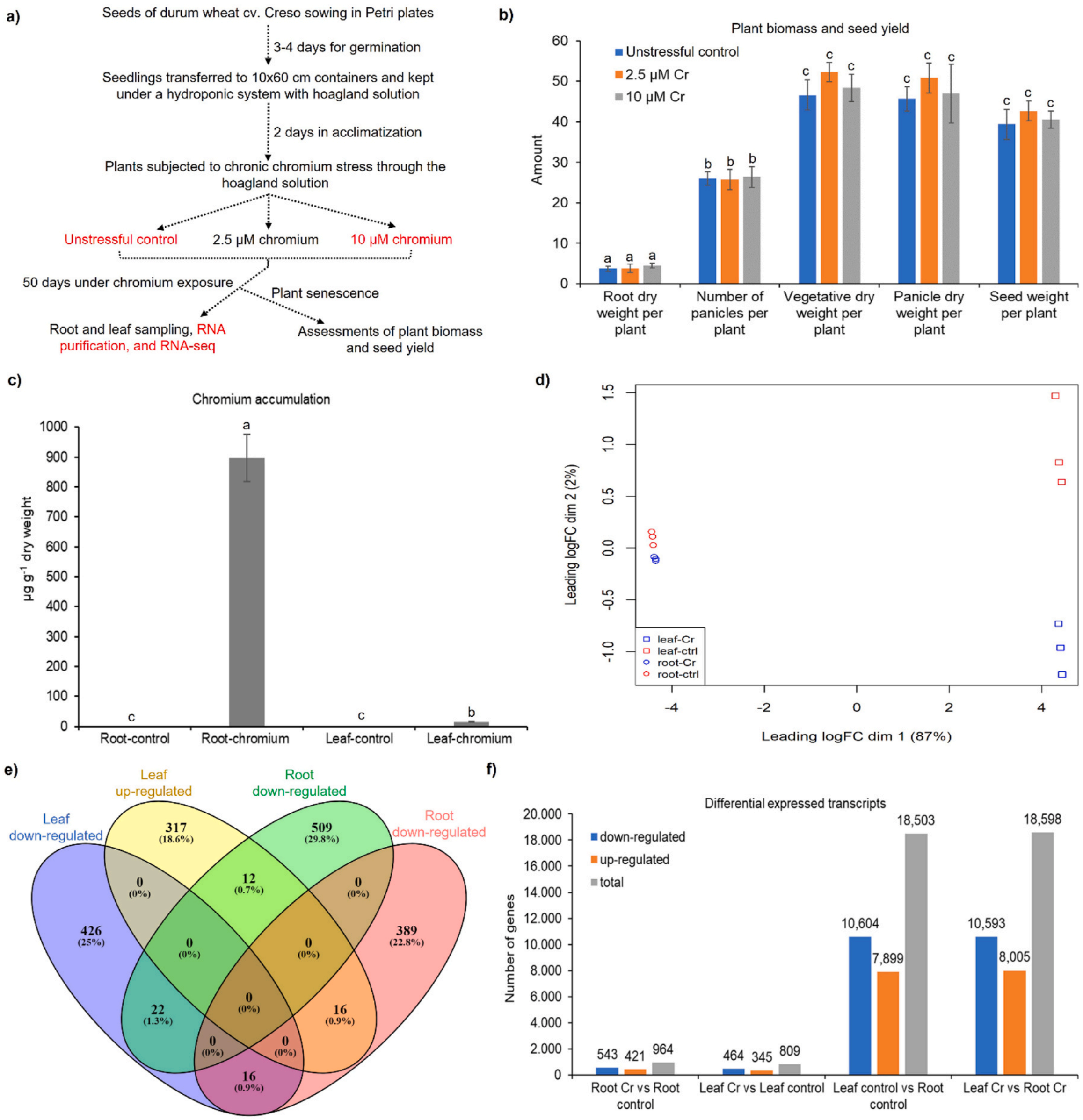

Fig. 1. Plant biomass under chromium (Cr) stress, design experimental, and correlation analysis among different samples based on transcript expression values and the number of differentially expressed transcripts (DETs) in each pairwise comparison for both leaf and root tissues. (A) Experimental design for RNA-seq analysis from plants kept for 50 days under 10 μM chromium (Cr) stress and unstressful control plants. (B) Biomass and seed yield after senescence of durum wheat plants kept under 2.5 and 10 μM chromium (Cr) stress compared to unstressful control plants. Seed yield is represented as fresh weight. Different letters on the bars indicate significant statistical differences according to Tukey's test at a 95 % significance level. Error bars represent the standard deviation of three biological replicates. (C) Chromium accumulation in roots and leaves of durum wheat plants kept under 10 μM chromium stress compared to unstressful control plants. Different letters on the bars indicate significant statistical differences according to Tukey's test at a 95 % significance level. Error bars represent the standard deviation of three biological replicates. (D) Multi-dimensional scaling analysis (MDS) analysis of the normalized read counts of the 12 RNA-seq libraries generated from durum wheat leaf and root samples under chromium stress and unstressful control conditions. Percentages represent variance captured by each principal component 1 and 2 in each analysis. (E) Venn diagrams of the overlapped DETs by comparing the contrast between different pairwise comparisons. The number and percentage of commonly and uniquely DETs were indicated. (F) Comparison between of number of DETs for each pairwise comparison. Only transcripts with log(fold change) lower than −1 or greater than +1, *p*-value <0.05, and FDR <0.05 were considered as DETs.

and seed weight per plant were determined. For chromium concentration, 100 mg dried root and leaf material from 10 μM chromium treated and control samples were digested using 10 mL of 69 % HNO_3 in a microwave digestion system (Mars 6, CEM), as described by Bettarini et al. (2019). In digest reactions, concentrations of total chromium were determined by flame atomic absorption spectroscopy using a PinAAcle 500 spectrometer (Perkin Elmer, Waltham, MA, USA).

2.3. RNA isolation and sequencing

Root and leaf tissues were harvested from 10 μM chromium stressed and unstressful control plants 50 days after sprouting, frozen in liquid nitrogen, and then stored at −80°C. Three biological replicates were used for each of the three conditions for both tissues. For leaf analysis, each biological replicate was composed of a pool of 3–5 fully expanded and green leaves per plant (1 biological replicate = 1 plant). For root analysis, each biological replicate was composed of a pool of 0.5 g of roots for each plant (1 biological replicate = 1 plant). RNA extraction was performed from 50 to 100 mg of leaves or roots ground with a pestle and mortar in liquid nitrogen using the RNeasy Plant Mini kit (Qiagen, Hilden, Germany). RNA quality was determined using the RNA 6000 nano kit on the Agilent 2100 Bioanalyzer System (Bio-Rad, Hercules, California, USA). Then, nine sequencing libraries were obtained following the procedure of TruSeq Stranded mRNA Library Prep (Illumina, San Diego, California, USA) using TruSeq RNA Single Indexes (three libraries for each of the experimental conditions or tissues, a total of 12 libraries). The RNA concentration of each library was determined using the dsDNA High Sensitivity Kit on Qubit™ 4 Fluorometer (Invitrogen, Waltham, Massachusetts, USA). Library sequencing was performed using the NovaSeq 6000 S1 Reagent Kit (100 cycles, 2 ×100+10+10 bp parameters) on the Illumina NovaSeq 6000 sequencing platform. All the libraries were run in a single flow cell following Illumina's standard procedure in XP mode.

2.4. RNA-Seq data elaboration and differential expression analysis

The RNA-seq raw read data in *.fastq* format were obtained from BCL files using the bcl2fastq2 v2.20 tool (Illumina). After assessing the quality of sequenced libraries with FastQC v0.11.9 (Andrews, 2010), adaptor sequences and low-quality bases were removed using Trimmomatic v0.39 (Bolger et al., 2014) with the following parameters: ILLUMINACLIP:adapters.fa:2:30:10 HEADCROP:1 LEADING:3 TRAILING:3 SLIDINGWINDOW:4:18 MINLEN:40. The filtered RNA reads were aligned to the *Triticum aestivum* cv. Svevo genome assembly (Maccaferri et al., 2019) using HiSat2 v2.2.1 (Kim et al., 2019) with default parameters. Reads counts were generated from alignment files using featureCounts v1.6.0 (Liao et al., 2013) with default parameters, according to the 'exon' feature and 'transcript_id' meta-feature of *Triticum_turgidum* Svevo v1.56 annotation file retrieved from Ensembl Plants website (https://plants.ensembl.org/Triticum_turgidum). Differential expression analyses were carried out using Bioconductor EdgeR v3.28.1 (Robinson et al., 2009). The EdgeR package was used to filter out the not-expressed or poorly expressed transcripts (a transcript was considered 'active' if reads per million mapping to that gene was >1 in at least two libraries), to normalize the RNA libraries, and to differential expression analyses with Likelihood-Ratio Test (LTR). Transcripts with a log2(fold change) lower than -1 or higher than $+1$, p-value <0.05, and false discovery rate (FDR) <0.05 were considered differentially expressed.

2.5. Functional data mining and enrichment analyses

According to the differential expression analysis results, transcripts with the same type of expression trend (up- or down-regulation) for the two pairwise comparisons (10 µM chromium stress *versus* unstressful control) were determined for both root and leaf tissues. The gene set enrichment analysis was performed with the same list of differentially expressed transcripts (DETs) using PageMan software (Usadel et al., 2006). The PageMan analysis was performed using the Wilcoxon test without correction and with a cutoff value = 1 (Wilcoxon, 1945). The DAVID database v.6.8 (Dennis et al., 2003) was used to get the gene ontology (GO) information related to the biological processes in the two pairwise comparisons. In addition, ShinyGO 0.80 software using the Benjamini-Hochberg method and with an FDR threshold of 0.05 was also used to carry out the GO enrichment analyses (Ge et al., 2020). MapMan 3.6.0RC1 software was used with the available *A. thaliana* mapping file (http://mapman.gabipd.org/;) to identify and visualize genes in functional overviews of cell pathways and gene categories (Thimm et al., 2004; Usadel et al., 2005). KEGG pathway enrichment analyses were carried out on DET sets to identify relevant pathways enriched for each pairwise comparison. As *Triticum durum* is not included in the list of KOBAS annotated species so, the *Triticum turgidum* Svevo proteome was annotated with eggNOG mapper v2.1.8 (Cantalapiedra et al., 2021) on the Galaxy platform using Poaceae taxonomic scope. Then, the annotated proteome was used as the background for the KEGG enrichment analyses, carried out using the clusterProfiler v4.4.4 R package (Yu et al., 2012). Significantly enriched KEGG categories (adjusted p-value <0.05) for both root and leaf DETs were generated. The bubble diagrams were plotted with ggplot2 v3.4.3 R visualization package (Wickham, 2016). Protein-protein interaction and gene co-expression networks among up- or down-regulated transcripts (log2(fold change) <-1 and $>+1$, p-value <0.05, and FDR <0.05; File S1) with partner proteins were predicted by the STRING database using the *A. thaliana* dataset as a reference, and those with >0.700 interaction scores and 1 % FDR stringency were selected for further examination (Szklarczyk et al., 2021). The location of the main DETs involved primary defense response toward chromium (Table 1) along the chromosomes of *Triticum aestivum* cv. Svevo genome assembly (Maccaferri et al., 2019) was performed using MapGene2-Chrom v2 (Jiangtao et al., 2015).

3. Results

3.1. Plant biomass, seed yield, and chromium accumulation

Plants kept under continuous 2.5 and 10 µM chronic chromium exposure showed similar root, panicle, and vegetative biomass yield, number of panicles, and seed yield per plant after the senescence phase compared to unstressful control plants (Fig. 1B). Meanwhile, chromium concentration was below the detection limit in control samples, whereas plants grown in the presence of 10 µM chromium showed metal accumulation levels of 896 ± 79 and 15 ± 1 $\mu g\ g^{-1}$ dry weight, in roots and leaves, respectively (Fig. 1C). Therefore, the level of chromium exposure used was low enough to activate plant defense responses without resulting in a yield penalty such as plant growth.

3.2. RNA sequencing and transcriptomic data analysis

In total, 12 libraries were constructed and sequenced, six libraries for root samples and six for leaf samples (chromium stress condition x unstressful control condition x three biological replicates). The raw sequences of the RNA libraries were deposited on the EMBL-EBI ArrayExpress database (https://www.ebi.ac.uk/biostudies/arrayexpress) under the accession number E-MTAB-13952. Overall, taking together the RNA-seq raw reads generated from the 12 libraries, 91.03–92.95 % of these paired reads passed quality control and filtering steps. In total, 8027,312 to 13,927,649 filtered reads were obtained, of which 90.90–96.83 % were mapped to the transcript dataset of the reference genome (Table S1). The number of reads per library mapped to the durum wheat reference transcripts was estimated and only 34,528 resulted as active transcripts and were used for further bioinformatic analyses. The 12 RNA libraries were normalized according to the amounts of filtered reads. Then, filtered and normalized counts were plotted in a multidimensional scaling (MDS) graph. The graph showed sample groups partially separated by type of plant tissue and treatment condition, confirming the high reproducibility among biological replicates (Fig. 1D). A total of 964 and 809 DETs were identified for root (Root chromium stress *versus* Root unstressful control condition) and leaf (Leaf chromium stress *versus* Leaf unstressful control condition), respectively (File S1). Among DETs, a total of 543 were down-regulated and 421 were up-regulated (Fig. 1E and F). In contrast, in leaf DETs, a total of 464 were down-regulated and 345 were up-regulated (Fig. 1E and F).

3.3. PageMan, GO, and KEGG pathway enrichment analyses

PageMan enrichment analysis showed that different pathways were impacted by DETs of roots and leaves in response to chromium exposure. In root datasets, photosynthesis chain respiration NADH dehydrogenase-like (NDH) subcomplex B component, RNA biosynthesis

Table 1

The main differentially expressed transcripts involved primary defense response toward chromium (Cr), such as in malate-derived organic acid biosynthesis, trace metal transport/detoxification/chelating, and vacuolar compartmentalization. Some of these main genes are suggested as powerful biotechnological assets to the genetic engineering of durum wheat to improve plant tolerance toward chromium stress.

Mechanism	Transcript ID	Root Cr *vs* Root control logFC	Leaf Cr *vs* Leaf control logFC	TAIR	Functional description	Genetic bioengineering suggested
Metal detoxification	TRITD7Av1G185570.2	ns	1.04	At1g06330	Heavy metal transport/detoxification superfamily protein (PF00403, PTHR22814, HMA domain)	Leaf-directed overexpression
	TRITD0Uv1G058320.1	ns	2.71	At2g36950	Heavy metal transport/detoxification or Heavy metal-associated proteins (HMA domain, PF00403, PTHR22814)	Leaf-directed overexpression
	TRITD0Uv1G136080.1	3.20	ns	At4g05030	Heavy metal transport/detoxification superfamily protein, putative G (PTHR22814, copper transport protein ATOX1)	Root-directed overexpression
	TRITD1Bv1G020100.1	1.26	ns	At4g05030	Heavy metal transport/detoxification superfamily protein, putative G (PTHR22814, copper transport protein ATOX1)	Root-directed overexpression
	TRITD2Av1G201840.1	ns	1.11	At4g05030	Heavy metal transport/detoxification protein G (PTHR22814, copper transport protein ATOX1-related)	Leaf-directed overexpression
	TRITD2Av1G201950.1	ns	3.07	At4g05030	Heavy metal transport/detoxification superfamily protein (PTHR22814, copper transport protein ATOX1-related)	Leaf-directed overexpression
	TRITD2Av1G201970.1	−2.72	1.03	At5g48290	Heavy metal transport/detoxification protein G (PTHR22814, copper transport protein ATOX1-related)	Leaf-directed overexpression
	TRITD2Av1G201990.1	1.26	ns	At5g48290	Heavy metal transport/detoxification protein G (PTHR22814, copper transport protein ATOX1-related)	Root-directed overexpression
	TRITD2Av1G202010.1	1.49	ns	At4g05030	Heavy metal transport/detoxification protein G (PTHR22814, copper transport protein ATOX1-related)	Root-directed overexpression
	TRITD2Av1G202010.8	1.70	ns	At3g20180	Heavy metal transport/detoxification protein G (PTHR22814, copper transport protein ATOX1-related)	Root-directed overexpression
	TRITD2Bv1G164870.1	−2.80	1.18	At3g20180	Heavy metal transport/detoxification protein G (PTHR22814, copper transport protein ATOX1-related)	Leaf-directed overexpression
	TRITD2Bv1G165040.1	1.60	ns	At4g05030	Heavy metal transport/detoxification protein G (PTHR22814, copper transport protein ATOX1-related)	Root-directed overexpression
	TRITD3Bv1G278120.1	ns	1.23	At2g37390	Heavy metal transport/detoxification superfamily protein (HMA domain, PF00403)	Leaf-directed overexpression
	TRITD1Av1G017040.1	1.93	ns	At4g05030	Heavy metal transport/detoxification protein (PTHR22814, copper transport protein ATOX1-related)	Root-directed overexpression
	TRITD1Av1G123180.1	2.68	ns	At4g29140	MATE-like detoxification protein (PF01554, PTHR11206)	Root-directed overexpression
Metal transport	TRITD3Bv1G278650.1	1.24	ns	At5g59520	Zinc transporter, putative ZIP2 (PF02535, PTHR11040, Zinc/iron permease)	Root-directed overexpression
	TRITD1Bv1G168120.1	ns	2.87	At1g05300	Zinc transporter ZIP5 (PF02535, PTHR11040, Zinc/iron permease)	Leaf-directed overexpression
	TRITD7Bv1G184110.1	1.07	ns	At4g37270	Metal-transporting ATPase (PR00119, PF00122, P-type ATPase, PTHR24093)	Root-directed overexpression
	TRITD3Bv1G229690.1	1.24	ns	At4g21250	Sulfite exporter TauE/SafE family protein, putative (PF01925, PTHR14255, transmembrane protein TauE-like)	Root-directed overexpression
	TRITD4Bv1G190110.1	3.50	ns	At1g67940	Phosphate import transmembrane ATP-binding protein PstB (PF00005, ABC transporter-like, PTHR24220, SM00382, AAA+ ATPase domain)	Root-directed overexpression
Vacuolar endosome - chromium compartmentalization?	TRITD3Av1G256800.1	ns	1.08	No hits	Vps51/Vps67 family (components of vacuole vesicular transport) protein G (PTHR48245, family unnamed)	Constitutive overexpression
	TRITD3Bv1G255460.1	ns	1.01	No hits	Vps51/Vps67 family (components of vacuole vesicular transport) protein G (PTHR48245, family unnamed)	Constitutive overexpression
	TRITD2Av1G268510.1	−5.10	ns	No hits	Heme transporter hrg1-A G for metal recycling in microphage (PTHR34483, family unnamed)	
Metal binding	TRITD3Bv1G173850.1	1.63	ns	At5g63140	Inactive purple acid phosphatase-like protein G (PF00149, calcineurin-like phosphoesterase domain, SSF56300, metallo-dependent phosphatase-like)	Root-directed overexpression

(*continued on next page*)

Table 1 (*continued*)

Mechanism	Transcript ID	Root Cr *vs* Root control logFC	Leaf Cr *vs* Leaf control logFC	TAIR	Functional description	Genetic bioengineering suggested
	TRITD0Uv1G129700.1	ns	2.01	At5g39110	RmlC-like cupins superfamily protein manganese ion binding (PR00325, Germin, PF07883, Cupin 1, SSF51182)	
	TRITD0Uv1G134940.1	ns	2.07	At5g39150	RmlC-like cupins superfamily protein manganese ion binding (PR00325, Germin, PF07883, Cupin 1, SSF51182)	
	TRITD5Bv1G094790.1	1.30	ns	At2g40300	Ferritin (PF00210, Ferritin/DPS protein domain, PTHR11431)	Constitutive overexpression
Organic acids - malate accumulation, chromium chelating?	TRITD2Av1G086060.1	2.05	ns	At3g21720	Isocitrate lyase (PF00463, SSF51621, pyruvate/phosphoenolpyruvate kinase-like domain)	Constitutive overexpression
	TRITD3Bv1G026020.1	3.46	ns	At2g44350	Citrate synthase (PR00143, TIGR01793)	Constitutive overexpression
	TRITD5Av1G255900.1	−3.83	−2.24	At5g58330	Malate dehydrogenase (PF00056, TIGR01757, PF02866, PTHR23382)	
Resilience toward stress	TRITD1Bv1G186850.1	1.14	1.20	At2g16060	Non-symbiotic hemoglobin (PF00042, PR00188, SSF46458, globin)	Constitutive overexpression
	TRITD1Av1G178640.1	ns	−4.87	At1g47710	Serpin family protein involved in the regulation of PCD induction (PF00079, PTHR11461)	Knockdown or knockout
	TRITD4Av1G056120.1	ns	3.34	At5g05350	Zinc transporter and plant cadmium resistance protein (PF04749, PLAC8 motif-containing protein, PF11204)	Constitutive overexpression
	TRITD5Av1G178430.1	3.32	2.34	At4g25480	CRT-binding factor CBF3/DREB1A (SSF54171, DNA-binding domain, PF00847, AP2/ERF domain)	Stress-induced overexpression
	TRITD2Av1G218770.1	2.72	2.91	At4g02550	Myb/SANT-like DNA-binding domain protein (PF12776, PTHR31704)	Stress-induced overexpression

ns: not significantly modulated.

transcriptional regulation mediated by C2H2 and HSF transcription factors, nutrient uptake and homeostasis (nitrogen, sulfur, and sulfate), and enzyme glycosyl-transferase (EC_2.4) were up-regulated, while that phytohormone action (gibberellin), RNA biosynthesis transcriptional regulation mediated by R2R3-MYB and bHLH transcription factors, protein modification (glycosylation, phosphorylation, and protease), solute transporter and metal chelation, nutrient uptake and homeostasis, and enzymes associated with oxidative stress defense and methylation (EC_1.14, EC_2, EC_2.5, and EC_2.7) were down-regulated in response to chromium stress (Fig. 2; File S2). In addition, a gene (TRITD7Bv1g193150.1) involved in auxin transport was up-regulated, some genes involved in brassinosteroid conjugation and degradation were up-regulated while other genes involved in signaling were down-regulated, several genes involved in cytokinin and ethylene biosynthesis, degradation, and signaling were up- or down-regulated, genes involved in gibberellin and jasmonic acid biosynthesis, degradation, signaling, and transport were down-regulated, and several genes encoding cysteine-rich- and non-cysteine-rich-peptides involved in signaling were also differentially modulated by the chromium stress in roots (File S2). Likewise, in leaf datasets, phytohormone action (abscisic acid and gibberellin), RNA biosynthesis transcriptional regulation mediated by AP2-ERF transcription factors, protein modification mediated by G-lectin protein kinases, and transferase enzyme (EC_2.7, transferring phosphorus-containing group) were up-regulated, while that lipid metabolism (fatty acid), cell division (cytokinesis and endoplasmic reticulum reorganization), protein modification (glycosylation), cell wall organization and degradation (hemicellulose and xylan), nutrient uptake and metal chelation, multi-process regulation of unfolded protein response and IRE1-bZIP60 signaling pathway, and acyltransferase enzyme (EC_2.3) were down-regulated (Fig. 2; File S2). In addition, two genes (TRITD1Bv1g168260.1 and TRITD7Bv1g164340.4) involved in abscisic acid (ABA) signaling and degradation were up-regulated, two genes (TRITD5Bv1g195070.1 and TRITD2Bv1g073930.1) involved in auxin signaling and degradation were respectively down- and up-regulated, two genes (TRITD2Bv1g225900.1 and TRITD3Bv1g059760.1) involved in cytokinin degradation were up-regulated, a gene (TRITD4Bv1g010840.1) involved in ethylene signaling was down-regulated, several genes involved in gibberellin degradation were differentially modulated, and several genes encoding cysteine-rich- and non-cysteine-rich-peptides involved in signaling were also differentially modulated by the chromium stress in leaves (File S2).

The GO enrichment analysis of DETs from root datasets showed that among the most important enriched GO-terms, we found terms related to nicotianamine metabolism (GO:0030417, GO0030418 and GO:0030410), tricarboxylic metabolism (GO:0072351 and GO:0072350), fatty acid (GO:0005504), amine biosynthesis (GO:0042401, GO:0009309, GO:0006576, and GO:0009308), carboxylic acid binding and metabolism (GO:0033293, GO:0031046, and GO:0046394), systemic acquired resistance (GO:0009627), transmembrane transporter (GO:0035673, GO:1904680, and GO:0022857), transferase (GO:0016765), and organic acid (GO:0016053) (Fig. 3A). These GO-terms from up-regulated genes are involved in the cell wall, response to oxidative stress, nutrient transport, metal transport and chelation, transcriptional regulation, and defense response, while GO-terms from down-regulated genes were related to the plasma membrane, protein phosphorylation, defense response, metal binding, transcriptional regulation, and vesicular transport (File S3).

Likewise, GO enrichment analysis of DETs from leaf datasets showed that the most important enriched GO-terms were related to the cell wall (GO:0045492, GO:0044038, GO:0070592, GO:0010410, GO:0010383, GO:0044036, GO:0042546, and GO:0071554), cellular component (GO:0070589), peptidase inhibitor (GO:0004867, GO:0004866, GO:0030414, GO:0061135, and GO:0061134), lipid transport (GO:00068698 and GO:0010876), extracellular region (GO:0005576), and transferase (GO:0016747, GO:0016746, and GO:0008194) (Fig. 3B). These GO-terms from up-regulated genes are involved in heme and metal binding, oxidoreductase activity, response to oxidative stress, protein phosphorylation, plasma membrane, and carbohydrate-binding, while the ones from down-regulated genes were related to transferase activity, plasma membrane, cell wall, defense response, carbohydrate, protein modification, response to oxidative stress, chloroplast, and heme binding were down-regulated (File S3).

The KEGG enrichment analysis of DETs from root datasets showed

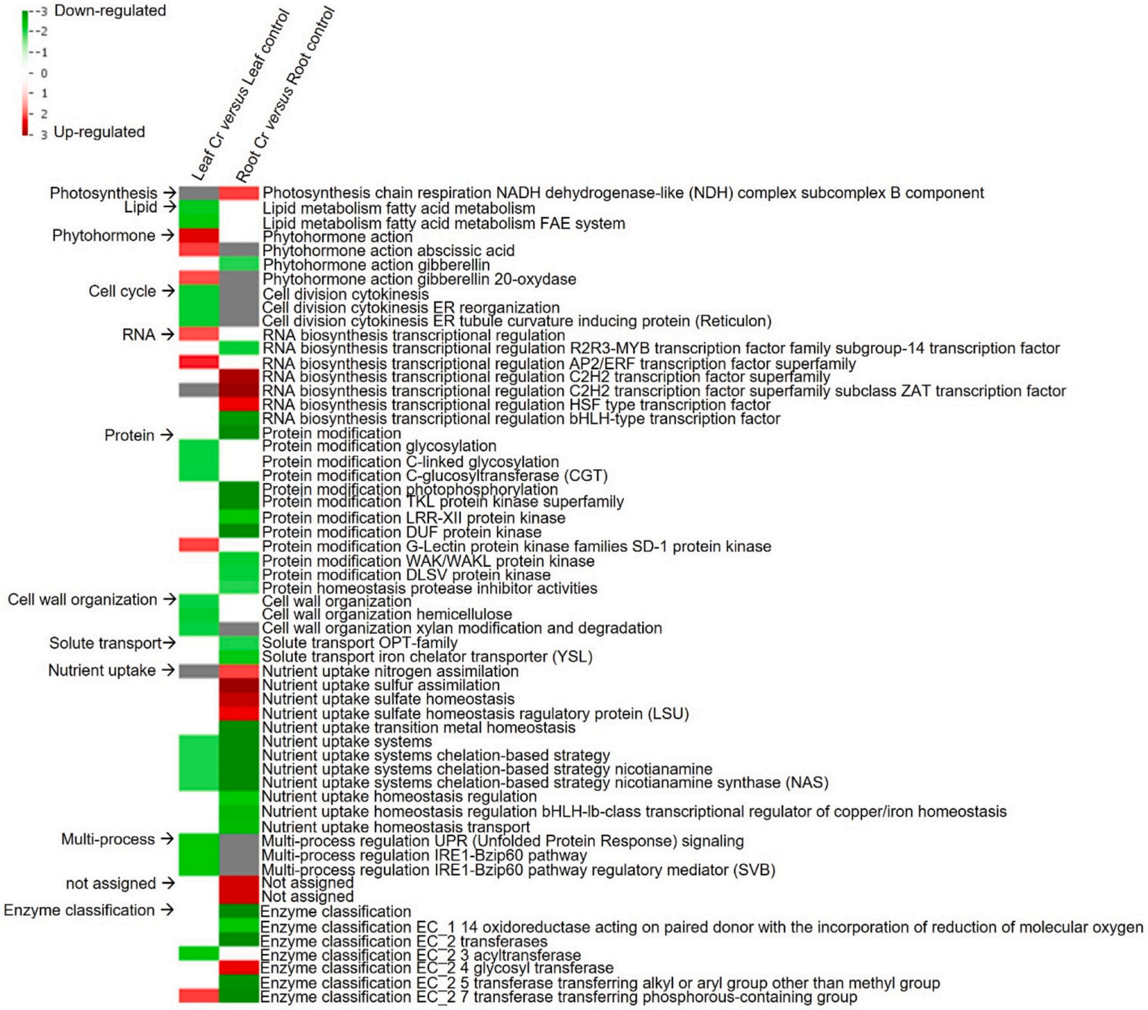

Fig. 2. PageMan pathway enrichment analysis from differentially expressed transcript (DET) sets in pairwise comparisons. The green (down-regulated) and red (up-regulated) extremes represent the metabolic pathways differentially modulated between each pairwise comparison for both root and leaf datasets from durum wheat plants under chromium (Cr) stress and unstressful control conditions. Only DETs with log(fold change) lower than −1 or greater than +1, p-value <0.05, and FDR <0.05 were considered in the PageMan analysis. The color intensity is correlated with the statistical significance based on the Wilcoxon test default implemented in the PageMan software.

that the main enriched KEGG categories were related to plant hormone signal transduction, cysteine and methionine metabolism, α-Linolenic acid metabolism, phenylpropanoid biosynthesis, ferroptosis, mineral absorption, MAPK signaling pathway, and zeatin biosynthesis (Fig. 3C; File S4). Likewise, the enriched KEGG categories in the leaf were related to flavonoid biosynthesis, plant hormone signal transduction, plant-pathogen interaction, phenylpropanoid biosynthesis, MAPK signaling pathway, antigen processing and presentation, and stilbenoid, diarylheptanoid and gingerol biosynthesis (Fig. 3D; File S4).

3.4. *Protein-protein interactions and co-expression network*

The protein-protein interactions and co-expression network analysis revealed that, from the root DETs dataset (root under chromium stresses *versus* unstressful control conditions), the network constructed with up-regulated transcripts resulted in four main clusters encompassing the following biological functions, such as group I: metal transport, transmembrane, defense response to metal, metal storage, plant defense to oxidative stress, and oxidoreductase; group II: plant defense to oxidative stress and transmembrane transport; group III: resistance toward abiotic stress, ABC transporter, and plant defense toward metal stress; group IV: signal transduction, transmembrane, and metal homeostasis (Fig. S1). Likewise, the network constructed with down-regulated transcripts resulted in four main clusters, such as group I: Metal transport, metal homeostasis, plant defense toward metal stress, membrane transport, metal storage, vacuolar transport, and metal starvation; group II: oxidoreductase, methyltransferase, secondary metabolism, and plant defense to oxidative stress; group III: pathogenesis-related, biotic disease resistance, LRR receptor, and signal transduction; group IV: signal transduction, biotic disease resistance, and TIR-NBS-LRR (Fig. S1).

Similarly, from the leaf DETs (root under chromium stresses *versus* unstressful control conditions), the network constructed with up-regulated transcripts resulted in four main clusters encompassing the following biological functions, such as group I: lignin metabolism; group II: metal transport, trace metal binding, and lipid metabolism; group III: RNA processing; group IV: protein ubiquitin, glycoprotein, and defense response to pathogens (Fig. S2). Likewise, the network constructed with down-regulated transcripts resulted in three main clusters, such as group I: secondary metabolism and lignin metabolism; group II: secondary metabolism and glycosyltransferase; group III: heat-shock protein (Fig. S2). Therefore, these protein-protein interactions and gene co-expression network data suggested that the greatest changes at the molecular level associated with plant defense toward chromium stress occur in the roots, while the impact at the molecular level is

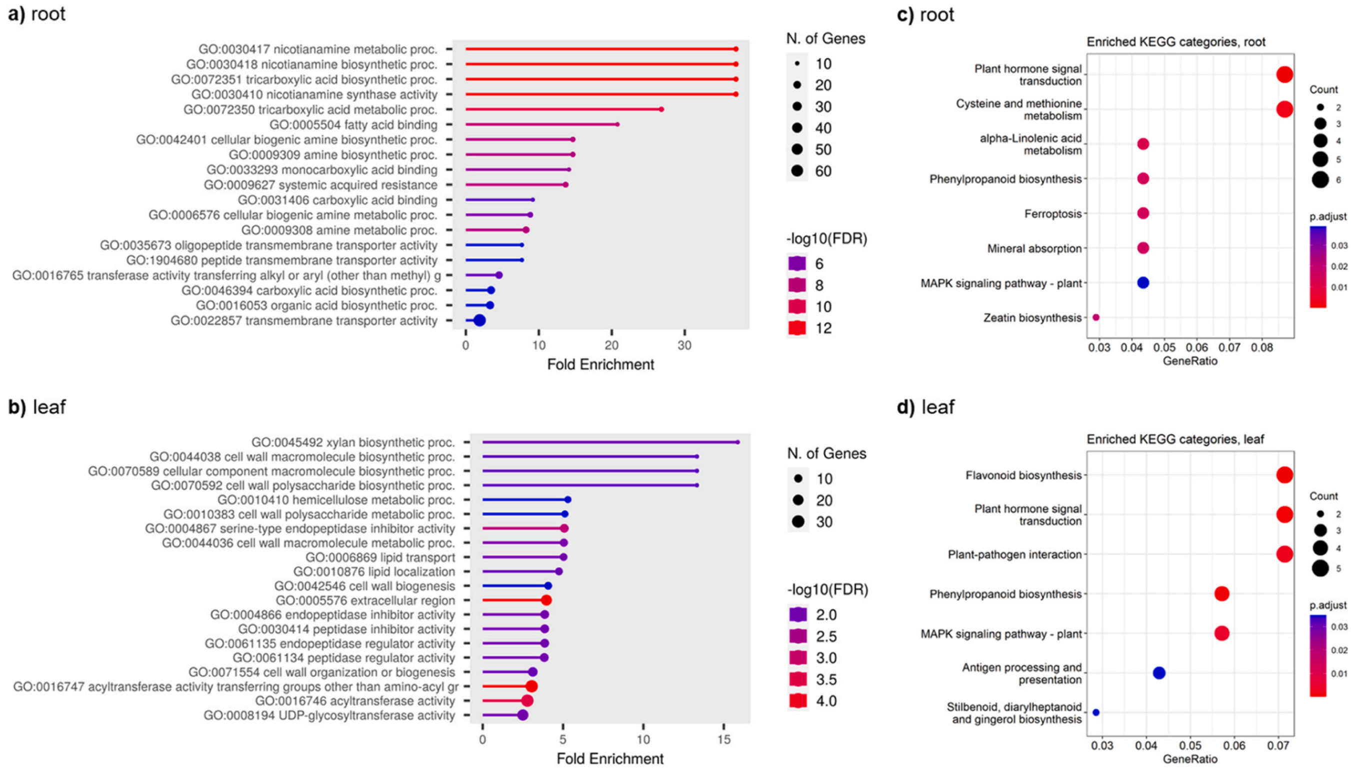

Fig. 3. GO and KEGG pathway enrichment analysis of differentially expressed transcript (DET) sets in pairwise comparisons. Top GO terms over-represented in (A) root chromium stress *versus* root unstressful control conditions. Top GO terms over-represented in (B) leaf chromium stress *versus* leaf unstressful control conditions. Plots of the most significant pathways were generated using ShinyGO 0.80 software with a 0.05 FDR threshold. KEGG category over-represented in (C) root chromium stress *versus* root unstressful control conditions. KEGG category over-represented in (D) leaf chromium stress *versus* leaf unstressful control conditions. KEGG enrichment bubble diagrams display the top 10 enriched KEGG categories. The X-axis represents the enrichment ratio based on the number of DETs belonging to the KEGG category/number of DETs belonging to the same KEGG category in the background genome. The Y-axis represents the enriched KEGG categories ordered by the number of detected DETs. The size of the dots represents the number of DETs included in each KEGG category. The dots color represents the adjusted *p*-values for each KEGG category enrichment.

comparatively smaller in leaves.

3.5. *Modulation of biological pathways in roots and leaves*

3.5.1. *Primary and secondary metabolism*

The implications caused by DETs on the primary and secondary metabolism pathways were shown (Fig. 4). Focusing on these metabolic pathways supported by transcript expression (up-regulated or down-regulated), it was observed in roots that cell wall, lipids, carbohydrates, flavonoids, phenylpropanoids and phenolics, some amino acids (*e.g.*, Ser, Gly, Cys, and His), C-1 metabolism, fermentation, sucrose, ascorbate and glutathione, tetrapyrrole, NO_3, and SO_4, pathways were released in most categories, while that waxes, S-misc, TCA, starch, some amino acids (*e.g.*, Phe, Tyr, and Trp), nucleotides, and light reaction pathways were repressed in most categories (Fig. 4). Likewise, in the leaves was observed that carbohydrates, phenylpropanoids and phenolics, some amino acids (*e.g.*, Val, Leu, and Ile), and nucleotides pathways were released in most categories, while cell wall, lipids, S-misc, terpenes, flavonoids, some amino acids (*e.g.*, Asn, Thr, Met, and Lys), TCA, OPP, starch, ascorbate and glutathione, tetrapyrrole, and photorespiration were repressed in most categories (Fig. 4). Therefore, these data suggest that primary and secondary metabolism is differentially affected in both roots and leaves in response to chromium exposure.

3.5.2. *Abiotic stress response and redox reactions*

The implications caused by DETs on the biological pathways involved in plant defense against abiotic stress and redox reactions were shown (Fig. 5A). In roots, the pathways involved in response to heat stress were released, while pathways related to miscellaneous processes were repressed. In addition, pathways related to redox reactions such as ascorbate/glutathione were released, while heme was repressed (Fig. 5A). Likewise, in leaves, the pathways involved in response to touch/wounding stress were released, while pathways related to heat, drought/salt, and miscellaneous processes were repressed. In addition, pathways related to redox reactions such as heme and dismutase/catalase were released, while the ascorbate/glutathione pathway was repressed (Fig. 5A). These results suggest that pathways related to abiotic stress and redox reactions are differentially affected in roots and leaves in response to chromium exposure.

3.5.3. *Protein modification and degradation, signaling, and hormones*

The implications caused by DETs on the biological pathways involved in protein modification and degradation, signaling, and hormones were shown (Fig. 5B). In roots, several transcripts involved in protein modification and degradation were differentially modulated in a similar proportion of up- or down-regulated transcripts, while receptor kinases-mediated signaling pathways were repressed. In addition, pathways related to indole-3-acetic acid (IAA), benzyl adenine (BA), and salicylic acid (SA) hormones were released, while jasmonate and gibberellic acid (GA) hormones were repressed (Fig. 5B). Likewise, in leaves, numerous transcripts involved in protein modification pathways were mostly up-regulated, while several transcripts involved in pathways of protein degradation and receptor kinases-mediated signaling were differentially modulated in a similar proportion of up- or down-regulated transcripts. In addition, pathways related to ABA and BA hormones were released, while ethylene hormone was repressed (Fig. 5B). Therefore, these data suggest that pathways related to protein modification and degradation, receptor kinases-mediated signaling, and

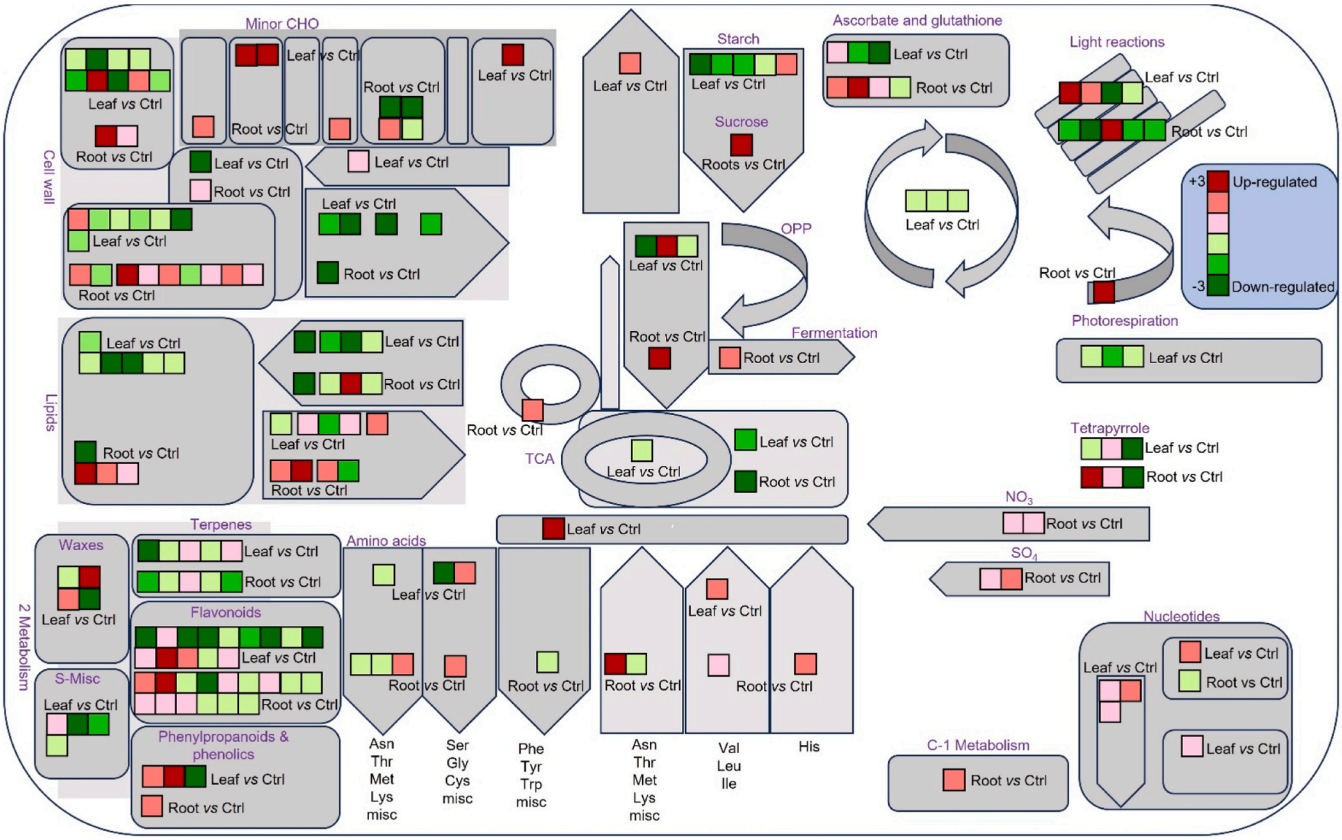

Fig. 4. MapMan pathway distribution based on differentially expressed transcripts (DETs) for the pairwise comparisons "Root under chromium stress *versus* Root under unstressful control conditions" [= Root *vs* Ctrl] and "Leaf under chromium stress *versus* Leaf under unstressful control conditions" [= Leaf *vs* Ctrl]. DETs involved in primary and secondary metabolism in response to chromium stress in leaves and root tissues. The log(fold change) of each DET (square) is expressed in a colorimetric scale (from red box = up-regulated to green box = down-regulated).

hormones are differentially affected in roots and leaves in response to chromium exposure.

3.6. Main DETs involved in chromium stress response

A set of top 31 DETs modulated in response to chromium exposure was particularly highlighted in the durum wheat RNA-seq datasets, such as being involved in metal detoxification, metal transport, vacuolar endosome for metal compartmentalization, metal binding, malate-derived organic acid biosynthesis, and related to the plant resilience toward trace metal stress (Table 1). Some of these selected genes had their expression up-regulated in the roots of plants kept under chromium stress while others were up-regulated specifically in leaves, indicating genes with tissue-specific activity triggered by chromium exposure. Based on the annotated biochemical function of the protein encoded by these transcripts, they are suggested as putative targets for further studies of specific functional validation associated with plant defense response toward chromium stress. Likewise, they are also suggested as powerful biotechnological assets to the genetic engineering of durum wheat to improve plant tolerance toward chromium stress. Consequently, it is suggested that their biotechnological use can be applied through their transgenic overexpression triggered by constitutive, tissue-specific, or abiotic stress-induced promoters. Furthermore, some DETs have the potential to be genetically modulated for their down-regulation through *in planta* RNA interference or plant genome editing. Analysis of the chromosomal location of these main genes suggested the presence of two gene clusters associated with plant defense against chromium stress (cluster #1: six genes closely grouped on chromosome 2 A; and cluster #2: three genes closely grouped on chromosome UN) (Fig. S3).

4. Discussion

The importance of agriculture in food production and ecosystem sustainability is highlighted worldwide (Basso et al., 2024c). The contamination of agricultural soils with high levels of chromium is a global reality and has raised concerns for human health and the ecosystem in general (Bertucci et al., 2018; Han et al., 2023). The mechanized industrialization growth and the disposal of contaminated waste material in the environment have contributed to the increase in chromium levels in the soil and effluents (Lunk, 2015; Prasad et al., 2021). Scientific studies reveal that the phytotoxic effect of chromium on different plant species disrupts several biological processes and negatively impacts their productivity and yield (Medda and Mondal, 2017; Kundu et al., 2018; Saud et al., 2022; Xu et al., 2022a; Ali et al., 2023; Colzi et al., 2023; Soni et al., 2023; Yu et al., 2023). Sharma et al. (1995) showed that Cr(VI) induced interveinal chlorosis in young wheat leaves which turned into necrosis at later growth stages, affected chlorophyll and Hill activity, protein nitrogen concentration was decreased, catalase activity in young leaves was impaired, and grain yield was severely affected and even no seed formation. The search for measures to mitigate these problems has been intensified in recent years, which are based on physical remediation, chemical reduction, bioreduction, microbial reduction, phytoremediation, and plant-based microbial fuel cells (Kumar, 2021; Albqmi et al., 2023; Fu et al., 2023; Yan et al., 2023). In particular, understanding how plants molecularly respond when exposed to chromium makes it possible to obtain insights and biotechnological assets to engineer biological mechanisms so that economically important crops can withstand this stress with minimal impact on their performance and yield (Colzi et al., 2023; Guarino et al., 2024). Therefore, through conventional plant breeding and genetic engineering

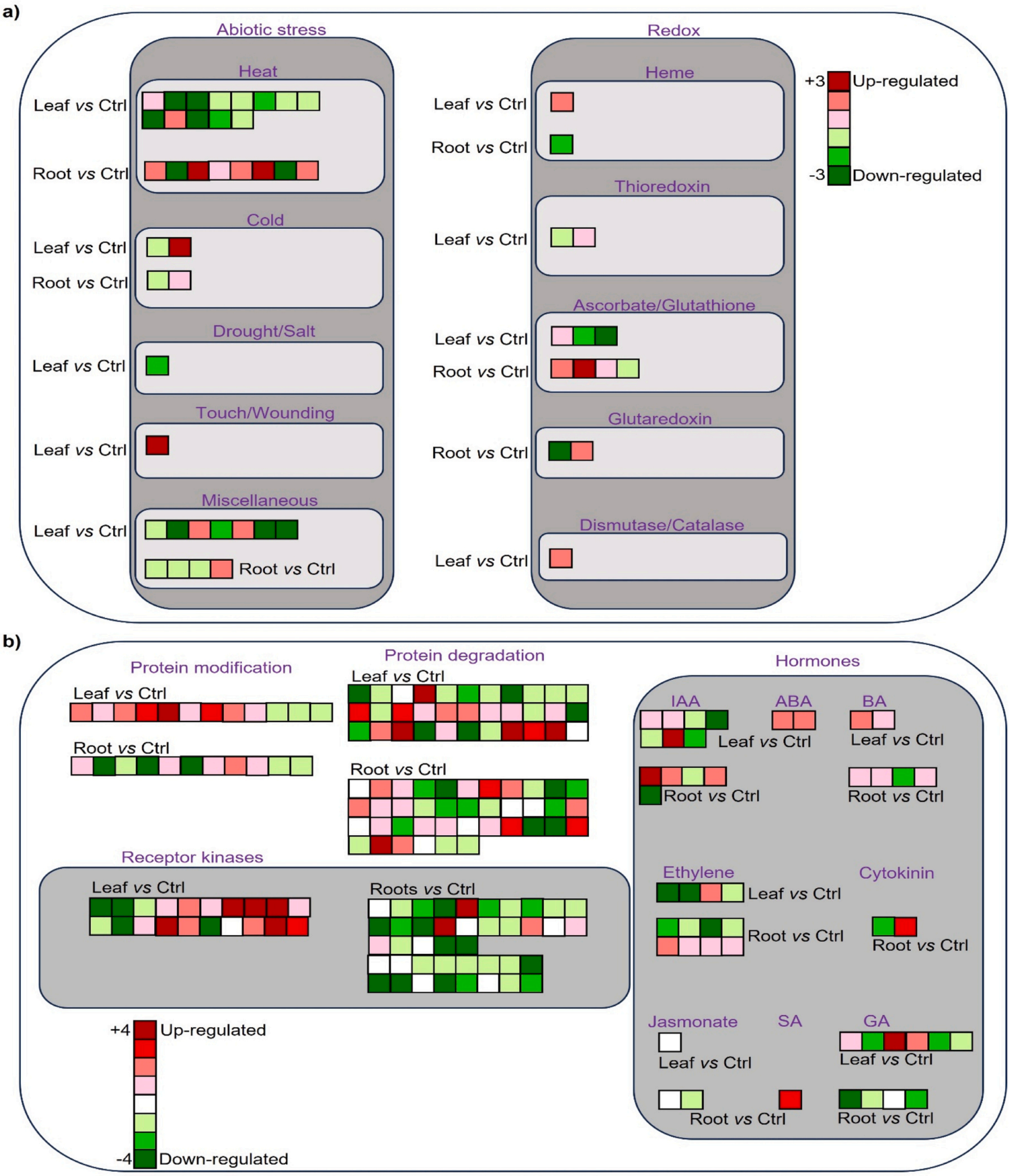

Fig. 5. MapMan pathway distribution based on differentially expressed transcripts (DETs) for the pairwise comparisons "Root under chromium stress *versus* Root under unstressful control conditions" [= Root *vs* Ctrl] and "Leaf under chromium stress *versus* Leaf under unstressful control conditions" [= Leaf *vs* Ctrl]. (A) DETs involved in plant defense pathways to abiotic stress and redox reactions. (B) DETs involved in protein modification and degradation, signaling, and hormone-related pathways. The log(fold change) of each DET (square) is expressed in a colorimetric scale (from red box = up-regulated to green box = down-regulated). IAA: indole-3-acetic acid; ABA: abscisic acid; BA: benzyl adenine; SA: salicylic acid; GA: gibberellic acid.

(such as genome editing, transgenesis, and RNA interference) it is possible to obtain conventional or transgenic traits associated with plant tolerance or reduced susceptibility to chromium exposure (Basso et al., 2019, 2020; Elango et al., 2022). This study aimed to provide insights into the molecular mechanisms of wheat plants kept under chromium exposure throughout their vegetative and productive cycle. Therefore, it sought to identify using RNA-seq biological processes, metabolic pathways, protein-protein interaction and gene co-expression networks, and major genes modulated by chromium presence involved in the signal perception and transduction, DNA transcription, protein translation, metal binding, transport, detoxifying and compartmentalization. To simulate an environment close to reality (Ertani et al., 2017), a chronic concentration of chromium was administered through an hydroponic system in hoagland solution for 50 days. At the phenotypic level, wheat plants did not show evident symptoms caused by chromium treatment and a significant reduction in biomass and seed yield, despite the chromium accumulation at levels considered toxic for plant shoots (5–30 μg g^{-1} according to Kabata-Pendias and Pendias, 2001). At the transcriptional level, stressed durum wheat plants showed a significant modulation in biological processes such as chlorophyll, photosynthesis, carbohydrate and amino acid metabolism, lipid metabolism, nicotianamine metabolism, phytohormone, chromatin organization, transcriptional regulation, protein biosynthesis, protein modification, cell division, cell wall organization, nutrient uptake, oxidative stress, secondary metabolism, external stimuli response, and multi-process regulation. In addition, significant changes in important mechanisms such as metal binding, transport and detoxification, organic acid biosynthesis, and defense response and resilience to abiotic stress were observed. These defense responses may be the reason for the absence of symptoms in the presence of relevant chromium concentrations in wheat plants. As expected, roots and leaves of wheat plants kept under prolonged chromium exposure showed significant differences in these molecular responses, mainly by differential expression of genes associated with malate biosynthesis, nutrient uptake, and metal transport. It has been suggested that the toxicity of trace metals hampers normal plant physiological processes through ROS production, inhibiting respiration and photosynthesis, changing ion homeostasis, causing metabolic imbalance, compromising the antioxidant defense system, disrupting membrane integrity, and enhancing lipid peroxidation (Emamverdian et al., 2015; Jalmi et al., 2018; Colzi et al., 2023; Guarino et al., 2024). Somewhat similar results were observed from microarray assay with roots of rice exposed to short- and long-term Cr(VI) stress, with 1181 genes modulated in plants exposed for a shorter period (1–3 hours) enriched mainly for kinases while 2097 genes were modulated for a more extended period (24 hours) enriched mainly for cytokinin signaling, the ubiquitin-proteasome system pathway, DNA repair, and copper transportation (Huang et al., 2014).

4.1. Nutrient uptake and oxidative stress

The nutrient uptake may have been severely altered in the roots of wheat plants kept under chromium exposure based on the down-regulation of 81 genes linked to metal chelation, reduction, assimilation, transport and homeostasis, and solute transport, and up-regulation of 25 genes linked to metal chelation, assimilation and transport, and solute transport. Likewise, nutrient uptake was also significantly altered in leaves based on the down-regulation of 24 genes linked to metal chelation and transport, solute transport, and metal homeostasis, and up-regulation of 16 genes linked to metal homeostasis and transport, and solute transporter. In turn, the redox homeostasis was also altered in roots by the down-regulation of six genes linked to glutathione S-transferases (GST)-based oxidative stress defense, ascorbate metabolism, tocopherol biosynthesis, and oxypilin carbonyl degradation, and up-regulation of seven genes linked to GST-based redox regulation and ascorbate metabolism. Likewise, the redox homeostasis was also altered in leaves by the down-regulation of three genes linked to thioredoxin, ascorbate, and lipid homeostasis, and up-regulation of four genes linked to ROS scavenging, GST-based redox regulation, and thioredoxin. Therefore, these collective data suggested that nutrient uptake and defense response to oxidative stress are severely modulated in both roots and leaves of wheat plants as a consequence of chronic chromium stress to maintain cellular homeostasis. In this sense, trace metals affect nutrient uptake primarily by interacting with other essential minerals by forming insoluble compounds, interfering with the metal transport and storage mechanism, and causing nutrient imbalance in roots (Dube et al., 2003; Sharma et al., 2020). Secondly, this chromium-induced disturbance in nutrient uptake results in plants with delayed growth and development and yield penalties (Passardi et al., 2004; Wakeel et al., 2018). Therefore, plants with high nutrient use efficiency and robust trace metal compartmentalization mechanisms are necessary to complete their reproductive cycle under abiotic stress (Liang et al., 2023). The cellular internalization of chromium ions results in ROS accumulation, lipid peroxidation, failure in the functioning of organelles, and imminent oxidative stress in the cytoplasm (Van de Mortel et al., 2008). Subsequently, to minimize the severe damage to plant cells, plant defense responses are triggered by the gene expression and accumulation of enzymatic and non-enzymatic antioxidant compounds, such as tocopherol, β-carotene, glutathione, ascorbate, GST, glutathione reductase (GR), ascorbate peroxidase (APX), superoxide dismutase (SOD), and catalase (CAT) (Geng et al., 2004; Mitsuhashi et al., 2005; Anjum et al., 2008; Hossain et al., 2010, 2015; Mishra et al., 2023). Previous studies showed that stress caused by different trace metals changed the amount and activity of APX, CAT, GST, SOD, and GR enzymes in different plant species (Halušková et al., 2009; Lyubenova et al., 2009; Anjum et al., 2012; Yilmaz et al., 2017; Acila et al., 2024). On another hand, phytochelatins are produced from glutathione, which binds with trace metals, and this complex is transferred into the vacuole by ABC transport proteins (Song et al., 2014; Komal et al., 2015). Considering the up-regulation of *GST* genes in response to chromium, was hypothesized that phytochelatins should be produced by durum wheat roots to sequester chromium in vacuoles (Roy et al., 2023). In addition, the up-regulation of genes involved in sulfur assimilation in wheat roots kept under chromium exposure may also result in increased production of metallothioneins, which are low molecular cysteine-rich proteins that interact with trace metals via their thiol groups, sequestering and improving metal homeostasis (Ernst et al., 2008; Sekhar et al., 2011).

4.2. External stimuli response and multi-process regulation

The external stimuli responses were changed in the roots of wheat plants kept under chromium exposure based on the down-regulation of 10 genes linked to metal detoxification, systemic acquired resistance, and defense response to abiotic and biotic stresses, and the up-regulation of seven genes linked to photoperception, metal detoxification, pathogen enzyme inhibitor, and symbiotic signaling pathway. Likewise, the external stimuli responses were also altered in leaves based on the down-regulation of two genes linked to pathogen enzyme inhibitors, and the up-regulation of eight genes linked to transmembrane oxygen sensor, multi-stress and symbiotic signaling pathways, and effector-triggered immunity. Plants are sensitive to diverse external or environmental stimuli such as optimal conditions, threats, and stressful conditions that modulate plant growth and development, signaling and activation of responses for adaptation to adverse conditions, and plant defense in any stress conditions, respectively (Tafforeau et al., 2006; Lamers et al., 2020). The quick perception of external stimuli and effective signaling allows the plant to adapt and defend itself from adverse conditions to minimize the damage and penalties caused to it (Sharma et al., 2022; Nawaz et al., 2023). In particular, trace metals-induced stress conditions are sensed primarily by transmembrane ion channels and cell surface receptors, which translate the environmental signal into a cellular response that culminates in the transcriptional modulation of defense genes (Singh et al., 2015). This cascade of gene expression modulation and signals leads to the modulation or reprogramming of multi-biological processes linked to detoxification, defense response, immunity, and plant growth (Movahedi et al., 2022). In this sense, the multi-process regulation was changed in the roots of wheat plants kept under chromium exposure based on the down-regulation of seven genes linked to vacuole-mediated cell death (MCP1-related proteins), circadian rhythms, and MAP3K-MEK/RAF signaling pathways, and up-regulation of eight genes linked to SnRK1, phosphoinositide, calcium-dependent and MAP3K-MEK signaling pathways, and vacuole-mediated cell death (MCP2-related protein). Likewise, the external stimuli response was also changed in leaves based on the down-regulation of six genes linked to programmed cell death, SnRK1, ROP-GTPase regulatory system, and IRE1-bZIP60 UPR pathway, and up-regulation of six genes linked to SnRK1, ROP-GTPase regulatory system, calcium-dependent signaling, Regulation of Ace2 and Morphogenesis (RAM/MOR) signaling, and MAPK cascade signaling. The perception of trace metals by transmembrane receptors and the disturbances caused by them in ion transport channels lead to triggering the first layer of defense signaling to immediately modulate multi-processes. The signaling pathways orchestrated by ROP-GTPases, MAPK and SnRK1 kinases, and Ca^{2+} ion lead to the effective activation of defense responses and broad adaptation of the plant to these adverse conditions (Feiguelman et al., 2018; Atif et al., 2019; Xu et al., 2022b). In turn, the RAM/MOR and phosphoinositides signaling pathways modulate cell differentiation, plant growth, fine-tuning stem cell maintenance, organ polarity, and pollen tube formation (Zermiani et al., 2015; Heilmann, 2016; Bizotto et al., 2018). Meanwhile, the IRE1-bZIP60 signaling pathway acts as a transmembrane stress sensor that catalyzes the splicing and active form of the bZIP60 transcription factor (Deng et al., 2011; Nagashima et al., 2011). Finally, the modulation of vacuole-mediated cell death aims to protect the plant from cytotoxic effects caused by trace metals (Gill and Tuteja, 2011; Huang et al., 2021). The maintenance of the cell's life and viable vacuole allows the transport and sequestration of the metal, while cellular death allows its elimination at the cellular level (Sharma et al., 2016). Therefore, these collective data suggested that the stimuli perception and signaling pathways were modulated in both roots and leaves of wheat plants to promote more effective recognition, signaling, adaptation, and defense against chronic chromium stress, as well as enhancing broad basal defense.

4.3. Phytohormones

The phytohormone profile was significantly changed in roots of plants kept under chromium exposure based on the down-regulation of 19 genes linked to brassinosteroid signaling, cytokinin biosynthesis and degradation, ethylene biosynthesis, gibberellin biosynthesis, jasmonic acid signaling, and non-cysteine-rich-peptide (NCRP) signaling peptides, while up-regulation of 10 genes linked to auxin transport, brassinosteroid and cytokinin degradation, ethylene biosynthesis, and NCRP signaling peptides. In contrast, a smaller number of these genes were differentially expressed in leaves, with five down-regulated genes linked to auxin and ethylene signaling, gibberellin biosynthesis, and NCRP signaling peptides, while 12 up-regulated genes linked to ABA signaling, auxin and cytokinin degradation, gibberellin biosynthesis, and NCRP signaling peptides. Therefore, these collective data suggested that wheat plants exhibit significant hormonal changes in response to chronic chromium stress to defend themselves, be resilient, and successfully promote their development and reproduction even under adverse conditions. It is well known that the exposure of plants to abiotic stress results in the modulation of multiple processes ranging from transcriptional to hormonal reprogramming, mainly hormones involved in defense, adaptation, development, senescence, and reproduction (Bücker-Neto et al., 2017; Yadav et al., 2022). Plant hormones are small chemical messengers that modulate phenological, physio-biochemical, and developmental aspects in plants (Waadt et al., 2022). Auxins and cytokinins are the major hormones involved in plant growth, development, and branching (Ongaro and Leyser, 2007; Ferguson and Beveridge, 2009; Müller and Leyser, 2011; Basso et al., 2024a). In contrast, ABA, jasmonic acid, and brassinosteroids act mainly in the modulation of defense responses to abiotic and biotic stresses, and indirectly on the modulation of plant development and growth (Wasternack, 2015; Yao and Finlayson, 2015; Deng et al., 2022). ABA is the major hormone involved in plant signaling to defense responses against abiotic stress (Rai et al., 2024). While ABA represses plant development under stress conditions (Teng et al., 2023), brassinosteroids modulate the uptake and accumulation of ions and trace metals, induce antioxidant and phytochelatin biosynthesis, and modulate plant growth under stress conditions (Bajguz, 2002; Sharma and Bhardwaj, 2007; Choudhary et al., 2010). In addition, brassinosteroids act by promoting an increase in cell volume in the meristem and control multiple processes related to bud outgrowth, branching, and apical dominance under unstressful conditions (Chen et al., 2020; Wei and Li, 2020; Xia et al., 2021). Jasmonic acid limits the accumulation and enhances the plant tolerance to trace metals by modulating ion transport, antioxidant enzymes, and chelating capacity (Wang et al., 2020; Chen et al., 2021). In turn, ethylene acts mainly in the formation of axillary roots, inhibiting leaf and shoot growth, and regulating plant senescence and cell wall, while gibberellin acts in seed germination, root and shoot elongation, flowering, fruit patterning, and positively or negatively regulating the axillary bud development (Bicalho et al., 2015; Dubois et al., 2018; Katyayini et al., 2020). On the other hand, NCRP peptides act in signaling pathways controlling vegetative development and interactions with the environment in response to abiotic and biotic stress (Bircheneder and Dresselhaus, 2016; Kim et al., 2021). Overall, all these plant hormones and signaling peptides work in a complex signaling network depending on the environmental context, plant species, stage, and tissue (Müller and Leyser, 2011; Corbineau et al., 2014; Kebrom, 2017).

4.4. Secondary metabolism

The secondary metabolism was changed in the roots of wheat plants kept under chromium exposure based on the down-regulation of five genes linked to terpenoid and p-coumaroyl-CoA biosynthesis, and the up-regulation of three genes linked to carotenoid and flavonoid biosynthesis. Likewise, the secondary metabolism was also altered in leaves based on the down-regulation of nine genes and up-regulation of four genes both linked to terpenoid, p-coumaroyl-CoA, and flavonoid biosynthesis. Therefore, these collective data suggested that the secondary metabolism is modulated in both roots and leaves of wheat plants to promote protective functions in response to chronic chromium stress. Secondary metabolites are multifunctional compounds that act as regulators, signals, or hormones of plant growth and development, as well as biomolecules that defend and adapt plants against abiotic and biotic stresses (Erb and Kliebenstein, 2020; Zandalinas et al., 2022). For example, glucosinolates, terpenoids, flavonoids, carotenoids, phenolic acids, and alkaloids are involved in protective, chelating, and antioxidant functions in plant defense response to abiotic and biotic stresses (Ramakrishna and Ravishankar, 2011; Anjitha et al., 2021; Nicolas-Espinosa et al., 2023). In turn, another example is that carotenoids are secondary metabolites derived from the isoprenoid pathway that gives rise to ABA and strigolactones hormones (Basso et al., 2023, 2024b). Likewise, the stress-induced accumulation of secondary metabolites such as flavonoids, terpenoids, and carotenoids enhances plant antioxidant efficiencies by mitigating oxidative stress (Al-Sammarraie et al., 2020; Boncan et al., 2020; Shomali et al., 2022; Muthusamy and Lee, 2024). In turn, trace metals-induced phytotoxicity can also result in penalties in the biosynthesis and accumulation of these secondary metabolites, negatively impacting plant development and defense (Ramakrishna and Ravishankar, 2011).

4.5. Chlorophyll, photosynthesis, carbohydrate and amino acid metabolism

Chlorophyll-related metabolism was little altered in roots. In contrast, the chlorophyll-related metabolism in leaves was most affected, with eight down-regulated genes linked to chlorophyll biosynthesis and catabolism, while three up-regulated genes linked to chlorophyll catabolism and iron-sulfur assembly machinery. In agreement with these data, the photosynthesis machinery was also little affected in durum wheat roots. However, in leaves was observed a most significant impact in photosynthesis, with four up-regulated genes linked to photophosphorylation and photosystem II, while 12 down-regulated genes linked to photophosphorylation, RuBisCo, and NADP-dependent malate dehydrogenase. These data suggested an impact on chlorophyll pigments and photosynthesis efficiency in wheat leaves as a consequence of chronic chromium exposure. However, it was not reflected in the wheat biomass, panicles, and seed yield, possibly due to the chromium concentration and exposure level used in this study being chronic rather than acute. The trace metal toxicity in the chlorophyll, photosynthetic apparatus, and photosynthesis is well-known, which compromises the photochemical efficiency, and carbohydrate and amino acid metabolism (Chandra and Kang, 2016; Giannakoula et al., 2021; Singh et al., 2022). In addition to disturbance in nutrient uptake, chromium exposure induces oxidative stress that results in degradation of photosynthetic pigments and disruption of chloroplast ultrastructure (Panda and Choudhury, 2005; Sharma et al., 2020). This abiotic stress negatively impacts these parameters, bringing considerable penalties to plants, meanwhile, plants seek to reorganize their physiological activities in the face of this stress to withstand, improve, complete their life cycle, and reproduce (Figlioli et al., 2019; Yang et al., 2020). Based on differential expression results, the carbohydrate metabolism was not severely affected in roots of plants kept under chromium exposure. However, significant changes were observed in leaves, with five up-regulated genes linked to sucrose degradation and starch, UDP-D-xylose, and trehalose biosynthesis, while four down-regulated genes were linked to sucrose biosynthesis and starch degradation. These data suggested a higher starch, UDP-D-xylose, and trehalose accumulation in leaves as a consequence of chronic chromium stress. trace metals strongly impact plant metabolic processes and carbohydrate metabolism, such as soluble sugars, starch, the ratio among them, and the accumulation of structural and non-structural carbohydrates (Yang et al., 2024). Consequently, the carbon partitioning is also altered impacting plant growth and development (Prado et al., 2010; Rosa et al., 2017). Overall, trace metal phytotoxicity results in growth inhibition and increased carbohydrate accumulation, enhancing plant tolerance (Wang et al., 2021b). Also, the amino acid metabolism in roots of plants under chromium exposure was severely affected, with 12 down-regulated genes linked to amino acid biosynthesis and degradation, and one up-regulated gene linked to serine hydroxymethyltransferase. Likewise, the amino acid metabolism in leaves was less affected compared to roots, with two up-regulated genes linked to serine biosynthesis and GABA degradation, and one down-regulated gene linked to amino acid racemization. These data suggested a considerable reduction of amino acid biosynthesis in roots while minor changes were observed in leaves after chronic chromium stress. Indeed, the amino acid metabolism is altered in plants by trace metal phytotoxicity, which can act together with organic acids in metal binding, transport and chelation, as well as enhance adaptation and tolerance and improve plant development (Rai, 2002; Sharma and Dietz, 2006; Rath et al., 2019; Ali et al., 2023; Kocaman, 2023). For example, proline and cysteine accumulation is induced by abiotic stresses and has a positive correlation with plant tolerance through osmotic adjustment and protein synthesis (Sharma et al., 2020; Terzi and Yıldız, 2021).

4.6. *Cell wall organization*

The cell wall organization was significantly altered in roots of wheat plants kept under chromium exposure based on the down-regulation of five genes linked to xyloglucan and pectin modification, monolignol, and cuticular lipid formation (cutin and suberin), and up-regulation of nine genes linked to xyloglucan and homogalacturonan modification, cell wall proteins/enzymes, and cuticular lipid formation. Likewise, the cell wall organization was comparatively more altered in the leaves based on the down-regulation of 23 genes linked to cellulose biosynthesis, xyloglucan, xylan, homogalacturonan, cell wall proteins/enzymes, and cuticular lipid formation, while up-regulation of seven genes linked to cell wall proteins/enzymes, callose biosynthesis, and cuticular lipid formation. Collectively, these data suggested that wheat plants have their cell wall damaged in consequence or reorganized to support the chronic chromium stress. It is known that oxidative stress caused by trace metals alters the structure, composition, esterification, recalcitrance, and rigidity of the primary and secondary cell walls (Berni et al., 2019; Zhang et al., 2020). The cell wall modifications triggered by ROS results in lignification and pectin remodeling by increasing low methyl-esterified pectins that sequester trace metals (Van de Mortel et al., 2008; Moura et al., 2010; Le Gall et al., 2015; Parrotta et al., 2015). The main storage sites of chromium are in vacuoles after complexing with metallothioneins and phytochelatins to prevent its short- and long-distance translocation, but they also can be transported back out of the cell via H^+ ATPase influx transport and bind with the cell wall to prevent its retention in the cytoplasm and reduce ROS production and oxidative stress (Wakeel et al., 2020; Saud et al., 2022; Abdullah et al., 2024). Although chromium causes negative effects on the cell wall organization, these cell wall modifications also contribute to increasing plant tolerance to abiotic stresses but interfere with plant growth and development (Passardi et al., 2004).

4.7. *Cell division*

The cell division was little affected in the roots of plants kept under chromium exposure based on up-regulation of one gene linked to sister chromatid separation, while the most significant changes were observed in leaves by the down-regulation of four genes linked to cyclin regulatory proteins and cytokinesis, and up-regulation of two genes linked to DNA replication fork maintenance complex and asymmetric cell division. These collective data suggested that wheat plants need to reorganize their cell cycle in response to chronic chromium stress to successfully promote their growth, development, and reproduction at an appropriate time even under this adverse condition. In addition, changes caused by trace metals damage from genome stability to cell cycle progression and dynamic, negatively impacting cell division and proliferation (Schuppler et al., 1998; Jiang et al., 2000; Dutta et al., 2018). Therefore, these cell cycle changes caused by chromium exposure may result in a reduction in plant development and yield (Wakeel et al., 2019). In accordance, Gashi et al. (2024) showed that nickel, chromium, and lead caused cell cycle arrest in maize exposed to treated soil. Srivastava and Jain (2011) showed that chromium cytotoxicity caused aberrant mitotic phases in sugarcane root meristems. Similarly, Zou et al. (2006) and Wakeel et al. (2018) showed that chromium inhibits primary root elongation by regulating cell cycle-related genes in *A. thaliana* and *Amaranthus viridis*. Overall, hormonal, transcriptional, translational, and proteolytic modulation observed in durum wheat under chromium stress conditions supports the changes in cell division.

4.8. *Chromatin organization, transcriptional regulation, protein biosynthesis, and post-translational modification*

The chromatin organization was slightly altered in roots of plants kept under chromium exposure based on the down-regulation of one gene linked to histone post-translational modification and the up-regulation of one gene linked to H2A histones. However, more significant changes were observed in leaves with the down-regulation of eight genes linked to histones, chromatin remodeling, and DNA methylation regulation, while up-regulation of two genes linked to histone modification. These data align with what is expected from hormonal changes under stress conditions, where the plant seeks to modulate or reprogram the tissue-specific gene expression and its biological activities to adapt to these conditions (Probst and Mittelsten Scheid, 2015; Bäurle and Trindade, 2020; Bhadouriya et al., 2020; Halder et al., 2022). This way, chromatin is converted from a transcriptionally inactive state to an active state or vice versa (Bhadouriya et al., 2020). In this regard, the DNA transcriptional regulation and RNA biosynthesis were also highly altered in roots of plants kept under chromium stress based on the down-regulation of 32 genes and the up-regulation of 34 genes both linked to C2C2, MYB, GARP, HD-ZIP I/II, AP2/ERF, C2H2, EIL, HSF, MDAS, NAC, SBP, TCP, WRKY, AS2/LOB, CAMTA, bHLH, TIFY, and mTERF transcription factors and RNA polymerase II-dependent transcription. Likewise, the DNA transcriptional regulation and RNA biosynthesis were highly altered in leaves based on the down-regulation of 18 genes and up-regulation of 26 genes both linked to C2C2, MYB, GARP, bZIP, B3, AP2/ERF, C2H2, GRAS, NAC, TCP, WRKY, AS2/LOB, bHLH, DPB3, and mTERF transcription factors and RNA polymerase II-dependent transcription. These collective data suggested that transcriptional regulation was modulated in both roots and leaves of wheat plants in response to chronic chromium stress to successfully promote their defense, resilience, development, and reproduction even under this adverse condition. The expression data of transcription factor-related genes obtained in durum wheat are also in agreement with what was observed with the modulation of genes involved in phytohormones. Transcription factors play pivotal roles by transcriptionally suppressing or activating genes in a tissue-specific manner in plants under different stress and cross-stress conditions (Joshi et al., 2016; Javed et al., 2020). Hormonal signaling is started from the transmembrane receptors and transmitted by phosphorylation to the transcription factors in the nucleus, mitochondria, and chloroplast, which trigger the transcription of numerous genes involved in multiple processes (McSteen and Zhao, 2008; Wang et al., 2021a; Xin et al., 2021). Several transcription factor families are directly linked to the activation of defense responses to certain abiotic or abiotic stresses, while other families are interconnected or are influenced by this first group and modulate other mechanisms such as plant growth and development in the face of adverse conditions (Jiang et al., 2017; Meraj et al., 2020; Ma et al., 2024). Therefore, the transcription factor families identified as differentially modulated in durum wheat are involved in multiple processes, from defense, adaptation, and resilience, to the modulation of plant growth and development.

In turn, the protein biosynthesis was enhanced in roots of plants kept under chromium exposure based on the up-regulation of three genes linked to ribosomal RNA maturation, elongation factor, and peptide deformylation. Likewise, the protein biosynthesis was also altered in leaves based on the down-regulation of five genes linked to tRNA ligase and plastidial ribosome biogenesis, and the up-regulation of two genes linked to mitochondrial ribosome biogenesis. It is well documented that plants under abiotic stress show significant changes from the alternative splicing, microRNA, and ribosome biogenesis to protein abundance, cellular localization, post-transcriptional and post-translational modifications, protein-protein interactions, and protein biological functions (Kosová et al., 2018; Nie et al., 2021a; Dias-Fields and Adamala, 2022; Hu et al., 2022; Son and Park, 2023). In addition, plant ribosomes remodel the protein translation process to reduce energy consumption and adapt to stresses (Fakih et al., 2023). On the other hand, the abiotic stresses negatively impact the efficiency of protein biosynthesis machinery (Darriere et al., 2022).

In the same way, the protein post-translational modifications were also altered in roots of plants kept under chromium exposure based on the down-regulation of 15 genes linked to phosphorylation and S-glutathionylation, and the up-regulation of 10 genes linked to glycosylation and phosphorylation. Likewise, the ubiquitination and proteolysis were also altered in roots based on the down-regulation of 11 genes and the up-regulation of 13 genes. Similarly, it was observed in leaves the down-regulation of 13 genes linked to glycosylation, S-acylation, and phosphorylation, while up-regulation of 18 genes linked to phosphorylation and protein folding. In addition, the ubiquitination and proteolysis were also altered in leaves based on the down-regulation of 19 genes linked to protein quality control, protein folding, and protease inhibitor, and the up-regulation of 14 genes linked to protein folding and protease inhibitor. Therefore, these collective data suggested that protein biosynthesis and post-translational modifications were strongly modulated in both roots and leaves of wheat plants in response to chronic chromium exposure. In accordance, the protein post-translational modifications are mandatory for regulating the protein function, subcellular localization, activity, and stability in plants under unstressful conditions, but also essential for the adjustment of biological processes and defense mechanisms when under stresses (Zhao et al., 2011; Wu et al., 2016, 2023; Singh et al., 2023). Likewise, protein ubiquitination and proteolysis are also essential for the adjustment of multiple processes, elimination of abnormal proteins, adaptation and defense response to abiotic stress conditions (Lyzenga and Stone, 2012; Hasan et al., 2017; Yu et al., 2020; Mo et al., 2021).

4.9. *Lipid metabolism*

The lipid metabolism was significantly altered in roots of plants kept under chromium exposure, with six down-regulated genes linked to fatty acid, glycerolipid, and phytosterol metabolism, most of them being related to desaturation and degradation, while nine up-regulated genes also linked to fatty acid, glycerolipid, and phytosterol metabolism. Likewise, the lipid metabolism was most severely affected in leaves, with 13 down- and eight up-regulated genes both linked to fatty acid, glycerolipid, and phytosterol metabolism, but most of the down-regulated genes were related to fatty acid metabolism while most of the up-regulated was related to phytosterol metabolism. These data suggested an alteration in lipid biosynthesis and degradation in roots and more severely in leaves as a consequence of chronic chromium stress. The trace metals cause lipid peroxidation as a consequence of oxidative stress, resulting in alterations of the membrane structure, function, permeability, and fluidity (Elloumi et al., 2014; Chaffai and Cherif, 2020; Wakeel et al., 2020). Changes in lipid metabolism may also be associated with the maintenance of membrane homeostasis (Wu et al., 2021, 2022). For example, sterols are positively correlated with plant tolerance, and their diversity and conjugated forms allow plants to adapt to environmental stresses (Rogowska and Szakiel, 2020; Du et al., 2022). Likewise, some lipids such as phosphatidic acid, oxylipins, phosphoinositides, lysophospholipids, sphingolipids, and N-acylethanolamines act as signaling molecules for the defense response toward stresses (Hou et al., 2016; Sharma et al., 2023).

4.10. *Nicotianamine metabolism*

The nicotianamine metabolism was severely altered in roots of plants kept under chromium exposure based on the down-regulation of 11 genes linked to its biosynthesis and one down-regulated gene linked to metal transport complexed with nicotianamine. No significant changes were observed in leaves. In particular, nicotianamine is a metal chelator and phytosiderophore found in all plant organs that acts in the long-distance transport, homeostasis, hyperaccumulation in storage sites, and remobilization of metals, including chelation of some trace metals (Douchkov et al., 2005; Deinlein et al., 2012; Aung et al., 2019; Seregin and Kozhevnikova, 2023). Based on the up-regulation of *nicotianamine synthase* genes, nicotianamine was associated with the improved tolerance of durum wheat plants against cadmium (Aprile et al., 2018). Similarly, the transgenic overexpression of these genes increased nicotianamine biosynthesis and enhanced plant tolerance to high levels of nickel and iron (Kim et al., 2005; Pianelli et al., 2005; Yang et al., 2015). Based on our data, nicotianamine does not seem to have a direct involvement in the detoxification or chelation of hexavalent chromium in durum wheat. Nevertheless, the down-regulation of these genes suggested that the plant can prevent the long-distance or root-to-leaf transport of possible traces of reduced chromium (such as trivalent chromium).

4.11. *Main genes suggested as involved in this primary defense response and resilience*

Among the main DETs modulated by chromium exposure, 14 genes coding trace metal transport/detoxification superfamily proteins and one gene (TRITD1Av1G123180) coding a MATE-like detoxification protein were identified with greater prominence. The first transcript subset encodes metalloproteins or metallochaperone-like proteins containing a typical trace metal-associated (HMA, PF00403) domain (Li et al., 2020; Yang et al., 2022). The HMA is a highly conserved domain, comprises ∼30 amino acid residues, and is found in a number of trace metal transport or detoxification proteins, which contain two conserved cysteines (CxxC core motif) that are involved in metal binding (He et al., 2020; Li et al., 2020; Barr et al., 2023). The HMA-containing proteins are organized into four groups: trace metal-associated plant proteins

(HPPs), trace metal-associated isoprenylated plant proteins (HIPPs), ATOX1/ATX1-like proteins, and P1B-ATPase proteins (Abreu-Neto et al., 2013; Li et al., 2020). In particular, the ATOX1/ATX1-related proteins (PTHR22814) play key roles in trace metal transport and detoxification, which are associated with metal homeostasis and detoxification from the cytoplasm, preventing the metal ion from binding to more essential proteins or organelles (Himelblau et al., 1998; Robinson and Winge, 2010; Abreu-Neto et al., 2013). For example, *atox1/atx1* mutant plants are sensitive to copper deficiency while *ATOX1/ATX1* overexpression enhanced copper tolerance and improved the tolerance to copper deficiency, improving copper homeostasis (Shin et al., 2012). Other members of this HMA-related gene family are responsive to trace metal stress, as well as other types of abiotic and biotic stresses, and their transgenic expression in susceptible-mutant yeasts and A. *thaliana* plants restore or improve tolerance to trace metals (Tehseen et al., 2010; Imran et al., 2016; Tan et al., 2017; Wei et al., 2023; Zhou et al., 2023). Importantly, some of these genes act specifically for a certain trace metal, conferring tolerance, while others are associated with plant susceptibility to a specific toxic metal (Suzuki et al., 2002; Tehseen et al., 2010; Abreu-Neto et al., 2013).

In contrast, MATE-like detoxification protein (At4g29140, ortholog of TRITD1Av1G123180) was previously identified as ADS1 (*activated disease susceptibility 1*) (Sun et al., 2011), ADP1 (*altered development program 1*) (Li et al., 2014), and ABS3 (*Arabidopsis abnormal shoot 3*) (Wang et al., 2015). It was designed as a putative MATE efflux protein with drug transmembrane transporter activity that negatively regulates plant resistance to biotic stress and controls hypocotyl cell elongation and shoot apical meristem development (Sun et al., 2011; Li et al., 2014; Wang et al., 2015). In general, MATE-related proteins act in plant tolerance toward aluminum phytotoxicity, mediating organic acid efflux from the root exudates for metal chelating (Ribeiro et al., 2017). In particular, *A. thaliana ads1/adp1/abs3* mutant plants displayed pleiotropic phenotypes, while gain-of-function mutants by transgenic overexpression of this gene driven by 4x enhanced CaMV 35S promoter showed from reestablishing the normal phenotype to accelerated growth rate and bushy phenotypes (Sun et al., 2011; Li et al., 2014). Likewise, its overexpression driven by the CaMV 35S promoter in wild-type background *A. thaliana* also resulted in strong bushy phenotypes (Wang et al., 2015). Our RNA-seq data revealed that this gene was up-regulated only in roots, thus, it is suggested that its transgenic overexpression triggered by root-specific promoter may confer improved tolerance to chromium stress. Therefore, as this gene has canonical expression in different tissues and is involved also in plant development, its constitutive or non-root-specific overexpression can result in a pleiotropic phenotype.

Another two DETs (TRITD3Bv1G278650 and TRITD1Bv1G168120) encode zinc transporter (putative ZIP2 and ZIP5) proteins, which contain a zinc/iron permease domain (PF02535, PTHR11040) and belong to the ZIP/ZRT/IRT multigenic family (Lin et al., 2009; Tan et al., 2019). This protein group mediates zinc uptake from the rhizosphere across the plasma membrane, but may also transport copper and cadmium ions, metal cations, as well as other trace metals outside the cell, within an intracellular compartment, or its loading through the xylem (Li et al., 2013; An et al., 2021; Yang et al., 2022; Ahmad et al., 2023). In addition, some ZIP proteins were suggested as trace metal transporters and associated with cellular homeostasis and plant tolerance to this stress (Angulo-Bejarano et al., 2021; Ullah et al., 2023). The transgenic overexpression or mutant plants of *ZIP/ZRT/IRT* genes showed improved or deficient metal uptake or influx transport (Lin et al., 2009; Lee et al., 2010; Tan et al., 2019). Likewise, a trace metal transporter (TRITD7Bv1G184110) of the P-type ATPase family (PR00119, PF00122, and PTHR24093) was identified as up-regulated in response to chromium and associated as an essential protein for metal homeostasis (Eren and Argüello, 2004; Hussain et al., 2004). In addition, a sulfite exporter TauE/SafE family transmembrane protein (TRITD3Bv1G229690) was also identified as up-regulated by chromium exposure and associated with the transport of sulfur and anions across the cytoplasmic membrane, which are commonly modulated by stress caused by metal phytotoxicity (Yokosho et al., 2014; Guo et al., 2017; Xu et al., 2017). Similarly, a phosphate import transmembrane ATP-binding protein (TRITD4Bv1G190110) containing ABC transporter-like and AAA+ ATPase domains (PF00005, PTHR24220, and SM00382) was also highlighted. It is suggested as involved in the transmembrane transport, which can also be associated with plant defense response toward metal-induced stress (Hwang et al., 2016; Tan et al., 2017; Angulo-Bejarano et al., 2021; Yang et al., 2022).

In the same sense, two genes encoding the Vps51/Vps67-like protein (TRITD3Av1G256800 and TRITD3Bv1G255460) were identified as up-regulated in leaves, and are functionally annotated as components of vacuole vesicular transport. They are suggested to participate in endo- or lysosomal vesicular transport to the vacuolar compartmentalization or sequestration triggered by cytoplasmatic accumulation of chromium (Pahari et al., 2014). In addition, another gene (TRITD2Av1G268510) encoding a heme transporter hrg1-A-G-like protein was down-regulated in roots, which is putatively associated with the metal recycling compartmentalized into plant cells, and its down-regulation can prevent metal decompartmentalization (White et al., 2013). The modulation of the expression of these genes as a result of chromium exposure suggests that they may be involved in the compartmentalization or vacuolarization process of this trace metal in durum wheat.

Another four genes encoding metal-binding proteins were up-regulated in root or leaf (TRITD3Bv1G173850, TRITD0Uv1G129700, TRITD0Uv1G134940, and TRITD5Bv1G094790). These include proteins such as inactive purple acid phosphatase-like protein (PF00149), RmlC-like cupins superfamily protein (PR00325 and PF07883), and ferritin (PF00210). These proteins are associated with the nutrient deprivation response, modulated by oxidative stress to improve plant resilience, important for metal homeostasis, and oxidoreduction (Li et al., 2002; Liao et al., 2003; Dunwell et al., 2004; Bournier et al., 2013; Vergata et al., 2023; Zhang et al., 2023). Possibly, the modulation of these genes is in response to the deficiency and disturbance of essential metals induced by chromium (Lešková et al., 2017). Meanwhile, the chelating activity of these proteins directly on the chromium ion has not been reported.

In this same sense, the positive regulation of *isocitrate lyase* (TRITD2Av1G086060) and *citrate synthase* (TRITD3Bv1G026020) genes and the negative regulation of *malate dehydrogenase* (TRITD5Av1G255900) gene were observed. This glyoxalate pathway modulation leads to increased malate accumulation, a precursor of malate-derived organic acids, such as malic acid and maleic acid, involved in the trace metals chelating (Angulo-Bejarano et al., 2021; Kocaman, 2023). Root exudation of these organic acids has been associated with increased plant tolerance to trace metals, including chromium (Chen et al., 2015; Angulo-Bejarano et al., 2021; Zulkernain et al., 2023), as well as exogenous application (Hawrylak-Nowak et al., 2015; Mahmud et al., 2017; Mahey et al., 2019). Therefore, genetic engineering of durum wheat using transgenesis or genome editing approaches can be suggested to increase the accumulation of malate-derived organic acids and improve plant tolerance to chromium exposure.

Finally, another five genes were highlighted as differentially expressed and associated with improved plant resilience toward chromium exposure. These genes encode for a non-symbiotic hemoglobin protein (TRITD1Bv1G186850), cell death-related protein (TRITD1Av1G178640), plant cadmium resistance protein (TRITD4Av1G056120), CBF3/DREB1A transcription factor (TRITD5Av1G178430), and Myb/SANT-related transcription factor (TRITD2Av1G218770). The non-symbiotic globin proteins of class #1 (Glb1) are commonly induced by hypoxia or anoxia and have a high affinity to oxygen and nitric oxide (Igamberdiev and Hill, 2004; Perazzoli et al., 2004; Garrocho-Villegas et al., 2007). Some *Glb1* genes have been proven as induced by abiotic and biotic stresses and were associated with improved plant tolerance to abiotic stress and reduced susceptibility to biotic stress (Nakayama et al., 2017; Basso et al., 2022;

Koltun et al., 2022). Meanwhile, another gene encoding a serpin family protein (TRITD1Av1G178640) was identified as down-regulated in leaves, which is annotated as an inhibitor of pro-apoptotic proteases and involved in the regulation of programmed cell death (PF00079 and PTHR11461) (Vercammen et al., 2006). Therefore, the down-regulation or even knockout of this gene is suggested to carry out a proof-of-concept in durum wheat aiming to improve plant tolerance to chromium stress. Another three genes (TRITD4Av1G056120, TRITD5Av1G178430, and TRITD2Av1G218770) were also identified as up-regulated in roots and leaves, which encodes a cadmium resistance protein, CBF3/DREB1A transcription factor, and Myb/SANT-like transcription factor, respectively. Based on functional analysis of orthologs, these three proteins are suggested to have a positive correlation between protein accumulation and improved plant tolerance to abiotic stresses (Sakuma et al., 2006; Roy, 2016; Wang et al., 2019; Kidokoro et al., 2020). Therefore, it is suggested that these five genes are associated with the plant defense against chromium stress, and the use of genetic engineering aimed at their transcriptional modulation based on overexpression or down-regulation can lead to improved resilience of durum wheat. Furthermore, the exploration of two putative gene clusters associated with plant resistance to chromium stress in durum wheat breeding is also suggested.

5. Conclusions

Herein, we hypothesize that chromium is sensed in the roots by membrane receptors whose nature is still unknown (Fig. 6) despite several gene candidates have been selected by studying chromium stress in *A. thaliana* (Colzi et al., 2023). The adsorption of chromium should activate a protein phosphorylation cascade where calcium-calmodulin plays a key role. This should activate the biosynthesis and response of key hormones such as brassinosteroids as shown by the upregulation of several brassinosteroid-associated genes in the roots. That is expected to be linked to the induction of C2H2 and HSF transcription factors which also were higher in abundance in roots. These small regulating molecules would trigger the upregulation of transcription factors which are known to induce two types of beneficial responses against trace metal stress: (i) the enhancement of antioxidant responses through GSTs, GSH, SOD, and other ROS detoxifying enzymes, (ii) the induction of chelators formed from sulfur as predicted by the induction of several genes involved in sulfur assimilation. A negative effect played by chromium presence seems to be the reduction of expression of genes involved in

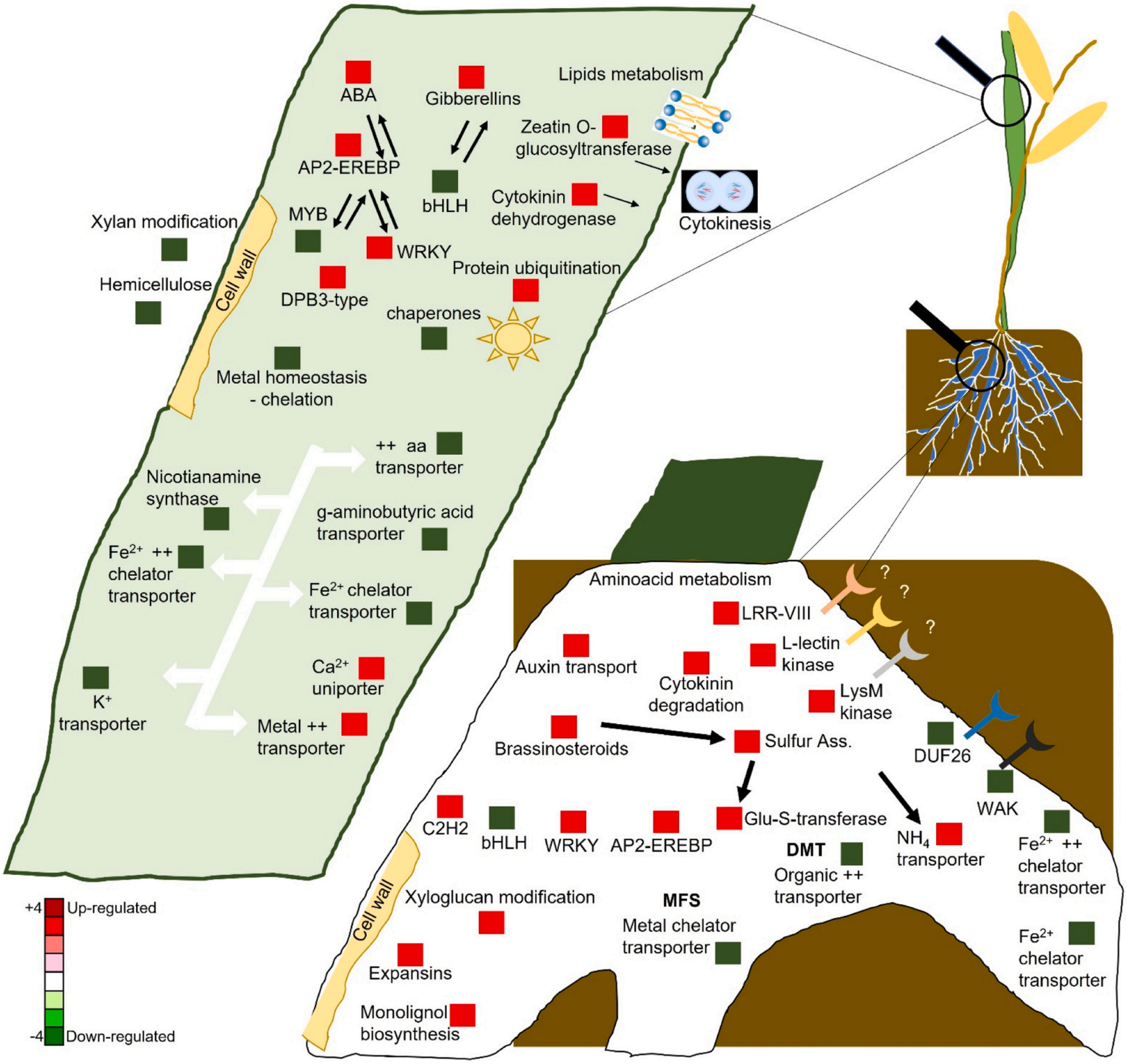

Fig. 6. A hypothetical model of transcriptional regulation of defense response to chromium stress in durum wheat plants. Main transcriptional changes (transcripts, transcript categories, and pathways) were shown. Green arrows and squares mean repression while red ones mean general induction.

nutrient uptake (micro- and macro-elements). Indeed, ABA-related genes were induced probably activating physiological responses against chromium uptake such as reduction of transpiration and closure of stomata. Leaves are less expanded as shown by the repression of genes involved in cell division and lipid biosynthesis and metabolism. Further research is needed to validate these shown findings identifying key players involved in chromium molecular response. In addition, it would be certainly important to understand possible transgenerational effects due to chromium presence due to epigenomic changes which should modulate the transcription of key genes in the signal transduction and defense response to this toxic trace metal. In particular, focusing on the primary defense response based on chromium import, detoxification, and compartmentalization, among the DETs was selected a gene/protein set involved in (i) metal detoxification, (ii) metal transport, (iii) vacuolar endosome for metal compartmentalization, (iv) metal binding, (v) malate-derived organic acid biosynthesis, and (vi) plant resilience to chromium exposure. At least 31 genes involved in this primary defense response are suggested as powerful biotechnological assets for future proof-of-concept studies aimed at the genetic engineering of durum wheat to improve plant tolerance toward chromium exposure.

Funding

This work was supported by the Partnership for Research and Innovation in the Mediterranean Area. The PRIMA program is an Art.185 initiative supported and funded under Horizon 2020, the European Union's Framework Program for Research and Innovation. Project No. 1432-LEGU-MED2-Legumes in biodiversity-based farming systems in Mediterranean basin project funded. In addition, FM was also supported by the Israeli Ministry of Science and Technology and the Italian Ministry of Foreign Affairs and International Cooperation (Grant # 3-17924) (project name: Resilient Hummus).

CRediT authorship contribution statement

Ida Pernice: Investigation. **Carla Lo Passo:** Investigation. **Cristina Gonnelli:** Investigation. **Alessandro Frontini:** Investigation. **Alessio Aprile:** Investigation, Formal analysis, Conceptualization. **Miriam Negussu:** Investigation. **Ilaria Colzi:** Investigation. **Luigi De Bellis:** Supervision, Funding acquisition, Conceptualization. **Matteo Buti:** Writing – review & editing, Formal analysis, Data curation. **Federico Martinelli:** Supervision, Project administration, Funding acquisition, Conceptualization. **Marcos Fernando Basso:** Writing – original draft, Formal analysis.

Authors contributions

LDB and AA provided plant material, physiology, and total RNA samples. MB performed bioinformatic analyses. MN, IC, and CG performed the elemental analysis. AF, IP, and CLP provide inputs in carrying out the experiments. MFB analyzed the data and wrote the original manuscript. FM, LDB, AA, CG, and MB provided intellectual input in the final version. FM provided financial resources. All authors approved the final version.

Consent for publication or Ethics approval and consent to participate

Not applicable.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Federico Martinelli reports financial support was provided by the University of Florence. Federico Martinelli reports a relationship with the University of Florence that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

MFB is grateful to CNPq for a postdoctoral research fellowship (process number: 108646/2024–6).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envexpbot.2024.105953.

References

Abdullah, Wani, K.I., Naeem, M., Jha, P.K., Jha, U.C., Aftab, T., Prasad, P.V.V., 2024. Systems biology of chromium-plant interaction: insights from omics approaches. Front. Plant Sci. 14, 1305179 https://doi.org/10.3389/fpls.2023.1305179.

Abreu-Neto, J.B., Turchetto-Zolet, A.C., de Oliveira, L.F.V., Bodanese Zanettini, M.H., Margis-Pinheiro, M., 2013. Heavy metal-associated isoprenylated plant protein (HIPP): characterization of a family of proteins exclusive to plants. FEBS J. 280 (7), 1604–1616. https://doi.org/10.1111/febs.12159.

Acila, S., Derouiche, S., Allioui, N., 2024. Embryo growth alteration and oxidative stress responses in germinating *Cucurbita pepo* seeds exposed to cadmium and copper toxicity. Sci. Rep. 14 (1), 8608. https://doi.org/10.1038/s41598-024-58635-1.

Ahmad, I., Rawoof, A., Priyanka, Islam, K., Momo, J., Anju, T., Kumar, A., Ramchiary, N., 2023. Diversity and expression analysis of ZIP transporters and associated metabolites under zinc and iron stress in *Capsicum*. Plant Physiol. Biochem. 196, 415–430. https://doi.org/10.1016/j.plaphy.2023.01.060.

Albqmi, M., Selim, S., Yaghoubi Khanghahi, M., Crecchio, C., Al-Sanea, M.M., Alnusaire, T.S., Almuhayawi, M.S., Al Jaouni, S.K., Hussein, S., Warrad, M., AbdElgawad, H., 2023. Chromium(VI) toxicity and active tolerance mechanisms of wheat plant treated with plant growth-promoting Actinobacteria and olive solid waste. ACS Omega 8 (36), 32458–32467. https://doi.org/10.1021/acsomega.3c02447.

Ali, S., Mir, R.A., Tyagi, A., Manzar, N., Kashyap, A.S., Mushtaq, M., Raina, A., Park, S., Sharma, S., Mir, Z.A., Lone, S.A., Bhat, A.A., Baba, U., Mahmoudi, H., Bae, H., 2023. Chromium toxicity in plants: signaling, mitigation, and future perspectives. Plants 12 (7), 1502. https://doi.org/10.3390/plants12071502.

Al-Sammarraie, O.N., Alsharafa, K.Y., Al-limoun, M.O., Khleifat, K.M., Al-Sarayreh, S.A., Al-Shuneigat, J.M., Kalaji, H.M., 2020. Effect of various abiotic stressors on some biochemical indices of *Lepidium sativum* plants. Sci. Rep. 10 (1), 21131 https://doi.org/10.1038/s41598-020-78330-1.

An, Q., Cui, C., Muhammad Khan, N., Zhou, G., Wan, Y., 2021. Genome-wide investigation of ZINC-IRON PERMEASE (ZIP) genes in *Areca catechu* and potential roles of ZIPs in Fe and Zn uptake and transport. Plant Signal. Behav. 16 (12), 1995647 https://doi.org/10.1080/15592324.2021.1995647.

Andrews, S., 2010. FastQC: A quality control tool for high throughput sequence data [online], 〈http://www.bioinformatics.babraham.ac.uk/projects/fastqc/〉.

Angulo-Bejarano, P.I., Puente-Rivera, J., Cruz-Ortega, R., 2021. Metal and metalloid toxicity in plants: an overview on molecular aspects. Plants 10 (4), 635. https://doi.org/10.3390/plants10040635.

Anjitha, K.S., Sameena, P.P., Puthur, J.T., 2021. Functional aspects of plant secondary metabolites in metal stress tolerance and their importance in pharmacology. Plant Stress 2, 100038. https://doi.org/10.1016/j.stress.2021.100038.

Anjum, N.A., Ahmad, I., Mohmood, I., Pacheco, M., Duarte, A.C., Pereira, E., Umar, S., Ahmad, A., Khan, N.A., Iqbal, M., Prasad, M.N.V., 2012. Modulation of glutathione and its related enzymes in plants' responses to toxic metals and metalloids - A review. Environ. Exp. Bot. 75, 307–324. https://doi.org/10.1016/j.envexpbot.2011.07.002.

Anjum, N.A., Umar, S., Ahmad, A., Iqbal, M., 2008. Responses of components of antioxidant system in moongbean genotypes to cadmium stress. Commun. Soil Sci. Plant Anal. 39, 2469–2483. https://doi.org/10.1080/00103620802292871.

Aprile, A., Sabella, E., Francia, E., Milc, J., Ronga, D., Pecchioni, N., Ferrari, E., Luvisi, A., Vergine, M., De Bellis, L., 2019. Combined effect of cadmium and lead on durum wheat. Int. J. Mol. Sci. 20 (23), 5891. https://doi.org/10.3390/ijms20235891.

Aprile, A., Sabella, E., Vergine, M., Genga, A., Siciliano, M., Nutricati, E., Rampino, P., De Pascali, M., Luvisi, A., Miceli, A., Negro, C., De Bellis, L., 2018. Activation of a gene network in durum wheat roots exposed to cadmium. BMC Plant Biol. 18 (1), 238. https://doi.org/10.1186/s12870-018-1473-4.

Atif, R.M., Shahid, L., Waqas, M., Ali, B., Rashid, M.A.R., Azeem, F., Nawaz, M.A., Wani, S.H., Chung, G., 2019. Insights on calcium-dependent protein kinases (CPKs) signaling for abiotic stress tolerance in plants. Int. J. Mol. Sci. 20 (21), 5298. https://doi.org/10.3390/ijms20215298.

Aung, M.S., Masuda, H., Nozoye, T., Kobayashi, T., Jeon, J.-S., An, G., Nishizawa, N.K., 2019. Nicotianamine synthesis by OsNAS3 is important for mitigating iron excess stress in rice. Front. Plant Sci. 10, 660. https://doi.org/10.3389/fpls.2019.00660.

Bajguz, A., 2002. Brassinosteroids and lead as stimulators of phytochelatins synthesis in *Chlorella vulgaris*. J. Plant Physiol. 159 (3), 321–324. https://doi.org/10.1078/0176-1617-00654.

Barr, Z.K., Werner, T., Tilsner, J., 2023. Heavy metal-associated isoprenylated plant proteins (HIPPs) at plasmodesmata: exploring the link between localization and function. Plants 12 (16), 3015. https://doi.org/10.3390/plants12163015.

Basso, M.F., Arraes, F.B.M., Grossi-de-Sa, M., Moreira, V.J.V., Alves-Ferreira, M., Grossi-de-Sa, M.F., 2020. Insights into genetic and molecular elements for transgenic crop development. Front. Plant Sci. 11, 509. https://doi.org/10.3389/fpls.2020.00509.

Basso, M.F., Contaldi, F., Lo Celso, F., Karalija, E., Paz-Carrasco, L.C., Barone, G., Ferrante, A., Martinelli, F., 2023. Expression profile of the *NCED/CCD* genes in chickpea and lentil during abiotic stress reveals a positive correlation with increased plant tolerance. Plant Sci. 336, 111817 https://doi.org/10.1016/j.plantsci.2023.111817.

Basso, M.F., Contaldi, F., Lo Celso, F., Baratto, C.M., Grossi-de-Sa, M.F., Barone, G., Ferrante, A., Martinelli, F., 2024b. Identification and expression profile of the *SMAX/SMXL* family genes in chickpea and lentil provide important players of biotechnological interest involved in plant branching. Planta 259 (1), 1–23. https://doi.org/10.1007/s00425-023-04277-y.

Basso, M.F., Ferreira, P.C.G., Kobayashi, A.K., Harmon, F.G., Nepomuceno, A.L., Molinari, H.B.C., Grossi-de-Sa, M.F., 2019. MicroRNAs and new biotechnological tools for its modulation and improving stress tolerance in plants. Plant Biotechnol. J. 17 (8), 1482–1500. https://doi.org/10.1111/pbi.13116.

Basso, M.F., Giradin, G., Vergata, C., Buti, M., Martinelli, F., 2024a. Genome-wide transcript expression analysis reveals major chickpea and lentil genes associated with plant branching. Front. Plant Sci. 15, 1384237 https://doi.org/10.3389/fpls.2024.1384237.

Basso, M.F., Lourenço-Tessutti, I.T., Moreira-Pinto, C.E., Mendes, R.A.G., Paes-de-Melo, B., das Neves, M.R., Macedo, A.F., Figueiredo, V., Grandis, A., Macedo, L.L.P., Arraes, F.B.M., do Carmo Costa, M.M., Togawa, R.C., Enrich-Prast, A., Marcelino-Guimaraes, F.C., Gomes, A., Silva, M.C.M., Floh, E.I.S., Buckeridge, M.S., de Almeida Engler, J., Grossi-de-Sa, M.F., 2022. Overexpression of a soybean Globin (GmGlb1-1) gene reduces plant susceptibility to *Meloidogyne incognita*. Planta 256 (4), 83. https://doi.org/10.1007/s00425-022-03992-2.

Basso, M.F., Neves, M.F., Grossi-de-Sa, M.F., 2024c. Agriculture evolution, sustainability and trends, focusing on Brazilian agribusiness: a review. Front. Sustain. Food Syst. 7, 1296337 https://doi.org/10.3389/fsufs.2023.1296337.

Bäurle, I., Trindade, I., 2020. Chromatin regulation of somatic abiotic stress memory. J. Exp. Bot. 71 (17), 5269–5279. https://doi.org/10.1093/jxb/eraa098.

Becquer, T., Quantin, C., Sicot, M., Boudot, J.P., 2003. Chromium availability in ultramafic soils from New Caledonia. Sci. Total Environ. 301 (1-3), 251–261. https://doi.org/10.1016/s0048-9697(02)00298-x.

Beres, B.L., Rahmani, E., Clarke, J.M., Grassini, P., Pozniak, C.J., Geddes, C.M., Porker, K. D., May, W.E., Ransom, J.K., 2020. A systematic review of durum wheat: enhancing production systems by exploring genotype, environment, and management (G × E × M) synergies. Front. Plant Sci. 11, 568657 https://doi.org/10.3389/fpls.2020.568657.

Berni, R., Luyckx, M., Xu, X., Legay, S., Sergeant, K., Hausman, J.-F., Lutts, S., Cai, G., Guerriero, G., 2019. Reactive oxygen species and trace metal stress in plants: impact on the cell wall and secondary metabolism. Environ. Exp. Bot. 161, 98–106. https://doi.org/10.1016/j.envexpbot.2018.10.017.

Bertucci, A., Pierron, F., Gourves, P.-Y., Klopp, C., Lagarde, G., Pereto, C., Dufour, V., Gonzalez, P., Coynel, A., Budzinski, H., Baudrimont, M., 2018. Whole-transcriptome response to wastewater treatment plant and stormwater effluents in the Asian clam, *Corbicula fluminea*. Ecotoxicol. Environ. Saf. 165, 96–106. https://doi.org/10.1016/j.ecoenv.2018.08.090.

Bettarini, I., Colzi, I., Coppi, A., Falsini, S., Echevarria, G., Pazzagli, L., Selvi, F., Gonnelli, C., 2019. Unravelling soil and plant metal relationships in Albanian nickel hyperaccumulators in the genus *Odontarrhena* (syn. *Alyssum* sect. *Odontarrhena*, Brassicaceae). Plant Soil 440, 135–149. https://doi.org/10.1007/s11104-019-04077-y.

Bhadouriya, S.L., Mehrotra, S., Basantani, M.K., Loake, G.J., Mehrotra, R., 2020. Role of chromatin architecture in plant stress responses: an update. Front. Plant Sci. 11, 603380 https://doi.org/10.3389/fpls.2020.603380.

Bicalho, E.M., Pintó-Marijuan, M., Morales, M., Müller, M., Munné-Bosch, S., Garcia, Q. S., 2015. Control of macaw palm seed germination by the gibberellin/abscisic acid balance. Plant Biol. 17 (5), 990–996. https://doi.org/10.1111/plb.12332.

Bircheneder, S., Dresselhaus, T., 2016. Why cellular communication during plant reproduction is particularly mediated by CRP signalling. J. Exp. Bot. 67 (16), 4849–4861. https://doi.org/10.1093/jxb/erw271.

Bizotto, F.M., Ceratti, R.S., Braz, A.S.K., Masuda, H.P., 2018. Evolutionary history of *Mo25* gene in plants, a component of RAM/MOR signaling network. Mech. Dev. 153, 64–73. https://doi.org/10.1016/j.mod.2018.09.001.

Blake, N.K., Lehfeldt, B.R., Lavin, M., Talbert, L.E., 1999. Phylogenetic reconstruction based on low copy DNA sequence data in an allopolyploid: the B genome of wheat. Genome 42 (2), 351–360. https://doi.org/10.1139/g98-136.

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30 (15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

Boncan, D.A.T., Tsang, S.S.K., Li, C., Lee, I.H.T., Lam, H.-M., Chan, T.-F., Hui, J.H.L., 2020. Terpenes and terpenoids in plants: interactions with environment and insects. Int. J. Mol. Sci. 21 (19), 7382. https://doi.org/10.3390/ijms21197382.

Bournier, M., Tissot, N., Mari, S., Boucherez, J., Lacombe, E., Briat, J.F., Gaymard, F., 2013. Arabidopsis ferritin 1 (*AtFer1*) gene regulation by the phosphate starvation response 1 (AtPHR1) transcription factor reveals a direct molecular link between iron and phosphate homeostasis. J. Biol. Chem. 288 (31), 22670–22680. https://doi.org/10.1074/jbc.m113.482281.

Bücker-Neto, L., Paiva, A.L.S., Machado, R.D., Arenhart, R.A., Margis-Pinheiro, M., 2017. Interactions between plant hormones and trace metals responses. Genet. Mol. Biol. 40 (1), 373–386. https://doi.org/10.1590/1678-4685-gmb-2016-0087.

Cantalapiedra, C.P., Hernández-Plaza, A., Letunic, I., Bork, P., Huerta-Cepas, J., 2021. eggNOG-mapper v2: functional annotation, orthology assignments, and domain prediction at the metagenomic scale. Mol. Biol. Evol. 38 (12), 5825–5829. https://doi.org/10.1093/molbev/msab293.

Chaffai, R., Cherif, A., 2020. The cadmium-induced changes in the polar and neutral lipid compositions suggest the involvement of triacylglycerol in the defense response in maize. Physiol. Mol. Biol. Plants 26 (1), 15–23. https://doi.org/10.1007/s12298-019-00734-9.

Chandra, R., Kang, H., 2016. Mixed trace metal stress on photosynthesis, transpiration rate, and chlorophyll content in poplar hybrids. For. Sci. Technol. 12, 55–61. https://doi.org/10.1080/21580103.2015.1044024.

Chen, X., Jiang, W., Tong, T., Chen, G., Zeng, F., Jang, S., Gao, W., Li, Z., Mak, M., Deng, F., Chen, Z.H., 2021. Molecular interaction and evolution of jasmonate signaling with transport and detoxification of trace metals and metalloids in plants. Front. Plant Sci. 12, 665842 https://doi.org/10.3389/fpls.2021.665842.

Chen, K., Li, G.J., Bressan, R.A., Song, C.P., Zhu, J.K., Zhao, Y., 2020. Abscisic acid dynamics, signaling, and functions in plants. J. Integr. Plant Biol. 62 (1), 25–54. https://doi.org/10.1111/jipb.12899.

Chen, Z., Sun, L., Liu, P., Liu, G., Tian, J., Liao, H., 2015. Malate synthesis and secretion mediated by a manganese-enhanced malate dehydrogenase confers superior manganese tolerance in *Stylosanthes guianensis*. Plant Physiol. 167 (1), 176–188. https://doi.org/10.1104/pp.114.251017.

Choudhary, R., Chawala, V., Soni, N., Kumar, J., Vyas, R., 2010. Oxidative stress and role of antioxidants in male infertility. Pak. J. Physiol. 6 (2), 54–59. 〈https://pjp.pps.org.pk/index.php/PJP/article/view/814〉.

Christou, A., Georgiadou, E.C., Zissimos, A.M., Christoforou, I.C., Christofi, C., Neocleous, D., Dalias, P., Torrado, S., Argyraki, A., Fotopoulos, V., 2020. Hexavalent chromium leads to differential hormetic or damaging effects in alfalfa (*Medicago sativa* L.) plants in a concentration-dependent manner by regulating nitro-oxidative and proline metabolism. Environ. Pollut. 267, 115379 https://doi.org/10.1016/j.envpol.2020.115379.

Colzi, I., Gonnelli, C., Vergata, C., Golia, G., Coppi, A., Castellani, M.B., Giovino, A., Buti, M., Sabato, T., Capuana, M., Aprile, A., De Bellis, L., Cicatelli, A., Guarino, F., Castiglione, S., Ioannou, A.G., Fotopoulos, V., Martinelli, F., 2023. Transgenerational effects of chromium stress at the phenotypic and molecular level in *Arabidopsis thaliana*. J. Hazard. Mater. 442, 130092 https://doi.org/10.1016/j.jhazmat.2022.130092.

Corbineau, F., Xia, Q., Bailly, C., El-Maarouf-Bouteau, H., 2014. Ethylene, a key factor in the regulation of seed dormancy. Front. Plant Sci. 5, 539. https://doi.org/10.3389/fpls.2014.00539.

Darriere, T., Jobet, E., Zavala, D., Escande, M.L., Durut, N., de Bures, A., Blanco-Herrera, F., Vidal, E.A., Rompais, M., Carapito, C., Gourbiere, S., Sáez-Vásquez, J., 2022. Upon heat stress processing of ribosomal RNA precursors into mature rRNAs is compromised after cleavage at primary P site in *Arabidopsis thaliana*. RNA Biol. 19 (1), 719–734. https://doi.org/10.1080/15476286.2022.2071517.

Deinlein, U., Weber, M., Schmidt, H., Rensch, S., Trampczynska, A., Hansen, T.H., Husted, S., Schjoerring, J.K., Talke, I.N., Krämer, U., Clemens, S., 2012. Elevated nicotianamine levels in *Arabidopsis halleri* roots play a key role in zinc hyperaccumulation. Plant Cell 24 (2), 708–723. https://doi.org/10.1105/tpc.111.095000.

Deng, Y., Humbert, S., Liu, J.-X., Srivastava, R., Rothstein, S.J., Howell, S.H., 2011. Heat induces the splicing by IRE1 of a mRNA encoding a transcription factor involved in the unfolded protein response in Arabidopsis. PNAS 108 (17), 7247–7252. https://doi.org/10.1073/pnas.1102117108.

Deng, B., Zhang, W., Yang, H., 2022. Abscisic acid decreases cell death in *Malus hupehensis* Rehd. under cd stress by reducing root Cd^{2+} influx and leaf transpiration. J. Plant Growth Regul. 41, 639–646. https://doi.org/10.1007/s00344-021-10327-0.

Dennis, G., Sherman, B.T., Hosack, D.A., Yang, J., Gao, W., Lane, H.C., Lempicki, R.A., 2003. DAVID: database for annotation, visualization, and integrated discovery. Genome Biol. 4 (5), R60. https://doi.org/10.1186/gb-2003-4-5-p3.

Dias-Fields, L., Adamala, K.P., 2022. Engineering ribosomes to alleviate abiotic stress in plants: A perspective. Plants 11 (16), 2097. https://doi.org/10.3390/plants11162097.

Douchkov, D., Gryczka, C., Stephan, U.W., Hell, R., Bäumlein, H., 2005. Ectopic expression of *nicotianamine synthase* genes results in improved iron accumulation and increased nickel tolerance in transgenic tobacco. Plant, Cell Environ. 28 (3), 365–374. https://doi.org/10.1111/j.1365-3040.2005.01273.x.

Du, Y., Fu, X., Chu, Y., Wu, P., Liu, Y., Ma, L., Tian, H., Zhu, B., 2022. Biosynthesis and the roles of plant sterols in development and stress responses. Int. J. Mol. Sci. 23 (4), 2332. https://doi.org/10.3390/ijms23042332.

Dube, B.K., Tewari, K., Chatterjee, J., Chatterjee, C., 2003. Excess chromium alters uptake and translocation of certain nutrients in citrullus. Chemosphere 53 (9), 1147–1153. https://doi.org/10.1016/s0045-6535(03)00570-8.

Dubois, M., Van den Broeck, L., Inzé, D., 2018. The pivotal role of ethylene in plant growth. Trends Plant Sci. 23 (4), 311–323. https://doi.org/10.1016/j.tplants.2018.01.003.

Dunwell, J.M., Purvis, A., Khuri, S., 2004. Cupins: the most functionally diverse protein superfamily? Phytochemistry 65 (1), 7–17. https://doi.org/10.1016/j.phytochem.2003.08.016.

Dutta, S., Mitra, M., Agarwal, P., Mahapatra, K., De, S., Sett, U., Roy, S., 2018. Oxidative and genotoxic damages in plants in response to trace metal stress and maintenance of genome stability. Plant Signal. Behav. 13(8), e1460048. https://doi.org/10.1080/15592324.2018.1460048.

Elango, D., Devi, K.D., Jeyabalakrishnan, H.K., Rajendran, K., Thoomatti Haridass, V.K., Dharmaraj, D., Charuchandran, C.V., Wang, W., Fakude, M., Mishra, R., Vembu, K., Wang, X., 2022. Agronomic, breeding, and biotechnological interventions to mitigate trace metal toxicity problems in agriculture. J. Agric. Food Res. 10, 100374 https://doi.org/10.1016/j.jafr.2022.100374.

Elloumi, N., Zouari, M., Chaari, L., Jomni, C., Marzouk, B., Ben Abdallah, F., 2014. Effects of cadmium on lipids of almond seedlings (*Prunus dulcis*). Bot. Stud. 55 (1), 61. https://doi.org/10.1186/s40529-014-0061-7.

Emamverdian, A., Ding, Y., Mokhberdoran, F., Xie, Y., 2015. Trace metal stress and some mechanisms of plant defense response. TheScientificWorldJournal 2015, 756120. https://doi.org/10.1155/2015/756120.

Erb, M., Kliebenstein, D.J., 2020. Plant secondary metabolites as defenses, regulators, and primary metabolites: the blurred functional trichotomy. Plant Physiol. 184 (1), 39–52. https://doi.org/10.1104/pp.20.00433.

Eren, E., Argüello, J.M., 2004. Arabidopsis HMA2, a divalent heavy metal-transporting P (IB)-type ATPase, is involved in cytoplasmic Zn^{2+} homeostasis. Plant Physiol. 136 (3), 3712–3723. https://doi.org/10.1104/pp.104.046292.

Ernst, W.H.O., Krauss, G.-J., Verkleij, J.A.C., Wesenberg, D., 2008. Interaction of trace metals with the sulphur metabolism in angiosperms from an ecological point of view. Plant, Cell Environ. 31 (1), 123–143. https://doi.org/10.1111/j.1365-3040.2007.01746.x.

Ertani, A., Mietto, A., Borin, M., Nardi, S., 2017. Chromium in agricultural soils and crops: a review. Water Air Soil Pollut. 228, 190. https://doi.org/10.1007/s11270-017-3356-y.

Fakih, Z., Plourde, M.B., Germain, H., 2023. Differential participation of plant ribosomal proteins from the small ribosomal subunit in protein translation under stress. Biomolecules 13 (7), 1160. https://doi.org/10.3390/biom13071160.

Feiguelman, G., Fu, Y., Yalovsky, S., 2018. ROP GTPases structure-function and signaling pathways. Plant Physiol. 176 (1), 57–79. https://doi.org/10.1104/pp.17.01415.

Ferguson, B.J., Beveridge, C.A., 2009. Roles for auxin, cytokinin, and strigolactone in regulating shoot branching. Plant Physiol. 149 (4), 1929–1944. https://doi.org/10.1104/pp.109.135475.

Figlioli, F., Sorrentino, M.C., Memoli, V., Arena, C., Maisto, G., Giordano, S., Capozzi, F., Spagnuolo, V., 2019. Overall plant responses to Cd and Pb metal stress in maize: growth pattern, ultrastructure, and photosynthetic activity. Environ. Sci. Pollut. Res. 26 (2), 1781–1790. https://doi.org/10.1007/s11356-018-3743-y.

Fu, X., Mehmood, S., Ahmed, W., Ou, W., Suo, P., Zhang, Q., Fu, X., Sun, Z., Li, W., 2023. Reducing chromium toxicity in chinese cabbage through synergistic effects of silicon and selenium: a study of plant growth, chromium content, and biochemical parameters. Sustainability 15 (6), 5361. https://doi.org/10.3390/su15065361.

Garrocho-Villegas, V., Gopalasubramaniam, S.K., Arredondo-Peter, R., 2007. Plant hemoglobins: what we know six decades after their discovery. Gene 398 (1-2), 78–85. https://doi.org/10.1016/j.gene.2007.01.035.

Gashi, B., Buqaj, L., Vataj, R., Tuna, M., 2024. Chlorophyll biosynthesis suppression, oxidative level and cell cycle arrest caused by Ni, chromium and Pb stress in maize exposed to treated soil from the Ferronikel smelter in Drenas, Kosovo. Plant Stress 11, 100379. https://doi.org/10.1016/j.stress.2024.100379.

Ge, S.X., Jung, D., Yao, R., 2020. ShinyGO: a graphical gene-set enrichment tool for animals and plants. Bioinformatics 36 (8), 2628–2629. https://doi.org/10.1093/bioinformatics/btz931.

Geng, B., Chang, L., Pan, C., Qi, Y., Zhao, J., Pang, Y., Du, J., Tang, C., 2004. Endogenous hydrogen sulfide regulation of myocardial injury induced by isoproterenol. Biochem. Biophys. Res. Commun. 318 (3), 756–763. https://doi.org/10.1016/j.bbrc.2004.04.094.

Giannakoula, A., Therios, I., Chatzissavvidis, C., 2021. Effect of lead and copper on photosynthetic apparatus in citrus (*Citrus aurantium* L.) plants. The role of antioxidants in oxidative damage as a response to trace metal stress. Plants 10 (1), 155. https://doi.org/10.3390/plants10010155.

Gill, S.S., Tuteja, N., 2011. Cadmium stress tolerance in crop plants: probing the role of sulfur. Plant Signal. Behav. 6 (2), 215–222. https://doi.org/10.4161/psb.6.2.14880.

Guarino, F., Cicatelli, A., Nissim, W.G., Colzi, I., Gonnelli, C., Basso, M.F., Vergata, C., Contaldi, F., Martinelli, F., Castiglione, S., 2024. Epigenetic effects induced by chronic and acute chromium stress conditions in *Arabidopsis thaliana* identified by the MSAP-Seq. Chemosphere, 142642. https://doi.org/10.1016/j.chemosphere.2024.142642.

Guo, P., Qi, Y.-P., Yang, L.-T., Lai, N.-W., Ye, X., Yang, Y., Chen, L.-S., 2017. Root adaptive responses to aluminum-treatment revealed by RNA-seq in two citrus species with different aluminum-tolerance. Front. Plant Sci. 8, 330. https://doi.org/10.3389/fpls.2017.00330.

Halder, K., Chaudhuri, A., Abdin, M.Z., Majee, M., Datta, A., 2022. Chromatin-based transcriptional reprogramming in plants under abiotic stresses. Plants 11 (11), 1449. https://doi.org/10.3390/plants11111449.

Halušková, Lu, Valentovičová, K., Huttová, J., Mistrík, I., Tamás, L., 2009. Effect of abiotic stresses on glutathione peroxidase and glutathione S-transferase activity in barley root tips. Plant Physiol. Biochem. 47 (11-12), 1069–1074. https://doi.org/10.1016/j.plaphy.2009.08.003.

Han, L., Gu, H., Lu, W., Li, H., Peng, W.X., Ling Ma, N., Lam, S.S., Sonne, C., 2023. Progress in phytoremediation of chromium from the environment. Chemosphere 344, 140307. https://doi.org/10.1016/j.chemosphere.2023.140307.

Hasan, M.K., Cheng, Y., Kanwar, M.K., Chu, X.Y., Ahammed, G.J., Qi, Z.Y., 2017. Responses of plant proteins to trace metal stress - A review. Front. Plant Sci. 8, 1492. https://doi.org/10.3389/fpls.2017.01492.

Hawrylak-Nowak, B., Dresler, S., Matraszek, R., 2015. Exogenous malic and acetic acids reduce cadmium phytotoxicity and enhance cadmium accumulation in roots of sunflower plants. Plant Physiol. Biochem. 94, 225–234. https://doi.org/10.1016/j.plaphy.2015.06.012.

He, G., Qin, L., Tian, W., Meng, L., He, T., Zhao, D., 2020. Trace metal transporters-associated proteins in *S. tuberosum*: genome-wide identification, comprehensive gene feature, evolution and expression analysis. Genes (Basel) 11 (11), 1269. https://doi.org/10.3390/genes11111269.

Heilmann, I., 2016. Phosphoinositide signaling in plant development. Development 143 (12), 2044–2055. https://doi.org/10.1242/dev.136432.

Himelblau, E., Mira, H., Lin, S.J., Culotta, V.C., Peñarrubia, L., Amasino, R.M., 1998. Identification of a functional homolog of the yeast copper homeostasis gene ATX1 from Arabidopsis. Plant Physiol. 117 (4), 1227–1234. https://doi.org/10.1104/pp.117.4.1227.

Hossain, M.A., Bhattacharjee, S., Armin, S.-M., Qian, P., Xin, W., Li, H.-Y., Burritt, D.J., Fujita, M., Tran, L.-S.P., 2015. Hydrogen peroxide priming modulates abiotic oxidative stress tolerance: insights from ROS detoxification and scavenging. Front. Plant Sci. 6, 420. https://doi.org/10.3389/fpls.2015.00420.

Hossain, M.A., Hasanuzzaman, M., Fujita, M., 2010. Up-regulation of antioxidant and glyoxalase systems by exogenous glycinebetaine and proline in mung bean confer tolerance to cadmium stress. Physiol. Mol. Biol. Plants 16 (3), 259–272. https://doi.org/10.1007/s12298-010-0028-4.

Hou, Q., Ufer, G., Bartels, D., 2016. Lipid signalling in plant responses to abiotic stress. Plant, Cell Environ. 39 (5), 1029–1048. https://doi.org/10.1111/pce.12666.

Hu, J., Cai, J., Xu, T., Kang, H., 2022. Epitranscriptomic mRNA modifications governing plant stress responses: underlying mechanism and potential application. Plant Biotechnol. J. 20 (12), 2245–2257. https://doi.org/10.1111/pbi.13913.

Huang, Y., Chen, J., Zhang, D., Fang, B., YangJin, T., Zou, J., Chen, Y., Su, N., Cui, J., 2021. Enhanced vacuole compartmentalization of cadmium in root cells contributes to glutathione-induced reduction of cadmium translocation from roots to shoots in pakchoi (*Brassica chinensis* L.). Ecotoxicol. Environ. Saf. 208, 111616 https://doi.org/10.1016/j.ecoenv.2020.111616.

Huang, T.L., Huang, L.Y., Fu, S.F., Trinh, N.N., Huang, H.J., 2014. Genomic profiling of rice roots with short- and long-term chromium stress. Plant Mol. Biol. 86 (1-2), 157–170. https://doi.org/10.1007/s11103-014-0219-4.

Hussain, D., Haydon, M.J., Wang, Y., Wong, E., Sherson, S.M., Young, J., Camakaris, J., Harper, J.F., Cobbett, C.S., 2004. P-type ATPase trace metal transporters with roles in essential zinc homeostasis in Arabidopsis. Plant Cell 16 (5), 1327–1339. https://doi.org/10.1105/tpc.020487.

Hwang, J.U., Song, W.Y., Hong, D., Ko, D., Yamaoka, Y., Jang, S., Yim, S., Lee, E., Khare, D., Kim, K., Palmgren, M., Yoon, H.S., Martinoia, E., Lee, Y., 2016. Plant ABC transporters enable many unique aspects of a terrestrial plant's lifestyle. Mol. Plant 9 (3), 338–355. https://doi.org/10.1016/j.molp.2016.02.003.

Igamberdiev, A.U., Hill, R.D., 2004. Nitrate, NO and haemoglobin in plant adaptation to hypoxia: an alternative to classic fermentation pathways. J. Exp. Bot. 55 (408), 2473–2482. https://doi.org/10.1093/jxb/erh272.

Imran, Q.M., Falak, N., Hussain, A., Mun, B.-G., Sharma, A., Lee, S.-U., Kim, K.-M., Yun, B.-W., 2016. Nitric oxide responsive heavy metal-associated gene AtHMAD1 contributes to development and disease resistance in *Arabidopsis thaliana*. Front. Plant Sci. 7, 1712. https://doi.org/10.3389/fpls.2016.01712.

Jalmi, S.K., Bhagat, P.K., Verma, D., Noryang, S., Tayyeba, S., Singh, K., Sharma, D., Sinha, A.K., 2018. Traversing the links between trace metal stress and plant signaling. Front. Plant Sci. 9, 12. https://doi.org/10.3389/fpls.2018.00012.

Javed, T., Shabbir, R., Ali, A., Afzal, I., Zaheer, U., Gao, S.J., 2020. Transcription factors in plant stress responses: challenges and potential for sugarcane improvement. Plants 9 (4), 491. https://doi.org/10.3390/plants9040491.

Jiang, W., Liu, D., Li, H., 2000. Effects of Cu^{2+} on root growth, cell division, and nucleolus of *Helianthus annuus* L. Sci. Total Environ. 256 (1), 59–65. https://doi.org/10.1016/s0048-9697(00)00470-8.

Jiang, J., Ma, S., Ye, N., Jiang, M., Cao, J., Zhang, J., 2017. WRKY transcription factors in plant responses to stresses. J. Integr. Plant Biol. 59 (2), 86–101. https://doi.org/10.1111/jipb.12513.

Jiangtao, C., Yingzhen, K., Qian, W., Yuhe, S., Daping, G., Jing, L., Guanshan, L., 2015. MapGene2Chrom, a tool to draw gene physical map based on Perl and SVG languages. Yi Chuan = Hered. 37 (1), 91–97. https://doi.org/10.16288/j.yczz.2015.01.013.

Joshi, R., Wani, S.H., Singh, B., Bohra, A., Dar, Z.A., Lone, A.A., Pareek, A., Singla-Pareek, S.L., 2016. Transcription factors and plants response to drought stress: current understanding and future directions. Front. Plant Sci. 7, 1029. https://doi.org/10.3389/fpls.2016.01029.

Kabata-Pendias, A., Pendias, H., 2001. Trace elements in soils and plants, 3rd Edition. CRC Press, Boca Raton. 403 p.

Katyayini, N.U., Rinne, P.L.H., Tarkowská, D., Strnad, M., van der Schoot, C., 2020. Dual role of gibberellin in perennial shoot branching: inhibition and activation. Front. Plant Sci. 11, 736. https://doi.org/10.3389/fpls.2020.00736.

Kebrom, T.H., 2017. A growing stem inhibits bud outgrowth - The overlooked theory of apical dominance. Front. Plant Sci. 8, 1874. https://doi.org/10.3389/fpls.2017.01874.

Kidokoro, S., Kim, J.S., Ishikawa, T., Suzuki, T., Shinozaki, K., Yamaguchi-Shinozaki, K., 2020. DREB1A/CBF3 is repressed by transgene-induced DNA methylation in the Arabidopsis *ice1-1* mutant. Plant Cell 32 (4), 1035–1048. https://doi.org/10.1105/tpc.19.00532.

Kim, J.S., Jeon, B.W., Kim, J., 2021. Signaling peptides regulating abiotic stress responses in plants. Front. Plant Sci. 12, 704490 https://doi.org/10.3389/fpls.2021.704490.

Kim, D., Paggi, J.M., Park, C., Bennett, C., Salzberg, S.L., 2019. Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. Nat. Biotechnol. 37 (8), 907–915. https://doi.org/10.1038/s41587-019-0201-4.

Kim, S., Takahashi, M., Higuchi, K., Tsunoda, K., Nakanishi, H., Yoshimura, E., Mori, S., Nishizawa, N.K., 2005. Increased nicotianamine biosynthesis confers enhanced tolerance of high levels of metals, in particular nickel, to plants. Plant Cell Physiol. 46 (11), 1809–1818. https://doi.org/10.1093/pcp/pci196.

Kocaman, A., 2023. Combined interactions of amino acids and organic acids in trace metal binding in plants. Plant Signal. Behav. 18 (1), 2064072 https://doi.org/10.1080/15592324.2022.2064072.

Koltun, A., Bianca Fuhrmann-Aoyagi, M., Alexandra Cardoso Moraes, L., Lima Nepomuceno, A., Simões Azeredo Gonçalves, L., Marcia Mertz-Henning, L., 2022. Uncovering the roles of hemoglobins in soybean facing water stress. Gene 810, 146055. https://doi.org/10.1016/j.gene.2021.146055.

Komal, T., Mustafa, M., Ali, Z., Kazi, A.G., 2015. Trace metal uptake and transport in plants. In: Sherameti, I., Varma, A. (Eds.), Trace metal contamination of soils: Monitoring and remediation. Springer International Publishing, Cham, pp. 181–194. https://doi.org/10.1007/978-3-319-14526-6_10.

Kosová, K., Vítámvás, P., Urban, M.O., Prášil, I.T., Renaut, J., 2018. Plant abiotic stress proteomics: the major factors determining alterations in cellular proteome. Front. Plant Sci. 9, 122. https://doi.org/10.3389/fpls.2018.00122.

Kumar, P., 2021. Soil applied glycine betaine with Arbuscular mycorrhizal fungi reduces chromium uptake and ameliorates chromium toxicity by suppressing the oxidative stress in three genetically different sorghum (*Sorghum bicolor* L.) cultivars. BMC Plant Biol. 21 (1), 336. https://doi.org/10.1186/s12870-021-03113-3.

Kumar, P., Tokas, J., Singal, H.R., 2019. Amelioration of chromium VI toxicity in sorghum (*Sorghum bicolor* L.) using glycine betaine. Sci. Rep. 9 (1), 16020 https://doi.org/10.1038/s41598-019-52479-w.

Kundu, D., Dey, S., Raychaudhuri, S.S., 2018. Chromium (VI) - induced stress response in the plant *Plantago ovata* Forsk *in vitro*. Genes Environ. 40, 21. https://doi.org/10.1186/s41021-018-0109-0.

Lamers, J., van der Meer, T., Testerink, C., 2020. How plants sense and respond to stressful environments. Plant Physiol. 182 (4), 1624–1635. https://doi.org/10.1104/pp.19.01464.

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.

Lee, S., Kim, S.A., Lee, J., Guerinot, M.L., An, G., 2010. Zinc deficiency-inducible *OsZIP8* encodes a plasma membrane-localized zinc transporter in rice. Mol. Cells 29 (6), 551–558. https://doi.org/10.1007/s10059-010-0069-0.

Lešková, A., Giehl, R.F.H., Hartmann, A., Fargašová, A., von Wirén, N., 2017. Trace metals induce iron deficiency responses at different hierarchic and regulatory levels. Plant Physiol. 174 (3), 1648–1668. https://doi.org/10.1104/pp.16.01916.

Li, R., Li, J., Li, S., Qin, G., Novák, O., Pěnčík, A., Ljung, K., Aoyama, T., Liu, J., Murphy, A., Gu, H., Tsuge, T., Qu, L.J., 2014. ADP1 affects plant architecture by regulating local auxin biosynthesis. PLoS Genet. 10 (1), e1003954 https://doi.org/10.1371/journal.pgen.1003954.

Li, J., Zhang, M., Sun, J., Mao, X., Wang, J., Liu, H., Zheng, H., Li, X., Zhao, H., Zou, D., 2020. Trace metal stress-associated proteins in rice and arabidopsis: genome-wide identification, phylogenetics, duplication, and expression profiles analysis. Front. Genet. 11, 477. https://doi.org/10.3389/fgene.2020.00477.

Li, S., Zhou, X., Huang, Y., Zhu, L., Zhang, S., Zhao, Y., Guo, J., Chen, J., Chen, R., 2013. Identification and characterization of the zinc-regulated transporters, iron-regulated transporter-like protein (ZIP) gene family in maize. BMC Plant Biol. 13, 114. https://doi.org/10.1186/1471-2229-13-114.

Li, D., Zhu, H., Liu, K., Liu, X., Leggewie, G., Udvardi, M., Wang, D., 2002. Purple acid phosphatases of *Arabidopsis thaliana*: comparative analysis and differential regulation by phosphate deprivation. J. Biol. Chem. 277 (31), 27772–27781. https://doi.org/10.1074/jbc.m204183200.

Liang, B.W., Li, C., Bai, T.H., Wang, P., 2023. Editorial: nutrient use efficiency of plants under abiotic stress. Front. Plant Sci. 14, 1179842 https://doi.org/10.3389/fpls.2023.1179842.

Liao, Y., Smyth, G.K., Shi, W., 2013. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics 30 (7), 923–930. https://doi.org/10.1093/bioinformatics/btt656.

Liao, H., Wong, F.L., Phang, T.H., Cheung, M.Y., Li, W.Y., Shao, G., Yan, X., Lam, H.M., 2003. *GmPAP3*, a novel purple acid phosphatase-like gene in soybean induced by NaCl stress but not phosphorus deficiency. Gene 318, 103–111. https://doi.org/10.1016/s0378-1119(03)00764-9.

Lin, Y.F., Liang, H.M., Yang, S.Y., Boch, A., Clemens, S., Chen, C.C., Wu, J.F., Huang, J.L., Yeh, K.C., 2009. Arabidopsis IRT3 is a zinc-regulated and plasma membrane localized zinc/iron transporter. N. Phytol. 182 (2), 392–404. https://doi.org/10.1111/j.1469-8137.2009.02766.x.

Liu, J., Ding, G., Gai, Z., Zhang, W., Han, Y., Li, W., 2020. Changes in the gene expression profile of *Arabidopsis thaliana* under chromium stress. Ecotoxicol. Environ. Saf. 193, 110302 https://doi.org/10.1016/j.ecoenv.2020.110302.

Lunk, H.-J., 2015. Discovery, properties and applications of chromium and its compounds. ChemTexts 1, 6. https://doi.org/10.1007/s40828-015-0007-z.

Lyubenova, L., Nehnevajova, E., Herzig, R., Schröder, P., 2009. Response of antioxidant enzymes in *Nicotiana tabacum* clones during phytoextraction of heavy metals. Environ. Sci. Pollut. Res. 16 (5), 573–581. https://doi.org/10.1007/s11356-009-0175-8.

Lyzenga, W.J., Stone, S.L., 2012. Abiotic stress tolerance mediated by protein ubiquitination. J. Exp. Bot. 63 (2), 599–616. https://doi.org/10.1093/jxb/err310.

Ma, Z., Hu, L., Jiang, W., 2024. Understanding AP2/ERF transcription factor responses and tolerance to various abiotic stresses in plants: a comprehensive review. Int. J. Mol. Sci. 25 (2), 893. https://doi.org/10.3390/ijms25020893.

Maccaferri, M., Harris, N.S., Twardziok, S.O., Pasam, R.K., Gundlach, H., Spannagl, M., Ormanbekova, D., Lux, T., Prade, V.M., Milner, S.G., Himmelbach, A., Mascher, M., Bagnaresi, P., Faccioli, P., Cozzi, P., Lauria, M., Lazzari, B., Stella, A., Manconi, A., Gnocchi, M., Moscatelli, M., Avni, R., Deek, J., Biyiklioglu, S., Frascaroli, E., Corneti, S., Salvi, S., Sonnante, G., Desiderio, F., Marè, C., Crosatti, C., Mica, E., Özkan, H., Kilian, B., De Vita, P., Marone, D., Joukhadar, R., Mazzucotelli, E., Nigro, D., Gadaleta, A., Chao, S., Faris, J.D., Melo, A.T.O., Pumphrey, M., Pecchioni, N., Milanesi, L., Wiebe, K., Ens, J., MacLachlan, R.P., Clarke, J.M., Sharpe, A.G., Koh, C.S., Liang, K.Y.H., Taylor, G.J., Knox, R., Budak, H., Mastrangelo, A.M., Xu, S.S., Stein, N., Hale, I., Distelfeld, A., Hayden, M.J., Tuberosa, R., Walkowiak, S., Mayer, K.F.X., Ceriotti, A., Pozniak, C.J., Cattivelli, L., 2019. Durum wheat genome highlights past domestication signatures and future improvement targets. Nat. Genet. 51 (5), 885–895. https://doi.org/10.1038/s41588-019-0381-3.

Mahey, S., Kumar, R., Kumar, V., Sharma, R., Sharma, M., Bhardwaj, R., Thukral, A.K., 2019. Maleic acid and EDTA mediated extenuation of Co(II) stress in *Hordeum vulgare* seedlings. Biotechnol. Res. Innov. 3 (2), 221–230. https://doi.org/10.1016/j.biori.2019.07.002.

Mahmud, J.A., Hasanuzzaman, M., Nahar, K., Rahman, A., Hossain, M.S., Fujita, M., 2017. Maleic acid assisted improvement of metal chelation and antioxidant metabolism confers chromium tolerance in *Brassica juncea* L. Ecotoxicol. Environ. Saf. 144, 216–226. https://doi.org/10.1016/j.ecoenv.2017.06.010.

McSteen, P., Zhao, Y., 2008. Plant hormones and signaling: common themes and new developments. Dev. Cell 14 (4), 467–473. https://doi.org/10.1016/j.devcel.2008.03.013.

Medda, S., Mondal, N.K., 2017. Chromium toxicity and ultrastructural deformation of *Cicer arietinum* with special reference of root elongation and coleoptile growth. Ann. Agrar. Sci. 15 (3), 396–401. https://doi.org/10.1016/j.aasci.2017.05.022.

Meraj, T.A., Fu, J., Raza, M.A., Zhu, C., Shen, Q., Xu, D., Wang, Q., 2020. Transcriptional factors regulate plant stress responses through mediating secondary metabolism. Genes (Basel) 11 (4), 346. https://doi.org/10.3390/genes11040346.

Mishra, N., Jiang, C., Chen, L., Paul, A., Chatterjee, A., Shen, G., 2023. Achieving abiotic stress tolerance in plants through antioxidative defense mechanisms. Front. Plant Sci. 14, 1110622 https://doi.org/10.3389/fpls.2023.1110622.

Mitsuhashi, H., Yamashita, S., Ikeuchi, H., Kuroiwa, T., Kaneko, Y., Hiromura, K., Ueki, K., Nojima, Y., 2005. Oxidative stress-dependent conversion of hydrogen sulfide to sulfite by activated neutrophils. Shock (Augusta, Ga.) 24 (6), 529–534. https://doi.org/10.1097/01.shk.0000183393.83272.de.

Mo, F., Li, H., Li, Y., Chen, X., Wang, M., Li, Z., Deng, N., Yang, Y., Huang, X., Zhang, R., Deng, W., 2021. Physiological, biochemical, and transcriptional regulation in a leguminous forage *Trifolium pratense* L. responding to silver ions. Plant Physiol. Biochem. 162, 531–546. https://doi.org/10.1016/j.plaphy.2021.02.046.

Morris, R., Sears, E.R., 1967. The cytogenetics of wheat and its relatives. In: Quisenberry, K.S., Reitz, L.P. (Eds.), Wheat and wheat improvement. American Society of Agronomy, Madison, WI, USA, pp. 19–87.

Moura, J.C.M.S., Bonine, C.A.V., De Oliveira Fernandes Viana, J., Dornelas, M.C., Mazzafera, P., 2010. Abiotic and biotic stresses and changes in the lignin content and composition in plants. J. Integr. Plant Biol. 52 (4), 360–376. https://doi.org/10.1111/j.1744-7909.2010.00892.x.

Movahedi, A., Aghaei-Dargiri, S., Barati, B., Kadkhodaei, S., Wei, H., Sangari, S., Yang, L., Xu, C., 2022. Plant immunity is regulated by biological, genetic, and epigenetic factors. Agronomy 12 (11), 2790. https://doi.org/10.3390/agronomy12112790.

Müller, D., Leyser, O., 2011. Auxin, cytokinin and the control of shoot branching. Ann. Bot. 107 (7), 1203–1212. https://doi.org/10.1093/aob/mcr069.

Muthusamy, M., Lee, S.I., 2024. Abiotic stress-induced secondary metabolite production in *Brassica*: opportunities and challenges. Front. Plant Sci. 14, 1323085 https://doi.org/10.3389/fpls.2023.1323085.

Nagashima, Y., Mishiba, K.-I., Suzuki, E., Shimada, Y., Iwata, Y., Koizumi, N., 2011. Arabidopsis IRE1 catalyses unconventional splicing of bZIP60 mRNA to produce the active transcription factor. Sci. Rep. 1, 29. https://doi.org/10.1038/srep00029.

Nakayama, T.J., Rodrigues, F.A., Neumaier, N., Marcolino-Gomes, J., Molinari, H.B.C., Santiago, T.R., Formighieri, E.F., Basso, M.F., Farias, J.R.B., Emygdio, B.M., de Oliveira, A.C.B., Campos Â, D., Borém, A., Harmon, F.G., Mertz-Henning, L.M., Nepomuceno, A.L., 2017. Insights into soybean transcriptome reconfiguration under hypoxic stress: functional, regulatory, structural, and compositional characterization. PLoS ONE 12 (11), e0187920. https://doi.org/10.1371/journal.pone.0187920.

Nawaz, M., Sun, J., Shabbir, S., Khattak, W.A., Ren, G., Nie, X., Bo, Y., Javed, Q., Du, D., Sonne, C., 2023. A review of plants strategies to resist biotic and abiotic environmental stressors. Sci. Total Environ. 900, 165832 https://doi.org/10.1016/j.scitotenv.2023.165832.

Nicolas-Espinosa, J., Garcia-Ibañez, P., Lopez-Zaplana, A., Yepes-Molina, L., Albaladejo-Marico, L., Carvajal, M., 2023. Confronting secondary metabolites with water uptake and transport in plants under abiotic stress. Int. J. Mol. Sci. 24 (3), 2826. https://doi.org/10.3390/ijms24032826.

Nie, G., Liao, Z., Zhong, M., Zhou, J., Cai, J., Liu, A., Wang, X., Zhang, X., 2021a. MicroRNA-mediated responses to chromium stress provide insight into tolerance characteristics of *Miscanthus sinensis*. Front. Plant Sci. 12, 666117 https://doi.org/10.3389/fpls.2021.666117.

Nie, G., Zhong, M., Cai, J., Yang, X., Zhou, J., Appiah, C., Tang, M., Wang, X., Feng, G., Huang, L., Zhang, X., 2021b. Transcriptome characterization of candidate genes related to chromium uptake, transport and accumulation in *Miscanthus sinensis*. Ecotoxicol. Environ. Saf. 221, 112445 https://doi.org/10.1016/j.ecoenv.2021.112445.

Ongaro, V., Leyser, O., 2007. Hormonal control of shoot branching. J. Exp. Bot. 59 (1), 67–74. https://doi.org/10.1093/jxb/erm134.

Pahari, S., Cormark, R.D., Blackshaw, M.T., Liu, C., Erickson, J.L., Schultz, E.A., 2014. Arabidopsis UNHINGED encodes a VPS51 homolog and reveals a role for the GARP complex in leaf shape and vein patterning. Dev. (Camb., Engl.) 141 (9), 1894–1905. https://doi.org/10.1242/dev.099333.

Panda, S.K., Choudhury, S., 2005. Chromium stress in plants, 95-10 Braz. J. Plant Physiol. 17 (1). https://doi.org/10.1590/S1677-04202005000100008.

Parrotta, L., Guerriero, G., Sergeant, K., Cai, G., Hausman, J.-F., 2015. Target or barrier? The cell wall of early- and later-diverging plants vs cadmium toxicity: differences in the response mechanisms. Front. Plant Sci. 6, 133. https://doi.org/10.3389/fpls.2015.00133.

Passardi, F., Penel, C., Dunand, C., 2004. Performing the paradoxical: how plant peroxidases modify the cell wall. Trends Plant Sci. 9 (11), 534–540. https://doi.org/10.1016/j.tplants.2004.09.002.

Patra, D.K., Pradhan, C., Patra, H.K., 2019. Chromium bioaccumulation, oxidative stress metabolism and oil content in lemon grass *Cymbopogon flexuosus* (Nees ex Steud.) W. Watson grown in chromium rich over burden soil of Sukinda chromite mine, India. Chemosphere 218, 1082–1088. https://doi.org/10.1016/j.chemosphere.2018.11.211.

Paunov, M., Koleva, L., Vassilev, A., Vangronsveld, J., Goltsev, V., 2018. Effects of different metals on photosynthesis: cadmium and zinc affect chlorophyll fluorescence in durum wheat. Int. J. Mol. Sci. 19 (3), 787. https://doi.org/10.3390/ijms19030787.

Perazzolli, M., Dominici, P., Romero-Puertas, M.C., Zago, E., Zeier, J., Sonoda, M., Lamb, C., Delledonne, M., 2004. Arabidopsis nonsymbiotic hemoglobin AHb1 modulates nitric oxide bioactivity. Plant Cell 16 (10), 2785–2794. https://doi.org/10.1105/tpc.104.025379.

Pianelli, K., Mari, S., Marquès, L., Lebrun, M., Czernic, P., 2005. Nicotianamine over-accumulation confers resistance to nickel in *Arabidopsis thaliana*. Transgenic Res. 14 (5), 739–748. https://doi.org/10.1007/s11248-005-7159-3.

Prado, C., Rodríguez-Montelongo, L., González, J.A., Pagano, E.A., Hilal, M., Prado, F.E., 2010. Uptake of chromium by *Salvinia minima*: effect on plant growth, leaf respiration and carbohydrate metabolism. J. Hazard. Mater. 177 (1-3), 546–553. https://doi.org/10.1016/j.jhazmat.2009.12.067.

Prasad, S., Yadav, K.K., Kumar, S., Gupta, N., Cabral-Pinto, M.M.S., Rezania, S., Radwan, N., Alam, J., 2021. Chromium contamination and effect on environmental health and its remediation: a sustainable approaches. J. Environ. Manag. 285, 112174 https://doi.org/10.1016/j.jenvman.2021.112174.

Probst, A.V., Mittelsten Scheid, O., 2015. Stress-induced structural changes in plant chromatin. Curr. Opin. Plant Biol. 27, 8–16. https://doi.org/10.1016/j.pbi.2015.05.011.

Rai, V.K., 2002. Role of amino acids in plant responses to stresses. Biol. Plant. 45, 481–487. https://doi.org/10.1023/A:1022308229759.

Rai, G.K., Khanday, D.M., Choudhary, S.M., Kumar, P., Kumari, S., Martínez-Andújar, C., Martínez-Melgarejo, P.A., Rai, P.K., Pérez-Alfocea, F., 2024. Unlocking nature's stress buster: Abscisic acid's crucial role in defending plants against abiotic stress. Plant Stress 11, 100359. https://doi.org/10.1016/j.stress.2024.100359.

Ramakrishna, A., Ravishankar, G.A., 2011. Influence of abiotic stress signals on secondary metabolites in plants. Plant Signal. Behav. 6 (11), 1720–1731. https://doi.org/10.4161/psb.6.11.17613.

Rascio, I., Gattullo, C.E., Porfido, C., Allegretta, I., Spagnuolo, M., Tiziani, R., Celletti, S., Cesco, S., Mimmo, T., Terzano, R., 2023. Fire-induced effects on the bioavailability of potentially toxic elements in a polluted agricultural soil: implications for chromium uptake by durum wheat plants. Environ. Sci. Pollut. Res. 30 (3), 6358–6372. https://doi.org/10.1007/s11356-022-22471-5.

Rath, B.P., Hota, S., Subhadarshini, S., Dash, D., Das, P.K., 2019. Consequence of chromium-tainted soil on physical and biochemical responses of *Vigna radiata* L. J. Appl. Biol. Biotechnol. 7 (1), 35–41. https://doi.org/10.7324/JABB.2019.70107.

Ribeiro, A.P., de Souza, W.R., Martins, P.K., Vinecky, F., Duarte, K.E., Basso, M.F., da Cunha, B.A.D.B., Campanha, R.B., de Oliveira, P.A., Centeno, D.C., Cançado, G.M.A., de Magalhães, J.V., de Sousa, C.A.F., Andrade, A.C., Kobayashi, A.K., Molinari, H.B.C., 2017. Overexpression of *BdMATE* gene improves aluminum tolerance in *Setaria viridis*. Front. Plant Sci. 8, 865. https://doi.org/10.3389/fpls.2017.00865.

Robinson, M.D., McCarthy, D.J., Smyth, G.K., 2009. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26 (1), 139–140. https://doi.org/10.1093/bioinformatics/btp616.

Robinson, N.J., Winge, D.R., 2010. Copper metallochaperones. Annu. Rev. Biochem. 79, 537–562. https://doi.org/10.1146/annurev-biochem-030409-143539.

Rogowska, A., Szakiel, A., 2020. The role of sterols in plant response to abiotic stress. Phytochem. Rev. 19, 1525–1538. https://doi.org/10.1007/s11101-020-09708-2.

Rosa, M., Prado, C., Chocobar-Ponce, S., Pagano, E., Prado, F., 2017. Effect of seasonality and Cr(VI) on starch-sucrose partitioning and related enzymes in floating leaves of *Salvinia minima*. Plant Physiol. Biochem. 118, 1–10. https://doi.org/10.1016/j.plaphy.2017.05.014.

Roy, S., 2016. Function of MYB domain transcription factors in abiotic stress and epigenetic control of stress response in plant genome. Plant Signal. Behav. 11 (1), e1117723 https://doi.org/10.1080/15592324.2015.1117723.

Roy, S.K., Naimuzzaman, M., Rahman, F., 2023. Glutathione: a key frontier of heavy-metal detoxification and tolerance in plants. Plant Trends 1 (1), 33–42. https://doi.org/10.5455/pt.2023.05.

Sabella, E., Aprile, A., Tenuzzo, B.A., Carata, E., Panzarini, E., Luvisi, A., De Bellis, L., Vergine, M., 2022. Effects of cadmium on root morpho-physiology of durum wheat. Front. Plant Sci. 13, 936020 https://doi.org/10.3389/fpls.2022.936020.

Sabella, E., Luvisi, A., Genga, A., De Bellis, L., Aprile, A., 2021. Molecular responses to cadmium exposure in two contrasting durum wheat genotypes. Int. J. Mol. Sci. 22 (14), 7343. https://doi.org/10.3390/ijms22147343.

Sakuma, Y., Maruyama, K., Osakabe, Y., Qin, F., Seki, M., Shinozaki, K., Yamaguchi-Shinozaki, K., 2006. Functional analysis of an Arabidopsis transcription factor, DREB2A, involved in drought-responsive gene expression. Plant Cell 18 (5), 1292–1309. https://doi.org/10.1105/tpc.105.035881.

Saud, S., Wang, D., Fahad, S., Javed, T., Jaremko, M., Abdelsalam, N.R., Ghareeb, R.Y., 2022. The impact of chromium ion stress on plant growth, developmental physiology, and molecular regulation. Front. Plant Sci. 13, 994785 https://doi.org/10.3389/fpls.2022.994785.

Schuppler, U., He, P.-H., John, P.C.L., Munns, R., 1998. Effect of water stress on cell division and Cdc2-like cell cycle kinase activity in wheat leaves. Plant Physiol. 117 (2), 667–678 https://doi.org/10.1104%2Fpp.117.2.667.

Sekhar, K., Priyanka, B., Reddy, V.D., Rao, K.V., 2011. Metallothionein 1 (CcMT1) of pigeonpea (*Cajanus cajan*, L.) confers enhanced tolerance to copper and cadmium in *Escherichia coli* and *Arabidopsis thaliana*. Environ. Exp. Bot. 72 (2), 131–139. https://doi.org/10.1016/j.envexpbot.2011.02.017.

Seregin, I.V., Kozhevnikova, A.D., 2023. Nicotianamine: a key player in metal homeostasis and hyperaccumulation in plants. Int. J. Mol. Sci. 24 (13), 10822. https://doi.org/10.3390/ijms241310822.

Shanker, A.K., Cervantes, C., Loza-Tavera, H., Avudainayagam, S., 2005. Chromium toxicity in plants. Environ. Int. 31 (5), 739–753. https://doi.org/10.1016/j.envint.2005.02.003.

Sharma, P., Bhardwaj, R., 2007. Effect of 24-epibrassinolide on seed germination, seedling growth and trace metal uptake in *Brassica juncea* L. Gen. Appl. Plant Physiol. 33, 59–73.

Sharma, D.C., Chatterjee, C., Sharma, C.P., 1995. Chromium accumulation and its effects on wheat (*Triticum aestivum* L. cv. HD 2204) metabolism. Plant Sci. 111 (2), 145–151. https://doi.org/10.1016/0168-9452(95)04230-R.

Sharma, S.S., Dietz, K.J., 2006. The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to trace metal stress. J. Exp. Bot. 57 (4), 711–726. https://doi.org/10.1093/jxb/erj073.

Sharma, S.S., Dietz, K.-J., Mimura, T., 2016. Vacuolar compartmentalization as indispensable component of trace metal detoxification in plants. Plant Cell Environ. 39 (5), 1112–1126. https://doi.org/10.1111/pce.12706.

Sharma, A., Kapoor, D., Wang, J., Shahzad, B., Kumar, V., Bali, A.S., Jasrotia, S., Zheng, B., Yuan, H., Yan, D., 2020. Chromium bioaccumulation and its impacts on plants: an overview. Plants 9 (1), 100. https://doi.org/10.3390/plants9010100.

Sharma, M., Kumar, P., Verma, V., Sharma, R., Bhargava, B., Irfan, M., 2022. Understanding plant stress memory response for abiotic stress resilience: molecular insights and prospects. Plant Physiol. Biochem. 179, 10–24. https://doi.org/10.1016/j.plaphy.2022.03.004.

Sharma, P., Lakra, N., Goyal, A., Ahlawat, Y.K., Zaid, A., Siddique, K.H.M., 2023. Drought and heat stress mediated activation of lipid signaling in plants: a critical review. Front. Plant Sci. 14, 1216835 https://doi.org/10.3389/fpls.2023.1216835.

Shin, L.-J., Lo, J.-C., Yeh, K.-C., 2012. Copper chaperone antioxidant protein 1 is essential for copper homeostasis. Plant Physiol. 159 (3), 1099–1110 https://doi.org/10.1104%2Fpp.112.195974.

Shomali, A., Das, S., Arif, N., Sarraf, M., Zahra, N., Yadav, V., Aliniaeifard, S., Chauhan, D.K., Hasanuzzaman, M., 2022. Diverse physiological roles of flavonoids in plant environmental stress responses and tolerance. Plants 11 (22), 3158. https://doi.org/10.3390/plants11223158.

Singh, A., Gupta, M., Kumar, S., Chawla, H., Mehra, M., Kumar, K., Jat, B.S., Bhushan, B., 2023. Posttranslational modifications and metal stress tolerance in plants. In: Gill, S. S., Tuteja, N., Khan, N.A., Gill, R. (Eds.), Biostimulants in alleviation of metal toxicity in plants. Academic Press, pp. 511–531. https://doi.org/10.1016/B978-0-323-99600-6.00001-3. Chapter, 21.

Singh, H., Kumar, D., Soni, V., 2022. Performance of chlorophyll a fluorescence parameters in *Lemna minor* under trace metal stress induced by various concentration of copper. Sci. Rep. 12 (1), 10620 https://doi.org/10.1038/s41598-022-14985-2.

Singh, S., Parihar, P., Singh, R., Singh, V.P., Prasad, S.M., 2015. Trace metal tolerance in plants: Role of transcriptomics, proteomics, metabolomics, and ionomics. Front. Plant Sci. 6, 1143. https://doi.org/10.3389/fpls.2015.01143.

Son, S., Park, S.R., 2023. Plant translational reprogramming for stress resilience. Front. Plant Sci. 14, 1151587 https://doi.org/10.3389/fpls.2023.1151587.

Song, W.Y., Mendoza-Cózatl, D.G., Lee, Y., Schroeder, J.I., Ahn, S.N., Lee, H.S., Wicker, T., Martinoia, E., 2014. Phytochelatin-metal(loid) transport into vacuoles shows different substrate preferences in barley and Arabidopsis. Plant Cell Environ. 37 (5), 1192–1201. https://doi.org/10.1111/pce.12227.

Soni, S., Jha, A.B., Dubey, R.S., Sharma, P., 2023. Alleviation of chromium stress in plants using metal and metal oxide nanoparticles. Environ. Sci. Pollut. Res. 30 (35), 83180–83197. https://doi.org/10.1007/s11356-023-28161-0.

Srivastava, S., Jain, R., 2011. *In-situ* monitoring of chromium cytotoxicity in sugarcane. J. Environ. Biol. 32 (6), 759–763.

Sun, X., Gilroy, E.M., Chini, A., Nurmberg, P.L., Hein, I., Lacomme, C., Birch, P.R., Hussain, A., Yun, B.W., Loake, G.J., 2011. ADS1 encodes a MATE-transporter that negatively regulates plant disease resistance. N. Phytol. 192 (2), 471–482. https://doi.org/10.1111/j.1469-8137.2011.03820.x.

Suzuki, N., Yamaguchi, Y., Koizumi, N., Sano, H., 2002. Functional characterization of a trace metal binding protein CdI19 from Arabidopsis. Plant J. 32 (2), 165–173. https://doi.org/10.1046/j.1365-313x.2002.01412.x.

Szklarczyk, D., Gable, A.L., Nastou, K.C., Lyon, D., Kirsch, R., Pyysalo, S., Doncheva, N. T., Legeay, M., Fang, T., Bork, P., Jensen, L.J., von Mering, C., 2021. The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Res. 49 (D1), D605–D612. https://doi.org/10.1093/nar/gkaa1074.

Tafforeau, M., Verdus, M.C., Norris, V., Ripoll, C., Thellier, M., 2006. Memory processes in the response of plants to environmental signals. Plant Signal Behav. 1 (1), 9–14. https://doi.org/10.4161/psb.1.1.2164.

Tan, M., Cheng, D., Yang, Y., Zhang, G., Qin, M., Chen, J., Chen, Y., Jiang, M., 2017. Co-expression network analysis of the transcriptomes of rice roots exposed to various cadmium stresses reveals universal cadmium-responsive genes. BMC Plant Biol. 17 (1), 194. https://doi.org/10.1186/s12870-017-1143-y.

Tan, L., Zhu, Y., Fan, T., Peng, C., Wang, J., Sun, L., Chen, C., 2019. OsZIP7 functions in xylem loading in roots and inter-vascular transfer in nodes to deliver Zn/Cd to grain in rice. Biochem. Biophys. Res. Commun. 512 (1), 112–118. https://doi.org/10.1016/j.bbrc.2019.03.024.

Tehseen, M., Cairns, N., Sherson, S., Cobbett, C.S., 2010. Metallochaperone-like genes in *Arabidopsis thaliana*. Metallomics 2 (8), 556–564. https://doi.org/10.1039/c003484c.

Teng, Z., Lyu, J., Chen, Y., Zhang, J., Ye, N., 2023. Effects of stress-induced ABA on root architecture development: positive and negative actions. Crop J. 11 (4), 1072–1079. https://doi.org/10.1016/j.cj.2023.06.007.

Terzi, H., Yıldız, M., 2021. Proteomic analysis reveals the role of exogenous cysteine in alleviating chromium stress in maize seedlings. Ecotoxicol. Environ. Saf. 209, 111784 https://doi.org/10.1016/j.ecoenv.2020.111784.

Thimm, O., Bläsing, O., Gibon, Y., Nagel, A., Meyer, S., Krüger, P., Selbig, J., Müller, L.A., Rhee, S.Y., Stitt, M., 2004. MAPMAN: a user-driven tool to display genomics data sets onto diagrams of metabolic pathways and other biological processes. Plant J. 37 (6), 914–939. https://doi.org/10.1111/j.1365-313x.2004.02016.x.

Tóth, G., Hermann, T., Da Silva, M.R., Montanarella, L., 2016. Heavy metals in agricultural soils of the European Union with implications for food safety. Environ. Int. 88, 299–309. https://doi.org/10.1016/j.envint.2015.12.017.

Ullah, S., Liu, Q., Wang, S., Jan, A.U., Sharif, H.M.A., Ditta, A., Wang, G., Cheng, H., 2023. Sources, impacts, factors affecting chromium uptake in plants, and mechanisms behind phytoremediation of Cr-contaminated soils. Sci. Total Environ. 899, 165726 https://doi.org/10.1016/j.scitotenv.2023.165726.

Usadel, B., Nagel, A., Steinhauser, D., Gibon, Y., Bläsing, O.E., Redestig, H., Sreenivasulu, N., Krall, L., Hannah, M.A., Poree, F., Fernie, A.R., Stitt, M., 2006. PageMan: An interactive ontology tool to generate, display, and annotate overview graphs for profiling experiments. BMC Bioinforma. 7, 535. https://doi.org/10.1186/1471-2105-7-535.

Usadel, Br, Nagel, A., Thimm, O., Redestig, H., Blaesing, O.E., Palacios-Rojas, N., Selbig, J., Hannemann, J., Piques, M.Cao, Steinhauser, D., Scheible, W.-Rd, Gibon, Y., Morcuende, R., Weicht, D., Meyer, S., Stitt, M., 2005. Extension of the visualization tool MapMan to allow statistical analysis of arrays, display of coresponding genes, and comparison with known responses. Plant Physiol. 138 (3), 1195–1204. https://doi.org/10.1104/pp.105.060459.

Van de Mortel, J.E., Schat, H., Moerland, P.D., Van Themaat, E.V.L., Van Der Ent, S., Blankestijn, H., Ghandilyan, A., Tsiatsiani, S., Aarts, M.G.M., 2008. Expression differences for genes involved in lignin, glutathione and sulphate metabolism in response to cadmium in *Arabidopsis thaliana* and the related Zn/Cd-hyperaccumulator *Thlaspi caerulescens*. Plant, Cell Environ. 31 (3), 301–324. https://doi.org/10.1111/j.1365-3040.2007.01764.x.

Vercammen, D., Belenghi, B., van de Cotte, B., Beunens, T., Gavigan, J.A., De Rycke, R., Brackenier, A., Inzé, D., Harris, J.L., Van Breusegem, F., 2006. Serpin1 of *Arabidopsis thaliana* is a suicide inhibitor for metacaspase 9. J. Mol. Biol. 364 (4), 625–636. https://doi.org/10.1016/j.jmb.2006.09.010.

Vergata, C., Contaldi, F., Baccelli, I., Basso, M.F., Santini, A., Pecori, F., Buti, M., Mengoni, A., Vaccaro, F., Moura, B.B., Ferrini, F., Martinelli, F., 2023. How does particulate matter affect plant transcriptome and microbiome? Environ. Exp. Bot. 209, 105313 https://doi.org/10.1016/j.envexpbot.2023.105313.

Vergine, M., Aprile, A., Sabella, E., Genga, A., Siciliano, M., Rampino, P., Lenucci, M.S., Luvisi, A., Bellis, L.D., 2017. Cadmium concentration in grains of durum wheat (*Triticum turgidum* L. subsp. durum). J. Agric. Food Chem. 65 (30), 6240–6246. https://doi.org/10.1021/acs.jafc.7b01946.

Viehweger, K., 2014. How plants cope with heavy metals. Bot. Stud. 55 (1), 35. https://doi.org/10.1186/1999-3110-55-35.

Waadt, R., Seller, C.A., Hsu, P.-K., Takahashi, Y., Munemasa, S., Schroeder, J.I., 2022. Plant hormone regulation of abiotic stress responses. Nat. Rev. Mol. Cell Biol. 23 (10), 680–694. https://doi.org/10.1038/s41580-022-00479-6.

Wakeel, A., Ali, I., Upreti, S., Azizullah, A., Liu, B., Khan, A.R., Huang, L., Wu, M., Gan, Y., 2018. Ethylene mediates dichromate-induced inhibition of primary root growth by altering AUX1 expression and auxin accumulation in *Arabidopsis thaliana*. Plant Cell Environ. 41 (6), 1453–1467. https://doi.org/10.1111/pce.13174.

Wakeel, A., Ali, I., Wu, M., Raza Kkan, A., Jan, M., Ali, A., Liu, Y., Ge, S., Wu, J., liu, B., Gan, Y., 2019. Ethylene mediates dichromate-induced oxidative stress and regulation of the enzymatic antioxidant system-related transcriptome in *Arabidopsis thaliana*. Environ. Exp. Bot. 161, 166–179. https://doi.org/10.1016/j.envexpbot.2018.09.004.

Wakeel, A., Xu, M., Gan, Y., 2020. Chromium-induced reactive oxygen species accumulation by altering the enzymatic antioxidant system and associated cytotoxic, genotoxic, ultrastructural, and photosynthetic changes in plants. Int. J. Mol. Sci. 21 (3), 728. https://doi.org/10.3390/ijms21030728.

Wang, Y., Cui, T., Niu, K., Ma, H., 2021b. Comparison and characterization of oxidation resistance and carbohydrate content in Cd-tolerant and -sensitive kentucky bluegrass under Cd stress. Agronomy 11 (11), 2358. https://doi.org/10.3390/agronomy11112358.

Wang, R., Liu, X., Liang, S., Ge, Q., Li, Y., Shao, J., Qi, Y., An, L., Yu, F., 2015. A subgroup of *MATE* transporter genes regulates hypocotyl cell elongation in Arabidopsis. J. Exp. Bot. 66 (20), 6327–6343. https://doi.org/10.1093/jxb/erv344.

Wang, J., Song, L., Gong, X., Xu, J., Li, M., 2020. Functions of jasmonic acid in plant regulation and response to abiotic stress. Int. J. Mol. Sci. 21 (4), 1446. https://doi.org/10.3390/ijms21041446.

Wang, F., Tan, H., Han, J., Zhang, Y., He, X., Ding, Y., Chen, Z., Zhu, C., 2019. A novel family of PLAC8 motif-containing/PCR genes mediates Cd tolerance and Cd accumulation in rice. Environ. Sci. Eur. 31, 82. https://doi.org/10.1186/s12302-019-0259-0.

Wang, M., Zhou, F., Wang, H.M., Xue, D.X., Liu, Y.-G., Zhang, Q.Y., 2021a. A rice mTERF protein V14 sustains photosynthesis establishment and temperature acclimation in early seedling leaves. BMC Plant Biol. 21 (1), 406. https://doi.org/10.1186/s12870-021-03192-2.

Wasternack, C., 2015. How jasmonates earned their laurels: past and present. J. Plant Growth Regul. 34, 761–794. https://doi.org/10.1007/s00344-015-9526-5.

Wei, Z., Li, J., 2020. Regulation of brassinosteroid homeostasis in higher plants. Front. Plant Sci. 11, 583622 https://doi.org/10.3389/fpls.2020.583622.

Wei, Y., Peng, X., Wang, X., Wang, C., 2023. The heavy metal-associated isoprenylated plant protein (*HIPP*) gene family plays a crucial role in cadmium resistance and accumulation in the tea plant (*Camellia sinensis* L.). Ecotoxicol. Environ. Saf. 260, 115077 https://doi.org/10.1016/j.ecoenv.2023.115077.

White, C., Yuan, X., Schmidt, P.J., Bresciani, E., Samuel, T.K., Campagna, D., Hall, C., Bishop, K., Calicchio, M.L., Lapierre, A., Ward, D.M., Liu, P., Fleming, M.D., Hamza, I., 2013. HRG1 is essential for heme transport from the phagolysosome of macrophages during erythrophagocytosis. Cell Metab. 17 (2), 261–270. https://doi.org/10.1016/j.cmet.2013.01.005.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis, 2 ed. Springer Publishing Company, Incorporated. https://doi.org/10.1007/978-3-319-24277-4.

Wilcoxon, F., 1945. Individual comparisons by ranking methods. Biom. Bull. 1 (6), 80–83. https://doi.org/10.2307/3001968.

Wu, X., Gong, F., Cao, D., Hu, X., Wang, W., 2016. Advances in crop proteomics: PTMs of proteins under abiotic stress. Proteomics 16 (5), 847–865. https://doi.org/10.1002/pmic.201500301.

Wu, J., Guo, S., Wang, J., He, J., Li, X., Zhan, Y., 2023. The phosphoproteomic response of pepper (*Capsicum annuum* L.) seedlings to selenium stress. Horticulturae 9 (8), 935. https://doi.org/10.3390/horticulturae9080935.

Wu, S., Hu, C., Wang, X., Wang, Y., Yu, M., Xiao, H., Shabala, S., Wu, K., Tan, Q., Xu, S., Sun, X., 2022. Cadmium-induced changes in composition and co-metabolism of glycerolipids species in wheat root: glycerolipidomic and transcriptomic approach. J. Hazard. Mater. 423, 127115 https://doi.org/10.1016/j.jhazmat.2021.127115.

Wu, D., Saleem, M., He, T., He, G., 2021. The mechanism of metal homeostasis in plants: a new view on the synergistic regulation pathway of membrane proteins, lipids and metal ions. Membranes 11 (12), 984. https://doi.org/10.3390/membranes11120984.

Xia, X., Dong, H., Yin, Y., Song, X., Gu, X., Sang, K., Zhou, J., Shi, K., Zhou, Y., Foyer, C. H., Yu, J., 2021. Brassinosteroid signaling integrates multiple pathways to release apical dominance in tomato. PNAS 118 (11), e2004384118. https://doi.org/10.1073/pnas.2004384118.

Xin, K., Pan, T., Gao, S., Yan, S., 2021. A transcription factor regulates gene expression in chloroplasts. Int. J. Mol. Sci. 22 (13), 6769. https://doi.org/10.3390/ijms22136769.

Xu, J.M., Fan, W., Jin, J.F., Lou, H.Q., Chen, W.W., Yang, J.L., Zheng, S.J., 2017. Transcriptome analysis of Al-induced genes in buckwheat (*Fagopyrum esculentum* Moench) root apex: new insight into al toxicity and resistance mechanisms in an Al accumulating species. Front. Plant Sci. 8, 1141. https://doi.org/10.3389/fpls.2017.01141.

Xu, T., Niu, J., Jiang, Z., 2022b. Sensing mechanisms: calcium signaling mediated abiotic stress in plants. Front. Plant Sci. 13, 925863 https://doi.org/10.3389/fpls.2022.925863.

Xu, S., Yu, C., Wang, Q., Liao, J., Liu, C., Huang, L., Liu, Q., Wen, Z., Feng, Y., 2022a. Chromium contamination and health risk assessment of soil and agricultural products in a rural area in southern China. Toxics 11 (1), 27. https://doi.org/10.3390/toxics11010027.

Xynias, I.N., Mylonas, I., Korpetis, E.G., Ninou, E., Tsaballa, A., Avdikos, I.D., Mavromatis, A.G., 2020. Durum wheat breeding in the mediterranean region: current status and future prospects. Agronomy 10 (3), 432. https://doi.org/10.3390/agronomy10030432.

Yadav, M.R., Choudhary, M., Singh, J., Lal, M.K., Jha, P.K., Udawat, P., Gupta, N.K., Rajput, V.D., Garg, N.K., Maheshwari, C., Hasan, M., Gupta, S., Jatwa, T.K., Kumar, R., Yadav, A.K., Prasad, P.V.V., 2022. Impacts, tolerance, adaptation, and mitigation of heat stress on wheat under changing climates. Int. J. Mol. Sci. 23 (5), 2838. https://doi.org/10.3390/ijms23052838.

Yan, G., Gao, Y., Xue, K., Qi, Y., Fan, Y., Tian, X., Wang, J., Zhao, R., Zhang, P., Liu, Y., Liu, J., 2023. Toxicity mechanisms and remediation strategies for chromium exposure in the environment. Front. Environ. Sci. 11, 1131204 https://doi.org/10.3389/fenvs.2023.1131204.

Yang, G., Li, J., Liu, W., Yu, Z., Shi, Y., Lv, B., Wang, B., Han, D., 2015. Molecular cloning and characterization of *MxNAS2*, a gene encoding nicotianamine synthase in *Malus xiaojinensis*, with functions in tolerance to iron stress and misshapen flower in transgenic tobacco. Sci. Hortic. 183, 77–86. https://doi.org/10.1016/j.scienta.2014.12.014.

Yang, Y., Wang, S., Zhao, C., Jiang, X., Gao, D., 2024. Responses of non-structural carbohydrates and biomass in plant to trace metal treatment. Sci. Total Environ. 909, 168559 https://doi.org/10.1016/j.scitotenv.2023.168559.

Yang, Z., Yang, F., Liu, J.L., Wu, H.T., Yang, H., Shi, Y., Liu, J., Zhang, Y.F., Luo, Y.R., Chen, K.M., 2022. trace metal transporters: functional mechanisms, regulation, and application in phytoremediation. Sci. Total Environ. 809, 151099 https://doi.org/10.1016/j.scitotenv.2021.151099.

Yang, Y., Zhang, L., Huang, X., Zhou, Y., Quan, Q., Li, Y., Zhu, X., 2020. Response of photosynthesis to different concentrations of trace metals in *Davidia involucrata*. PLoS ONE 15 (3), e0228563. https://doi.org/10.1371/journal.pone.0228563.

Yao, C., Finlayson, S.A., 2015. Abscisic acid is a general negative regulator of arabidopsis axillary bud growth. Plant Physiol. 169 (1), 611–626. https://doi.org/10.1104/pp.15.00682.

Yilmaz, S.H., Kaplan, M., Temizgul, R., Yilmaz, S., 2017. Antioxidant enzyme response of sorghum plant upon exposure to aluminum, chromium and lead heavy metals. Turk. J. Biochem. 42 (4), 503–512. https://doi.org/10.1515/tjb-2016-0112.

Yokosho, K., Yamaji, N., Ma, J.F., 2014. Global transcriptome analysis of Al-induced genes in an Al-accumulating species, common buckwheat (*Fagopyrum esculentum* Moench). Plant Cell Physiol. 55 (12), 2077–2091. https://doi.org/10.1093/pcp/pcu135.

Yu, S.G., Kim, J.H., Cho, N.H., Oh, T.R., Kim, W.T., 2020. Arabidopsis RING E3 ubiquitin ligase JUL1 participates in ABA-mediated microtubule depolymerization, stomatal closure, and tolerance response to drought stress. Plant J. 103 (2), 824–842. https://doi.org/10.1111/tpj.14775.

Yu, G., Wang, L.-G., Han, Y., He, Q.-Y., 2012. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS: A J. Integr. Biol. 16 (5), 284–287. https://doi.org/10.1089/omi.2011.0118.

Yu, X., Yang, Z., Xu, Y., Wang, Z., Fan, C., Zeng, X., Liu, Y., Lei, T., Jiang, M., Li, J., Xiao, X., Yang, L., Li, X., Zhou, Y., Gao, S., 2023. Effect of chromium stress on metal accumulation and cell wall fractions in *Cosmos bipinnatus*. Chemosphere 315, 137677. https://doi.org/10.1016/j.chemosphere.2022.137677.

Zandalinas, S.I., Balfagón, D., Gómez-Cadenas, A., Mittler, R., 2022. Plant responses to climate change: metabolic changes under combined abiotic stresses. J. Exp. Bot. 73 (11), 3339–3354. https://doi.org/10.1093/jxb/erac073.

Zayed, A.M., Terry, N., 2003. Chromium in the environment: factors affecting biological remediation. Plant Soil 249, 139–156. https://doi.org/10.1023/A:1022504826342.

Zermiani, M., Begheldo, M., Nonis, A., Palme, K., Mizzi, L., Morandini, P., Nonis, A., Ruperti, B., 2015. Identification of the Arabidopsis RAM/MOR signalling network: adding new regulatory players in plant stem cell maintenance and cell polarization. Ann. Bot. 116 (1), 69–89. https://doi.org/10.1093/aob/mcv066.

Zhang, J., Qian, Y., Chen, Z., Amee, M., Niu, H., Du, D., Yao, J., Chen, K., Chen, L., Sun, J., 2020. Lead-induced oxidative stress triggers root cell wall remodeling and increases lead absorption through esterification of cell wall polysaccharide. J. Hazard. Mater. 385, 121524 https://doi.org/10.1016/j.jhazmat.2019.121524.

Zhang, H., Song, J., Dong, F., Li, Y., Ge, S., Wei, B., Liu, Y., 2023. Multiple roles of wheat ferritin genes during stress treatment and TaFER5D-1 as a positive regulator in response to drought and salt tolerance. Plant Physiol. Biochem. 202, 107921 https://doi.org/10.1016/j.plaphy.2023.107921.

Zhang, W., Zhang, M., Zhu, X., Cao, Y., Sun, Q., Ma, G., Chao, S., Yan, C., Xu, S.S., Cai, X., 2018. Molecular cytogenetic and genomic analyses reveal new insights into the origin of the wheat B genome. Theor. Appl. Genet. 131 (2), 365–375. https://doi.org/10.1007/s00122-017-3007-0.

Zhao, L., Sun, Y.L., Cui, S.X., Chen, M., Yang, H.M., Liu, H.M., Chai, T.Y., Huang, F., 2011. Cd-induced changes in leaf proteome of the hyperaccumulator plant *Phytolacca americana*. Chemosphere 85 (1), 56–66. https://doi.org/10.1016/j.chemosphere.2011.06.029.

Zhou, L., Ye, L., Pang, B., Hou, Y., Yu, J., Du, X., Gu, L., Wang, H., Zhu, B., 2023. Overexpression of *ApHIPP26* from the hyperaccumulator *Arabis paniculata* confers enhanced cadmium tolerance and accumulation to *Arabidopsis thaliana*. Int. J. Mol. Sci. 24 (20), 15052. https://doi.org/10.3390/ijms242015052.

Zohary, D., Feldman, M., 1962. Hybridization between amphiploids and evolution of polyploids in the wheat (Aegilops-Triticum) group. Evol. 16 (1), 44–61. https://doi.org/10.1111/j.1558-5646.1962.tb03197.x.

Zou, J.H., Wang, M., Jiang, W.S., Liu, D.H., 2006. Effects of hexavalent chromium (VI) on root growth and cell division in root tip cells of *Amaranthus viridis* L. Pak. J. Bot. 38 (3), 673–681.

Zulkernain, N.H., Uvarajan, T., Ng, C.C., 2023. Roles and significance of chelating agents for potentially toxic elements (PTEs) phytoremediation in soil: a review. J. Environ. Manag. 341, 117926 https://doi.org/10.1016/j.jenvman.2023.117926.