Transcriptomic analysis of cadmium stressed *Tamarix hispida* revealed novel transcripts and the importance of ABA network

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Abstract

Aim Cadmium (Cd) pollution is widely detected in soil and has been recognized as a major environmental problem. *Tamarix hispida* is a woody halophyte, which can form natural forest on desert and the soil with 0.5–1% salt content, making it an ideal plant for research investigating the effects of various stresses on plants. However, no systematic study has investigated the molecular mechanism of Cd tolerance in *T. hispida*.

Methods In this study, the RNA-seq technique was applied to analyze the transcriptomic changes in *T. hispida* treated with $150 \mu\text{mol L}^{-1}$ CdCl_2 for 24, 48 and 72 h compared with control.

Results In total, 72764 unigenes exhibited similar sequences in the NR database, while 41528 unigenes (36.3% of all the unigenes) did not exhibit similar sequences, which may be new transcripts. In addition, 6778, 8282 and 8601 DEGs were detected at 24, 48 and 72 h, respectively. Functional annotation analysis indicated that many genes may be involved in several aspects of the Cd stress response, including ion bonding, signal transduction, stress sensing, hormone responses and ROS metabolism. A *ThUGT* gene from the abscisic acid (ABA) signaling pathway can enhance the Cd resistance ability of *T. hispida* by regulating the production of reactive oxygen species under Cd stress and inhibiting *T. hispida* absorption of Cd.

Conclusion The new transcriptome resources and data that we present in this study for *T. hispida* may substantially facilitate molecular studies of the mechanisms governing Cd resistance.

Introduction

Heavy metal pollution in soil has become a worldwide problem. This pollution not only inhibits crop growth and reduces yield and quality but also poses a considerable threat to human health through the food chain (Wu et al., 2007). The heavy metal cadmium is a biologically nonessential element. Cd is highly toxic and migratory with a biological half-life of 10 to 30 years (Suwazono et al., 2001). Therefore, Cd can easily enter the human body through the food chain and accumulate in the body, causing injury to the kidneys, lungs, liver, testicles, brain, bones and blood system (Hamada et al., 1991).

A high concentration of Cd can cause a great toxic effect on plants, but some plants have been determined to grow under high Cd stress without exhibiting toxic phenotypes. Approximately 10%–33% of *Arabidopsis halleri* subsp. *gemmifera* can accumulate more than 100 ppm of Cd in contaminated soil (Bert et al., 2002). Yang et al. (2004) showed that no reduction in shoot and root dry matter yields was noted when *Sedum alfredii* Hance were grown at Cd supply levels of $200 \mu\text{mol L}^{-1}$ in nutrient solution. Under natural conditions, the aboveground part of *Noccaea caerulea* can accumulate up to 164 mg/kg Cd (Baker et al., 1994). *Thlaspi praecox* Wulfen is a hyperaccumulator plant of zinc, cadmium and lead, and the aboveground part can accumulate up to 5030 mg/kg of Cd (Vogel-Mikus et al., 2005). Under the Cd pollution level of 25 mg/kg, the Cd content of stems and leaves in *Solanum nigrum* L. exceeded 100 mg/kg. The Cd content in shoots was greater than that in roots, and the Cd enrichment factor in the

shoots was greater than 1 (Wei et al., 2005). These studies suggested that these superenriched plants have good cadmium tolerance, and these findings provide an important theoretical basis for the study of plant remediation of Cd-contaminated soil.

At present, it is generally believed that the accumulation of Cd in plants is primarily reflected in two aspects. On the one hand, from the cellular level, Cd primarily accumulates in the vacuoles and apoplasts of plants. On the other hand, at the organizational level, this process is manifested in the epidermal cells, subepithelial cells and epidermal hairs of plants. According to Küpper et al. (2000), mustard mesophyll cells are an important site for Cd accumulation. In addition, Salt and Wagner (1993) found that a large amount of Cd was found in the leaf epidermis and epidermis hairs in the mustard. Recently, Zhang's research team, through research on rapeseed and *Arabidopsis*, discovered that the regulation of the ion channel protein activity of NO_3^- and Cd in the vacuolar membrane provides a means of synergistically improving the NUE and Cd toxicity of rapeseed. At the same time, the vacuolar compartmentalization and cell wall fixation of Cd may be the main physiological reasons for the difference in Cd toxicity resistance between Cd-resistant cultivar Z11 and Cd-sensitive cultivar W10 of rapeseed (Zhang et al., 2019b). In *Arabidopsis*, the defensive protein AtPDF2.5 may chelate cytoplasmic Cd and mediate its efflux, promote Cd accumulation in apoplasts, and regulate plant detoxification and accumulation of Cd (Luo et al., 2019). Therefore, plant accumulation of Cd in cell walls, vacuoles, epidermal cells, or epidermal hair is likely to be one of the ways in which plants achieve detoxification.

In recent years, with the expansion of available transcriptional data, a growing body of knowledge regarding the genetic basis underlying Cd stress physiological processes has formed, which has greatly increased our understanding of the molecular mechanism of Cd transcription and toxicity in some Cd hyperaccumulating plants, such as *A. halleri* (Herbette et al., 2006), *Brassica juncea* (Farinati et al., 2010), *S. alfredii* (Gao et al., 2013), and *Noccaea caerulescens* (Halimaa et al., 2014; Milner et al., 2014). At the same time, the molecular mechanism of Cd affecting some cultivated plants was also studied. Such as cabbage (*Brassica oleracea subsp. capitata f. alba*) (Bączek-Kwinta et al., 2019), pea (*Pisum sativum* L.) (Rodriguez-Serrano et al., 2009), barley (*Hordeum vulgare* L.) (Cao et al., 2014), rice (*Oryza sativa* L.) (Oono et al., 2014), tobacco (*Nicotiana tabacum* L.) (Martin et al., 2012), ramie (*Boehmeria nivea* L.) (Liu et al., 2015) and pakchoi (*Brassica chinensis* L.) (Zhou et al., 2016).

Tamarix hispida is a woody halophyte that grows in arid and semiarid regions. In a previous study, the transcriptome of *T. hispida* treated with NaHCO_3 was constructed and analyzed to detect the response of *T. hispida* to alkaline treatment (Wang et al., 2013). Some transcript factor, including *ThNAC7*, *ThCRF1*, *ThZFP1* and *ThbHLH1*, were cloned and identified which involved in the process of reducing ROS and confer transgenic plant salt or osmotic tolerance (He et al., 2019; Qin et al., 2017; Zang et al., 2015; Ji et al., 2016). At the same time, recent studies have found that multiple *T. hispida* genes can enhance its tolerance to Cd. For example, the transfer of the metallothionein gene *ThMT3*, increases the resistance of transgenic tobacco and yeast to Cd (Zhou et al., 2014; Yang et al., 2011). Overexpression of vacuolar membrane H^+ -ATPase c subunit gene *ThVHAc1* improved the Cd tolerance of *Saccharomyces cerevisiae*, *Arabidopsis* and *T. hispida*. *ThWRKY7*, the possible upstream gene of *ThVHAc1*, exhibited similar

expression patterns as *ThVHAc1* under CdCl₂ treatment and improved Cd tolerance (Yang et al., 2016; Gao et al., 2011).

Therefore, studying the transcriptome of Cd-stressed leaves would be particularly useful for furthering the genetic improvement of *T. hispida* to Cd stress. To elucidate the initial perception mechanism in response to Cd stimuli in *T. hispida* leaves, we examined gene expression changes over 72 h (control, 24, 48 and 72 h) and identified Cd-specific regulatory networks. This study helps to elucidate the mechanism of Cd tolerance in *T. hispida* and provides a useful reference for further exploration in woody plants and using to remediation of heavy metals (Cd) from contamination soils.

Materials And Methods

Plant materials and Cd treatments

Seeds of *T. hispida* (The Turpan Desert Botanical Garden (Xinjiang, 293 China)) were germinated in plastic pots containing a mixture of turf peat and sand (1:1 v/v) under constant photoperiod conditions (14/10 h light/dark) with a light intensity of 1500 ~ 2000lx at temperature (24 ± 1 °C). After culturing for 3 months in a greenhouse, at least 800 healthy seedlings of similar size (9 cm in height) were selected for Cd treatment. Based on preliminary test results (Gao et al., 2011), 150 µmol L⁻¹ CdCl₂ was applied to the seedlings. At the same time, samples irrigated with fresh water were treated as controls. After treatment for 24, 48 or 72 h (each treatment contained three separate repeats with at least 200 seedlings), the leaves (the whole ground part) were washed with clean water and frozen in liquid nitrogen immediately, after which they were stored at -80 °C for subsequent experiments. Each sample contains three replicates.

Determination of Cd concentration and H₂O₂-related physiological indices

To detect the Cd concentration of the samples, leaves from samples (each sample contains at least 20 seedlings) containing three replicates for each control, 24, 48 and 72 h were dried at 72 °C to a constant weight and then digested with HNO₃. Subsequently, the Cd ion content was determined using ICP-OES 5110 VDV (Agilent Instruments Inc. State of California, USA). The H₂O₂ content was detected by a hydrogen peroxide assay kit (Nanjing Jiancheng Bioengineering Institute), and detailed operating procedures were carried out according to the instructions. At the same time, the hydrogen peroxide (H₂O₂) content in the leaves after Cd stress was detected by 3,3-diaminobenzidine (DAB) staining. Briefly, the above mentioned samples were placed in PBS (pH 7.0) solution containing 1 mg/mL DAB and treated in the dark for 12 h at 37 °C. After exposure for 1 h, samples were decolorized with ethanol, and finally, the seedlings were photographed. Each sample contains three separate biological replicates.

RNA extraction, sequencing and de novo assembly

Total RNA was extracted from the leaf tissues of each sample. The degradation degree and whether there is pollution of RNA samples was verified by RNase-free agarose gel electrophoresis, and the purity of RNA was detected by Nanodrop. The RNA concentration was detected by qubit, and the RNA integrity was

accurately detected with an Agilent 2100 Bioanalyzer. Equal quantities of high-quality RNA from the samples was employed for the subsequent RNA sequencing.

A cDNA library was constructed for each of the samples and sequenced on the Illumina HiSeq 2000 platform (Illumina Inc., CA, USA). For the original sequenced reads or raw reads containing reads with adapters or low quality, to ensure the quality of information analysis, the raw reads were filtered to obtain clean reads. Before assembly, reads with adapters were removed. Then, over 10% of unknown bases (N bases) and/or low-quality reads (the number of bases with a mass value of Qphred ≤ 20 accounts for more than 50% of the total reads) were removed from each data point to improve the reliability of the results. Then, the clean reads of the twelve samples with high quality were spliced using the Trinity package (Grabherr et al., 2011) to construct unique sequences as the reference sequences. At the same time, gene numbers estimated by FPKM (expected number of fragments per kilobase of transcript sequence per million base pairs sequenced) value to assess the quality of transcripts.

Normalization of gene expression levels and identification of differentially expressed genes

The clean reads of each sample were remapped to reference sequences using RSEM software (Li et al., 2009). RSEM counts the results of the bowtie comparison and further obtains the number of read counts for each sample that is aligned to each gene and performs FPKM conversion to analyze gene expression levels. For genes with more than one alternative transcript, the longest transcript was selected to calculate the FPKM.

To infer the transcriptional changes over time under Cd stress conditions, differentially expressed genes (DEGs) after 24, 48 and 72 h of Cd treatment were identified by comparing the expression levels of the control. The false discovery rate (FDR) was calculated to adjust the threshold of the p-value to correct for multiple testing (Rajkumar et al., 2015). Transcripts with a minimal 4-fold difference in expression ($|\log_2 \text{Ratio}| \geq 2$) and an $\text{FDR} \leq 0.001$ were considered differentially expressed between the two time points (Audic et al., 1997). For convenience, DEGs with higher expression levels at 24, 48 and 72 h than those of the control were denoted as “upregulated”, whereas those with the opposite were denoted as “downregulated”. At the same time, Venn diagrams of these differentially expressed genes were made to distinguish the differences between them using online software (<http://bioinfogp.cnb.csic.es/tools/venny/index2.0.2.html>).

Short Timeseries Expression Miner (STEM) version 1.3.8 was used to analyze expression pattern. (Ernst et al., 2006). To further explore the temporal expression patterns in the data, K-means clustering was applied to the identified DEGs. It is proposed that DEGs belonging to the same cluster have expression patterns similar to each other. For each genotype, the clustering profiles of DEGs with p-values < 0.05 were considered to be significantly different from the reference group.

Validation of DEGs with qRT-PCR

Eight genes were randomly selected for quantitative real-time RT-PCR (qRT-PCR) to identify different expression patterns revealed by RNA sequencing. RNA was extracted from the leaves of control, 24, 48

and 72 h samples. The PrimeScript™ RT reagent Kit (Takara) was used for first-strand cDNA synthesis. Primers for qRT-PCR analysis are listed in Table S2. *β-actin* (FJ618517) was used as an internal control (Vandesompele et al., 2002). qRT-PCR was performed using a real-time PCR instrument (qTOWER 2.0) (analytik jena). The reaction mixture (20 µl) consisted of 10 µl of TransStar^R Top Green qPCR SuperMix (TRANS), 2 µl of cDNA template (equivalent to 500 ng of total RNA), 0.5 µmol L⁻¹ of forward and reverse primers. The reaction procedure was as follows: one cycle at 95°C for 3 min, followed by 45 cycles of 95°C for 30 s, 58°C for 15 s, 72°C for 30 s. Three independent replicates were performed to ensure the reproducibility of results. The data were treated using the 2^{-ΔΔ(Ct)} method (Livak et al., 2001). Then, qRT-PCR results and sequencing results are analyzed together to verify the accuracy of the sequencing results.

Sequence annotation, functional classification, and biological pathway analysis

The obtained unigenes were analyzed for functional annotation and functional classification. After splicing, the unigene sequences were compared with the protein database by blast, and the annotation included the NCBI Nonredundant nucleic acid database (NR) and the Swiss-port protein sequence database (Swiss-Prot) with a threshold of e-value < 0.000 01. The GO annotation information was obtained by Blast2GO analysis based on the NR annotation information. (Conesa et al., 2005). The GO functional classification of all unigenes was performed by WEGO (Ye et al., 2006). At the same time, the Kyoto Encyclopedia of Genes and Genomes (KEGG) was performed to further characterize the metabolic pathways and biological functions of DEGs in the transcriptome.

Cloning of the *ThUGT* gene in the ABA signaling pathway and analysis of cadmium stress function

The abscisic acid (ABA) signaling pathway gene *ThUGT* was successfully cloned, and an overexpression vector was constructed, and the vector construction method reference Wang et al., (2020). Afterwards, the overexpression strain was transiently transformed into *T. hispida* according to the method of Ji et al. (2014) and treated with 100 µmol L⁻¹ CdCl₂ for 24 h (According to the pre-experimental results, under this CdCl₂ concentration and treatment time, the relative expression level of *ThUGT* gene had the largest difference (Fig. S1), and the pROKII empty vector transiently transformed seedlings were used as a control. Each treatment contained three separate repeats with at least 45 seedlings. Then the expression levels of *ThUGT* in transient overexpression *T. hispida* and the control seedlings were analyzed by qRT-PCR. At the same time, the Cd content, ABA content and H₂O₂ content of the samples were determined. DAB, NBT and Evans Blue staining were also performed on each sample (Zhang et al., 2011). Each experiment was repeated three times.

Results

Changes in Cd and H₂O₂ concentrations in *T. hispida* subjected to Cd stress

In this study, the Cd and H₂O₂ contents were detected after *T. hispida* was treated with 150 µmol L⁻¹ CdCl₂. The results showed that the Cd concentration showed an upward trend after Cd stress compared to the control. Especially at 72 h, the concentration reached a peak with an absolute value of 41.6 mg/kg, which was 227 times that of the control (Fig. 1A). The H₂O₂ concentration was also significantly increased after Cd stress and reached the highest level at 24 h (20.2 times that of the control) (Fig. 1B). At the same time, the results of DAB staining showed that the leaves of *T. hispida* were dyed the deepest at 24 h (Fig. 1C).

Pairwise comparisons of transcriptome between control and Cd stressed leaves

To determine the gene expression changes resulting from Cd stress, DEGs between control samples and each treated sample (24, 48 and 72 h) were identified. Using a cutoff of 4-fold difference in gene expression as methodological description, 3505, 3983 and 4443 upregulated genes and 3273, 4299 and 4158 downregulated genes were identified at 24, 48 and 72 h, respectively, compared with those in the control (Fig. 2A). Among these genes, 2673, 3058 and 3077 DEGs at 24, 48 and 72 h had no similar sequences in the NR database, respectively (Table S1). Interestingly, the expression of many DEGs changed significantly only at a certain time point, for example, there were 5012 DEGs at 72 h. And there were 1069 DEGs common to all three treatment time points compared with the control (Fig. 2B).

To validate the expression data obtained from RNA sequencing, eight genes were randomly selected from the identified DEGs to perform qRT-PCR analysis. The results showed a strong correlation between the RNA sequencing and qRT-PCR data (Fig. S2), which supports the reliability of the expression results generated by RNA sequencing.

Through GO and KEGG pathway enrichment analysis, the function of the DEGs was characterized. GO annotation suggested that biological processes and molecular functions related to reactive oxygen species (ROS) functions and biosynthetic and metabolic processes were enriched among the DEGs at different time points (Fig. 3A and 3B). In biological processes, biosynthetic and metabolic processes DEGs were enriched at all three time points (Fig. 3A). In addition, the molecular function term “phenylalanine ammonia-lyase activity” was enriched at 24 h and 72 h. The term “methylenetetrahydrofolate reductase (NAD(P)H) activity” occurred at all three time points (Fig. 3B). These results indicated that substances participate in hormone and ROS metabolism play crucial roles in the *T. hispida* response to Cd stress.

KEGG pathway enrichment analysis results showed that 90, 91 and 105 pathways were categorized from the pairwise comparisons between 24 h vs control, 48 h vs control, and 72 h vs control, respectively. Based on these results, the DEGs involved in the biosynthetic pathways of seven hormones and one pathway for “plant hormone signal transduction” were enriched (Fig. 3C). At the same time, we observed that the gene numbers of six hormone synthetic pathways were the highest at 72 h, whereas brassinosteroid biosynthesis pathways peaked at 24 h. Interestingly, six hormone synthetic pathways were included in the top 30 pathways from 24 h vs control (Table 1). In the top 20 pathways from 24 h vs

control, five hormone synthesis pathways were found. These results demonstrated that the expression of genes involved in hormone synthesis may play an important role in the Cd response in *T. hispida*.

Analysis of Gene Temporal Expression Pattern

Hierarchical clustering produced six groups with similar expression trends to those of K-means clustering. Specifically, 1069 qualifying genes were categorized into six groups (referred to as G1, G2, G3, G4, G5 and G6), comprising 240, 110, 384, 142, 164 and 29 genes, respectively (Fig. 4), of which the G2, G3, G4, G5 and G6 groups mainly showed an upregulated trend under Cd treatment.

The expression of the G1 group showed a down-regulated expression trend, and reaching its lowest value at 48 h, then slightly increased at 72 h. The G2 group mainly showed a waveform trend, reaching a peak at 24 h, then showing a downward trend and slightly increasing at 72 h. The G3 group showed an up-regulated expression trend at the beginning (24 h) followed by stable expression in the subsequent stage. The G4 group showed a continuous increasing trend at 24 h and 48 h. Interestingly, the G5 group showed an increasing expression trend during the whole stage and peaks at 72 h. The G6 group showed an up-regulated trend at 24 h and 48 h and a slight decrease at 72 h. From these results, we can assume that the G3 and G5 group genes can be rapidly induced by Cd stress, and the G2, G4 and G6 group genes show a time-dependent trend in the process. In contrast, the G1 group genes show inhibition trend in response to cadmium stress.

Furthermore, GO functional annotation analysis of the genes in the six groups was performed. The number of genes in each group based on their biological pathway, molecular function and cellular component were counted. The results showed that all selected DEGs were involved in 282 biological pathways. Among them, DEGs involved in protein binding, ATP binding, DNA binding were relatively large number (88, 52 and 45, respectively) (Table 2).

In total, the K-means/hierarchical clustering and GO function annotation results indicated that the genes related to hormones were significantly affected during Cd stress in *T. hispida* leaves. Hence, the DEGs involved in the metabolism of hormones and their signaling pathways were further explored systematically.

DEGs involved in hormone biosynthetic pathways

To further explore the genes involved in hormone biochemical pathways following Cd stress treatment, the DEGs involved in the seven hormone biosynthesis or metabolism KEGG pathways (ABA, ETH, IAA, SA, GA, BRs and JA) were analyzed (Table 1). Analysis results indicated that the number of genes in the ABA, SA, ETH, IAA, GA and JA biosynthetic or metabolism pathways primarily increased, while BR biosynthesis showed decreasing trends (Fig. 3C).

Under Cd stress, 7 DEGs were identified in the ABA-related pathway, including ABA β -glucosyltransferase, zeta-carotene desaturase (PLN02487), isoprenoid biosynthesis enzymes (IBe), carotene beta-ring hydroxylase (PLN02738), antheraxanthin epoxidase/zeaxanthin epoxidase (PLN02927) and cytochrome P450. Three of them were induced, especially *ThUGT* (ABA β -glucosyltransferase), which appeared at 24

h and 72 h with 3.7 and 4.2-fold increased, respectively. In contrast, the expression of PLN02487, PLN02738, PLN02927 and cytochrome P450 showed an inhibitory expression trend.

Thirty DEGs in the ethylene biosynthetic pathway were detected. Among the metabolic processes, nine genes were involved in beta-eliminating lyase, four genes were involved in hypothetical protein, two genes were involved in 1-aminocyclopropane-1-carboxylate oxidase and two genes were involved in 5-methyltetrahydropterin glutamate homocysteine methyltransferase. The expression of these 17 genes was upregulated. In contrast, genes involved in homocysteine S-methyltransferase, 1-aminocyclopropane-1-carboxylate synthase, ARD/ARD' family and S-adenosyl-methionine synthase were downregulated.

These results indicated that the key regulatory components of the biosynthetic pathways in ABA and ethylene changed significantly during the *T. hispida* response to Cd stress. In addition, there are also many genes participate in hormone processes, such as BRs, JA, GA, IAA and SA, which can respond to Cd stress.

ThUGT-overexpressing *T. hispida* increased ABA content and Cd tolerance

To further explore the genes involved in hormone pathways following Cd stress treatment, the ABA-related KEGG pathway after each stress treatment point was analyzed. The results showed that ABA pathway-related genes mainly participated in phytoene, lycopene, zeaxanthin, abscisate and lutein processes (Fig. S3). *ThUGT*, a predicted ABA β -glucosyltransferase gene, was one of the DEGs in the ABA signaling pathway, and the expression levels were obviously induced (3.7 and 4.2 times of the control at 24 h and 72 h, respectively). Therefore, *ThUGT* was selected and further cloned and constructed into an overexpression vector, which was transiently transformed into *T. hispida*. The results of qRT-PCR showed that the expression level of *ThUGT* was significantly higher in the overexpression plants than in the control plants (Fig. 5A), indicating the transient overexpression transgenic plants were successfully obtained.

The staining analysis showed that the overexpression plants were stained lighter after CdCl₂ stress treatment compared with the control plants (Fig. 5B). The results obtained with physiological indicators showed that the ABA content in *ThUGT*-overexpressing *T. hispida* and the control plants was effectively increased under 100 $\mu\text{mol L}^{-1}$ CdCl₂ treatment, while in the *ThUGT* transgenic plants, it increased less than in the control (Fig. 5C). At the same time, the Cd ion content in both the overexpression plants and the control plants increased significantly after Cd treatment, but the Cd ion content in the overexpression plants was significantly less than that of the control (Fig. 5C). The H₂O₂ content in overexpression plants was lower than that of the control after Cd treatment (Fig. 5C). These results suggested that the *ThUGT* transgenic plants eliminated more reactive oxygen species and inhibited the absorption of Cd to a certain extent by *T. hispida* under cadmium stress, thereby enhancing the cadmium resistance of *T. hispida*.

Discussion

When plants are under heavy metal stress, it can produce a series of corresponding functions to relieve the toxic effects of heavy metals. Some reports suggested that the production of glutathione, phytochelatin, and metal chelates or chaperones, which can bind to heavy metal ions and then transport them out of cells. In addition, antioxidant enzymes, such as superoxide dismutase (SOD), peroxidase (POD), glutathione reductase (GR), ascorbate peroxidase (APX) and glutathione peroxidase (GPX), were produced to scavenge oxygen free radicals generated by heavy metals (Emamverdian et al., 2015).

In this work, we used RNA-seq to explore the time course of the response mechanism in *T. hispida* under Cd stress. Our study showed that 114292 unigenes were obtained after the transcriptome data were spliced and 36.3% of the unigenes were novel transcripts under Cd stress. Further detailed analysis found that 6778 DEGs were detected at 24 h and then increased to 8282 and 8601 at 48 h and 72 h, respectively (Fig. 2A), which indicated that an increasing number of DEGs were induced or activated under Cd stress in *T. hispida*. Similarly, a large number of DEGs did not exhibit similar sequences in the NR database (39.4%, 36.9%, and 35.8% at 24, 48 and 72 h, respectively), indicating that the novel transcripts may play important roles in the response to Cd stress.

H₂O₂ is a product of aerobic metabolism of cells, and its production is increased under various stresses. It not only has the effect of damaging biological macromolecules and thereby harming cells, it is also an important signal molecule that induces the expression of a series of defense genes in the cell. And improve the activity of protective enzymes to remove active oxygen, prevent its excessive accumulation under adversity conditions and protect plants from damage (Mittler et al., 2002; Molleret al., 2001). Interestingly, the Cd content increased with increasing treatment time (Fig. 1A), while the H₂O₂ content peaked at 24 h (Fig. 1B and 1C). At the same time, we also found that many studies reported H₂O₂ functions a secondary messenger during plant development and defence to abiotic stress (Jiang et al., 2012; Saxena et al., 2016; Liu et al., 2016; Avshalumov et al., 2003; Khalili et al., 2014). Therefore, we speculate that H₂O₂ may serve as a secondary messenger to induce the expression of stress-related proteins in the early stage of cadmium stress and initiate the development of systemic acquired resistance in *T. hispida*. This plant synthesizes many ROS-clearing genes, thereby effectively eliminating excess H₂O₂ to help *T. hispida* tolerate Cd stress. Consistent with this property, the results of the DEGs at 24 h showed that 1/6 DEGs participated in the redox reaction, the transport of ions, and the synthesis of signal substances (Table S3), which have important functions in plant stress resistance.

In previous studies, multiple transcription factors or proteins were found which involved in the scavenging of ROS or increasing SOD and POD activities in *T. hispida* to improve the salt tolerance or osmotic stress ability of plants (He et al., 2019; Qin et al., 2017; Zang et al., 2015; Ji et al., 2016). Many studies on transcriptome analyses of Cd-treated plants have found that it is mainly related to the pathways of “ROS-scavenging enzymes” (Guo et al., 2017; Gupta et al., 2017), “cell wall alternation and strengthening” (Fan et al., 2011; Wan et al., 2012; Xu et al., 2015), “lipid oxidation” (Guo et al., 2017), “auxin biosynthesis and metabolism” (Yue et al., 2016), and “nitric oxide-mediated homeostasis” (Zuccarelli et al., 2017).

In *T. hispida*, the GO analysis showed in the biological pathway that “oxidation–reduction process” was the top enriched term, and “oxidoreductase activity” in the molecular function also ranked in the top 10 (Fig. 3A and 3B). These results indicated that the expression of antioxidant and redox homeostasis-related genes plays an important role in the response to Cd stress in *T. hispida*.

ABA, a widely known phytohormone involved in the plant response to abiotic stress, plays a vital role in mitigating Cd²⁺ toxicity in herbaceous species. Studies have found that when plants are exposed to Cd, endogenous ABA levels are increased in plant cells (Sharma et al., 2002). At the same time, several other studies demonstrated that the application of ABA can reduce Cd accumulation in crops (Uraguchi et al., 2009; Fan et al., 2014; Hsu et al., 2005; Hsu et al., 2003). Fan et al. (2014) reported that ABA treatment correlates with the downregulation of ABA-inhibited *IRON-REGULATED TRANSPORTER 1 (IRT1)* to decrease Cd accumulation. Through interaction with MYB49, the ABI5 represses MYB49 binding to the downstream genes *bHLH38*, *bHLH101*, *HIPP22* and *HIPP44*, which result in the inactivation of *IRT1* and reduced Cd uptake (Zhang et al., 2019a).

In this study, the results of KEGG pathway analysis showed that seven main phytohormones participate in Cd stress in *T. hispida*. Among the phytohormones, the input numbers vs background gene numbers for ABA pathways increased over time (Fig. 3C), with ratios of 0.9, 2.7 and 3.5% being observed at 24, 48 and 72 h, respectively. By GO function analysis, we found that seven DEGs were involved in the ABA signaling pathway, three of which were upregulated (Fig. S3). In particular, the expression of *ThUGT* gene was clearly induced. The ABA content in transient overexpression *ThUGT T. hispida* plants was significantly increased after cadmium stress treatment, but it increased less compared with the control (Fig. 5C). Moreover, the H₂O₂ content and Cd content increased lower in OE plants than the control after Cd stress (Fig. 5C), indicating that the *ThUGT* gene has the ability to remove ROS under Cd stress and enhance the tolerance of *T. hispida* to Cd.

Uridine diphosphate-glucosyltransferases (UGTs) are a family of proteins involved in physiological responses to the inactivation of many glycosylation hormones (Kleczkowski et al., 1995). The overexpression of the *UGT74E2* gene in Arabidopsis can improve the resistance of Arabidopsis to drought and salt stress by regulating the IBA dynamic balance (Tognetti et al., 2010). ABA β-glucosyltransferase belongs to the UGT family and is a key enzyme in the ABA catabolism binding pathway. This enzyme plays an important role in maintaining the normal physiological level of ABA. The ABA β-glucosyltransferase gene *AtUGT71B6* in *A. thaliana* regulates intracellular ABA balance (Priest et al., 2006; Dong et al., 2014). The *Phaseolus vulgaris* *PvABAGT* gene regulates the ABA balance and stress response in adversity during bean development (Xu et al., 2002).

In *Beta vulgaris*, the ROS produced by a plasma membrane NADPH oxidase may act as a signal to induce *BvGT* (UGT family gene) expression after wounding and bacterial infiltration (Sepulveda-Jimenez et al., 2004). Oxidative stress and conditions that promote cell death could induce the expression of glucosyltransferase genes and produce transportable glucosides that function as ROS scavengers (Mazel et al., 2002). Therefore, we predicted that *ThUGT* may play a crucial role in the ABA conjugation pathway

and in adaptation to Cd stress by inducing ROS scavengers to deduce H₂O₂. The specific mechanism governing this process warrants further research.

Conclusions

The present study identified novel transcripts, gene structures, and DEGs in *T. hispida* after Cd stress. In total, 114292 unigenes were identified. The large number of transcripts identified will serve as a global resource for future studies. Among these genes, a large number of genes related to ROS clearance and hormone signals were identified, which may facilitate the analysis of ABA signaling pathways and the overexpression of related gene *ThUGT* reduced the accumulation of Cd in *T. hispida* under high CdCl₂ stress. These results may help to establish a foundation for future research on cadmium tolerance in *T. hispida* and further use genetic engineering methods to improve plant cadmium resistance.

Abbreviations

ABA, Absciscic Acid; ABA, abscisic acid; APX, ascorbate peroxidase; Cd, Cadmium; cDNA, complementary DNA; DAB, 3,3-diaminobenzidine; DEGs, differentially expressed genes; ETH, Ethylene; FDR, false discovery rate; FPKM, expected number of fragments per kilobase of transcript sequence per million base pairs sequenced; GA, gibberellin; GO, Gene Ontology; GPX, glutathione peroxidase; GR, glutathione reductase; H₂O₂, hydrogen peroxide; IAA, indole-3-acetic acid; JA, jasmonic acid; KEGG, the Kyoto Encyclopedia of Genes and Genomes; N bases, unknown bases; NBT, Nitro blue tetrazolium; NR, Nonredundant nucleic acid database; POD, peroxidase; qRT-PCR, quantitative real-time polymerase chain reaction; RNA, ribonucleic acid; ROS, reactive oxygen species; SA, Salicylic acid; SOD, superoxide dismutase; STEM, Short Timeseries Expression Miner; Swiss-Prot, Swiss-port protein sequence database; *T. hispida*, *Tamarix hispida* Willd; UGTs, uridine diphosphate-glucosyltransferases.

Declarations

Declaration of competing interest

The authors declare no competing financial interest.

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References

Audic, S., Claverie, J.M., 1997. The significance of digital gene expression profiles. *Genome Res.* 7(10), 986-995.

- Avshalumov, M.V., Chen, B.T., Marshall, S.P., Pena, D.M., Rice, M.E., 2003. Glutamate-Dependent Inhibition of Dopamine Release in Striatum Is Mediated by a New Diffusible Messenger, H₂O₂. *The Journal of Neuroscience*. 23(7), 2744-2750.
- Bączek-Kwinta R., Juzoń K., Borek M., Antonkiewicz J., 2019. Photosynthetic response of cabbage in cadmium-spiked soil. *Photosynthetica*, 57(3)3, 731-739.
- Baker, B.J.M., Reeves, R.D., Hajar, A.S.M., 1994. Heavy metal accumulation and tolerance in British populations of the metallophyte *Thlaspi caerulescens* J. & C. Presl (Brassicaceae). *New Phytol.* 127, 61-68.
- Bert, V., Bonnin, I., Saumitou-Laprade, P., Patrick de Laguérie, P.d., Daniel Petit, D., 2002. Do *Arabidopsis halleri* from nonmetallicolous populations accumulate zinc and cadmium more effectively than those from metallicolous populations? *New Phytol.* 155, 47-57.
- Cao, F.B., Chen, F., Sun, H.Y., Zhang, G.P., Chen, Z.H., Wu, F.B., 2014. Genome-wide transcriptome and functional analysis of two contrasting genotypes reveals key genes for cadmium tolerance in barley. *BMC GENOMICS*. 15(1), 1-15.
- Conesa, A., Götz, S., García-Gómez, J.M., Terol, J., Talón, M., Robles, M., 2005. Blast2GO, a universal tool for annotation, visualization and analysis in functional genomics research. *BIOINFORMATICS*. 21(18), 3674-3676.
- Dong, T., Xu, Z.Y., Park, Y., Kim, D.H., Lee, Y., Hwang, I., 2014. Absciscic acid uridine diphosphate glucosyltransferases play a crucial role in absciscic acid homeostasis in *Arabidopsis*. *Plant PHYSIOL.* 165(1), 277-289.
- Emamverdian, A., Ding, Y.L., Mokhberdoran, F., Xie, Y.F., 2015. Heavy metal stress and some mechanisms of plant defense response. *THE SCIENTIFIC WORLD J.*
- Ernst, J., Bar-Joseph, Z., 2006. STEM, a tool for the analysis of short time series gene expression data. *BMC BIOINFORMATICS*. 7(1), 191-202.
- Fan, J.L., Wei, X.Z., Wan, L.C., Zhang, L.Y., Zhao, X.Q., Liu, W.Z., Hao, H.Q., Zhang, H.Y., 2011. Disarrangement of actin filaments and Ca²⁺ gradient by CdCl₂ alters cell wall construction in *Arabidopsis thaliana* root hairs by inhibiting vesicular trafficking. *J PLANT PHYSIOL.* 168, 1157-1167.
- Fan, S.K., Fang, X.Z., Guan, M.Y., Ye, Y.Q., Lin, X.Y., Du, S.T., Jin, C.W., 2014. Exogenous absciscic acid application decreases cadmium accumulation in *Arabidopsis* plants, which is associated with the inhibition of IRT1-mediated cadmium uptake. *FRONT PLANT SCI.* 721(5), 1-8.
- Farinati, S., DalCorso, G., Varotto, S., Furini, A., 2010. The *Brassica juncea* BjCdR15, an ortholog of *Arabidopsis* TGA3, is a regulator of cadmium uptake, transport and accumulation in shoots and confers

cadmium tolerance in transgenic plants. *New Phytol.* 185(4), 964-978.

Gao, C.Q., Wang, Y.C., Jiang, B., Liu, G.F., Yu, L.L., Wei, Z.G., Yang, C.P., 2011. A novel vacuolar membrane H⁺-ATPase c subunit gene (*ThVHAc1*) from *Tamarix hispida* confers tolerance to several abiotic stresses in *Saccharomyces cerevisiae*. *MOL BIOL REP.* 38, 957-963.

Gao, J., Sun, L., Yang, X.E., Liu, J.X., 2013. Transcriptomic analysis of cadmium stress response in the heavy metal hyperaccumulator *Sedum alfredii* Hance. *PLOS ONE.* 8(6), 1-10.

Grabherr, M.G., Haas, B.J., Yassour, M., Levin, J.Z., Thompson, D.A., Amit, I., Adiconis, X., Fan, L., Raychowdhury, R., Zeng, Q.D., Chen, Z.H., Mauceli, E., Hacohen, N., Gnirke, A., Rhind, N., di Palma, F., Birren, B.W., Nusbaum, C., Lindblad-Toh, K., Friedman, N., Regev, A., 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. *NAT BIOTECHNOL.* 29(7), 644-652.

Guo, Q., Meng, L., Zhang, Y.N., Mao, P.C., Tian, X.X., Li, S.S., Zhang, L., 2017. Antioxidative systems, metal ion homeostasis and cadmium distribution in *Iris lactea* exposed to cadmium stress. *ECOTOX ENVIRON SAFE.* 139, 50-55.

Gupta, D.K., Pena, L.B., Romero-Puertas, M.C., Hernández, A., Inouhe, M., Sandalio, L.M., 2017. NADPH oxidases differentially regulate ROS metabolism and nutrient uptake under cadmium toxicity. *PLANT CELL ENVIRON.* 40, 509-526.

Halimaa, P., Lin, Y.F., Ahonen, V.H., Blande, D., Clemens, S., Gyenesei, A., Häikiö, E., Kärenlampi, S.O., Laiho, A., Aarts, M.G., Pursiheimo, J.P., Schat, H., Schmidt, H., Tuomainen, M.H., Tervahauta, A.I., 2014. Gene expression differences between *Noccaea caerulea* ecotypes help identifying candidate genes for metal phytoremediation. *ENVIRON SCI TECHNOL.* 48(6), 3344-3353.

Hamada, T., Nakano, S., Iwai, S., Tanimoto, A., Ariyoshi, K., Koide O., 1991. Pathological study on beagles after long-term oral administration of cadmium. *TOXICOL PATHOL.* 19(2), 138-147.

He, Z., Li, Z., Lu, H., Huo, L., Wang, Z., Wang, Y., Ji, X., 2019. The NAC Protein from *Tamarix hispida*, ThNAC7, confers salt and osmotic stress tolerance by increasing reactive oxygen species scavenging capability. *Plants (Basel).* 8(7), 221.

Herbette, S., Taconnat, L., Hugouvieux, V., Piette, L., Magniette, M.L.M., Cuine, S., Auroy, P., Richaud, P., Forestier, C., Bourguignon, J., Renou, J.P., Vavasseur, A., Leonhardt, N., 2006. Genome-wide transcriptome profiling of the early cadmium response of *Arabidopsis* roots and shoots. *BIOCHIMIE.* 88(11), 1751-1765.

Hsu, Y.T., Kao, C.H., 2003. Role of abscisic acid in cadmium tolerance of rice (*Oryza sativa* L.) seedlings. *Plant, Cell and Environment.* 26, 867-874.

- Hsu, Y.T., Kao, C.H., 2005. Absciscic acid accumulation and cadmium tolerance in rice seedlings. *PHYSIOL PLANTARUM*. 124, 71-80.
- Ji, X., Nie, X., Liu, Y., Zheng, L., Zhao, H., Zhang, B., Huo, L., Wang, Y., 2016. A *bHLH* gene from *Tamarix hispida* improves abiotic stress tolerance by enhancing osmotic potential and decreasing reactive oxygen species accumulation. *Tree Physiol*. 36(2), 193-207.
- Ji, X.Y., Zheng, L., Liu, Y.J., Nie, X.G., Liu, S.N., Wang, Y.C., 2014. A transient transformation system for the functional characterization of genes involved in stress response. *PLANT MOL BIOL REP*. 32(3), 732-739.
- Jiang, Y.P., Cheng, F., Zhou, Y.H., Xia, X.J., Mao, W.H., Shi, K., Chen, Z.X., Yu, J.Q., 2013. Hydrogen peroxide functions as a secondary messenger for brassinosteroids-induced CO₂ assimilation and carbohydrate metabolism in *Cucumis sativus*. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*. 13(10), 811-823.
- Khalili, M., Hasanloo, T., Safdari, Y., 2014. Hydrogen peroxide Acts as a Secondary Messenger for Production of Silymarin in Ag⁺ Elicited *Silybum marianum* Hairy Root Cultures. *Journal of Medicinal Plants and By-products*. 1, 35-40.
- Kleczkowski, K., Schell, J., Bandur, R., 1995. Phytohormone conjugates, Nature and function. *CRIT REV PLANT SCI*. 14(4), 283-298.
- Küpper, H., Lombi, E., Zhao, F.J., McGrath, S.P., 2000. Cellular compartmentation of cadmium and zinc in relation to other elements in the hyperaccumulator *Arabidopsis halleri*. *PLANTA*. 212, 75-84.
- Li, R.Q., Yu, C., Li, Y.R., Lam, T.W., Yiu, S.M., Kristiansen, K., Wang, J., 2009. SOAP2, an improved ultrafast tool for short read alignment. *BIOINFORMATICS*. 25(15), 1966-1967.
- Liu, J.P., Zhang, C.C., Wei, C.C., Liu, X., Wang, M.G., Yu, F.F., Xie, Q., Tu, J.M., 2016. The RING Finger Ubiquitin E3 Ligase OsHTAS Enhances Heat Tolerance by Promoting H₂O₂-Induced Stomatal Closure in Rice. *Plant physiology*. 170, 429-443.
- Liu, T.M., Zhu, S.Y., Tang, Q.M., Tang, S.W., 2015. Genome-wide transcriptomic profiling of ramie (*Boehmeria nivea* L. Gaud) in response to cadmium stress. *Gene*. 558(1), 131-137.
- Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{-ΔΔCT} method. *Methods*. 25(4), 402-408.
- Luo, J.S., Yang, Y., Gu, T.Y., Wu, Z.M., Zhang, Z.H., 2019. The *Arabidopsis* defensin gene *AtPDF2.5* mediates cadmium tolerance and accumulation. *PLANT CELL ENVIRON*. 1-15.
- Martin, F., Bovet, L., Cordier, A., Stanke, M., Gunduz, I., Peitsch, M.C., Ivanov, N.V., 2012. Design of a tobacco exon array with application to investigate the differential cadmium accumulation property in two tobacco varieties. *BMC GENOMICS*. 13(1), 674-690.

- Mazel, A., Levine, L., 2002. Induction of glucosyltransferase transcription and activity during superoxide-dependent cell death in *Arabidopsis* plants. PLANT PHYSIOL BIOCH. 40, 133-140.
- Mittler R. 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7: 405-410.
- Moller IM. 2001. Plant Mitochondria and oxidative stress: Electron transport, NADPH turnover, and metabolism of reactive oxygen species. Annu Rev Plant Physiol Plant Mol Biol. 52: 561-591.
- Milner, M.J., Mitani-Ueno, N., Yamaji, N., Yokosho, K., Craft, E., Fei, Z.J., Ebbs, S., Clemencia Zambrano, M., Ma, J.F., Kochian, L.V., 2014. Root and shoot transcriptome analysis of two ecotypes of *Nocca caerulea* uncovers the role of *NcNramp1* in Cd hyperaccumulation. PLANT J. 78(3), 398-410.
- Oono, Y., Yazawa, T., Kawahara, Y., Kanamori, H., Kobayashi, F., Sasaki, H., Mori, S., Wu, J.Z., Handa, H., Itoh, T., Matsumoto, T., 2014. Genome-wide transcriptome analysis reveals that cadmium stress signaling controls the expression of genes in drought stress signal pathways in rice. PLOS ONE. 9(5), 1-14.
- Priest, D.M., Ambrose, S.J., Vaistij, F.E., Elias, L., Higgins, G.S., Ross, A.R.S., Abrams, S.R., Bowles, D.J., 2006. Use of the glucosyltransferase UGT71B6 to disturb abscisic acid homeostasis in *Arabidopsis thaliana*. PLANT J. 46(3), 492-502.
- Qin, L., Wang, L., Guo, Y., Li, Y., Ümüt, H., Wang, Y., 2017. An ERF transcription factor from *Tamarix hispida*, *ThCRF1*, can adjust osmotic potential and reactive oxygen species scavenging capability to improve salt tolerance. Plant Sci. 265, 154-166.
- Rajkumar, A.P., Qvist, P., Lazarus, R., Lescai, F., Ju, J., Nyegaard, M., Mors, O., Børglum, A.D., Li, Q.B., Christensen, J.H., 2015. Experimental validation of methods for differential gene expression analysis and sample pooling in RNA-seq. BMC GENOMICS. 16(1), 548-556.
- Rodriguez-Serrano, M., Romero-Puertas M.C., Pazmino, D.M., Testillano, P.S., Risueno, M.C., del Rio, L.A., Sandalio, L.M., 2009. Cellular response of pea plants to cadmium toxicity, cross talk between reactive oxygen species, nitric oxide, and calcium. Plant PHYSIOL. 150, 229-243.
- Salt, D.E., Wagner, G.J., 1993. Cadmium transport across tonoplast of vesicles from oat roots. Evidence for a $\text{Cd}^{2+}/\text{H}^{+}$ antiport activity. The journal of Biological Chemistry. 268(17), 12297-12302.
- Saxena, I., Srikanth, S., Chen, Z., 2016. Cross Talk between H_2O_2 and Interacting Signal Molecules under Plant Stress Response. Frontiers in Plant Science. 7, 1-16.
- Sepulveda-Jimenez, G., Rueda-Benitez, P., Porta, H., Rocha-Sosa, M., 2004. A red beet (*Beta vulgaris*) UDP-glucosyltransferase gene induced by wounding, bacterial infiltration and oxidative stress. J EXP BOT. 1-7.
- Sharma, S.S., Kumar, V., 2002. Responses of wild type and abscisic acid mutants of *Arabidopsis thaliana* to cadmium. J PLANT PHYSIOL. 159, 1323-1327.

- Suwazono, Y., Kido, T., Nakagawa, H., Nishijo, M., Nogawa, K., 2009. Biological half-life of cadmium in the urine of inhabitants after cessation of cadmium exposure. *Biomarkers*. 14(2), 77-81.
- Tognetti, V.B., Van Aken, O., Morreel, K., Vandenbroucke, K., Van de Cotte, B., De Clercq, I., Chiwocha, S., Fenske, R., Prinsen, E., Boerjan, W., Genty, B., Stubbs, K.A., Inze, D., Breusegema, F.V., 2010. Perturbation of indole-3-butyric acid homeostasis by the UDP- glucosyltransferase *UGT74E2* modulates *Arabidopsis* architecture and water stress tolerance. *PLANT CELL*. 22, 2660-2679.
- Uraguchi, S., Mori, S., Kuramata, M., Kawasaki, A., Arao, T., Ishikawa, S., 2009. Root-to-shoot Cd translocation via the xylem is the major process determining shoot and grain cadmium accumulation in rice. *J EXP BOT*. 60(9), 2677-2688.
- Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Roy, N.V., Paepe, A.D., Speleman, F., 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol Res*. 3(7), 1-12.
- Vogel-Mikus, K., Pongrac, P., Kump, P., Necemer, M., Regvar, M., 2005. Colonisation of a Z9(2), 362n, Cd and Pb hyperaccumulator *Thlaspi praecox* Wulfen with indigenous arbuscular mycorrhizal fungal mixture induces changes in heavy metal and nutrient uptake. *ENVIRON POLLUT*. 13-371.
- Wan, L.C., Zhang, H.Y., 2012. Cadmium toxicity, effects on cytoskeleton, vesicular trafficking and cell wall construction. *Plant Signaling & Behavior*. 7, 345-348.
- Wang, C., Gao, C., Wang, L., Zheng, L., Yang, C., Wang, Y., 2013. Comprehensive transcriptional profiling of NaHCO₃-stressed *Tamarix hispida* roots reveals networks of responsive genes. *Plant Mol Biol*. 84, 145-157.
- Wang, PL., Lei, XJ., Lü, JX., Gao, CQ., 2020. Overexpression of the *ThTPS* gene enhanced salt and osmotic stress tolerance in *Tamarix hispida*. *J For Res*.
- Wei, S.H., Zhou, Q.X., Wang, X., 2005. Cadmium Hyperaccumulator *Solanum nigrum* L. and its accumulating characteristics. *Environmental science*. 26(3), 167-171.
- Wu, F.B., Zhang, G.P., Dominy, P., Wu, H.X., Bachir, Dango.M.L., 2007. Differences in yield components and kernel Cd accumulation in response to Cd toxicity in four barley genotypes. *CHEMOSPHERE*. 70, 83-92.
- Xu, S.S., Lin, S.Z., Lai, Z.X., 2015. Cadmium impairs iron homeostasis in *Arabidopsis thaliana* by increasing the polysaccharide contents and the iron-binding capacity of root cell walls. *PLANT SOIL*. 392, 71-85.
- Xu, Z.J., Nakajima, M., Suzuki, Y., Yamaguchi, I., 2002. Cloning and characterization of the Absciscic acid-specific glucosyltransferase gene from Adzuki bean seedlings. *J PLANT PHYSIOL*. 129, 1285-1295.

- Yang, G.Y., Wang, C., Wang, Y.C., Guo, Y.C., Zhao, Y.L., Yang, C.P., Gao, C.Q., 2016. Overexpression of *ThVHAc1* and its potential upstream regulator, *ThWRKY7*, improved plant tolerance of Cadmium stress. *SCI REP-UK*. 1-17.
- Yang, J.L., Wang, Y.C., Liu, G.F., Yang, C.P., Li, C.H., 2011. *Tamarix hispida* metallothionein-like *ThMT3*, a reactive oxygen species scavenger, increases tolerance against Cd^{2+} , Zn^{2+} , Cu^{2+} , and NaCl in transgenic yeast. *MOL BIOL REP*. 38, 1567-1574.
- Yang, X.E., Long, X.X., Ye, H.B., He, Z.L., Calver, D.V., Stofflla, P.J., 2004. Cadmium tolerance and hyperaccumulation in a new Zn-hyperaccumulating plant species (*Sedum alfredii* Hance). *PLANT SOIL*. 259, 181-189.
- Ye, J., Fang, L., Zheng, H.K., Zhang, Y., Chen, J., Zhang, Z.J., Wang, J., Li, S.T., Li, R.Q., Bolund, L., Wang, J., 2006. WEGO, a web tool for plotting GO annotations. *NUCLEIC ACIDS RES*. 34, 293-297.
- Yue, R.Q., Lu, C.X., Qi, J.S., Han, X.H., Yan, S.F., Guo, S.L., Liu, L., Fu, X.L., Chen, N.N., Yin, H.Y., Chi, H.F., Tie, S.G., 2016. Transcriptome analysis of cadmium-treated roots in maize (*Zea mays* L.). *FRONT PLANT SCI*. 7, 1298-1309.
- Zang, D., Wang, C., Ji, X., Wang, Y., 2015. *Tamarix hispida* zinc finger protein ThZFP1 participates in salt and osmotic stress tolerance by increasing proline content and SOD and POD activities. *Plant Sci*. 235, 111-121.
- Zhang, P., Wang, R.L., Ju, Q., Li, W.Q., Tran, L.P., Jin, X., 2019a. The R2R3-MYB transcription factor MYB49 regulates cadmium accumulation. *Plant PHYSIOL*.
- Zhang, X., Wang, L., Meng, H., Wen, H., Fan, Y., Zhao, J., 2011. Maize ABP9 enhances tolerance to multiple stresses in transgenic Arabidopsis by modulating ABA signaling and cellular levels of reactive oxygen species. *PLANT MOL BIOL*. 75, 365-378.
- Zhang, Z.H., Zhou, T., Tang, T.J., Song, H.Q., Guan, C.Y., Huang, J.S., Hua, Y.P., 2019b. Multiomics landscapes uncover the pivotal role of subcellular reallocation of cadmium in regulating rapeseed resistance to cadmium toxicity. *J EXP BOT*. 1-19.
- Zhou, B.R., Yao, W.J., Wang, S.J., Wang, X.W., Jiang, T.B., 2014. The Metallothionein Gene, *TaMT3*, from *Tamarix androssowii* Confers Cd^{2+} Tolerance in Tobacco. *Int. J. Mol. Sci*. 15, 10398-10409.
- Zhou, Q., Guo, J.J., He, C.S., Shen, C., Huang, Y.Y., Chen, J.X., Guo, J.H., Yuan, J.G., Yang, Z.Y., 2016. Comparative Transcriptome Analysis between Low- and High- Cadmium -Accumulating Genotypes of Pakchoi (*Brassica chinensis* L.) in Response to Cadmium Stress. *ENVIRON SCI TECHNOL*. 50, 6485-6494.

Zuccarelli, R., Coelho, A.C.P., Peres, L.E.P., Freschi, L., 2017. Shedding light on NO homeostasis, light as a key regulator of glutathione and nitric oxide metabolisms during seedling deetiolation. NITRIC OXIDE-BIOL CH. 68, 77-90.

Tables

Table 1 TOP30 KEGG pathways based on the percentage of DEGs in 24 h vs control

Term	Total	24h vs control		48h vs control		72h vs control	
		DEGs	%	DEGs	%	DEGs	%
Glucosinolate biosynthesis	7	2	28.6	1	14.3	2	28.6
Photosynthesis - antenna proteins	48	10	20.8	1	2.1	10	20.8
Cutin, suberine and wax biosynthesis	77	7	9.1	2	2.6	9	11.7
Brassinosteroid biosynthesis(BRs)	31	2	6.5	0	0.0	1	3.2
Flavonoid biosynthesis	73	4	5.5	1	1.4	11	15.1
Phenylpropanoid biosynthesis	372	19	5.1	12	3.2	49	13.2
Stilbenoid, diarylheptanoid and gingerol biosynthesis	61	3	4.9	0	0.0	10	16.4
Cyanoamino acid metabolism	161	7	4.4	6	3.7	13	8.1
Phenylalanine metabolism(SA)	123	5	4.1	1	0.8	17	13.8
Nitrogen metabolism	104	4	3.9	4	3.9	11	10.6
Arachidonic acid metabolism	57	2	3.5	1	1.8	5	8.8
Phenylalanine, tyrosine and tryptophan biosynthesis	206	7	3.4	8	3.9	9	4.4
alpha-Linolenic acid metabolism(JA)	184	6	3.3	4	2.2	10	5.4
Tryptophan metabolism[IAA]	134	4	3.0	1	0.8	7	5.2
Alanine, aspartate and glutamate metabolism	241	7	2.9	4	1.7	22	9.1
Ascorbate and aldarate metabolism	214	6	2.8	7	3.3	19	8.9
Pentose phosphate pathway	215	6	2.8	7	3.3	15	7.0
Amino sugar and nucleotide sugar metabolism	408	11	2.7	6	1.5	24	5.9
Cysteine and methionine metabolism (ETH)	342	9	2.6	9	2.6	23	6.7
Terpenoid backbone biosynthesis	190	5	2.6	3	1.6	4	2.1
Taurine and hypotaurine metabolism	76	2	2.6	0	0.0	7	9.2
Steroid biosynthesis	123	3	2.4	1	0.8	8	6.5
Plant hormone signal transduction	633	15	2.4	11	1.7	30	4.7
Butanoate metabolism	128	3	2.3	1	0.8	2	1.6
Photosynthesis	131	3	2.3	3	2.3	12	9.2
Carbon fixation in photosynthetic organisms	306	7	2.3	8	2.6	28	9.2
Plant-pathogen interaction	482	11	2.3	13	2.7	20	4.2
Glutathione metabolism	264	6	2.3	8	3.0	18	6.8
Pentose and glucuronate interconversions	221	5	2.3	4	1.8	13	5.9
Sphingolipid metabolism	133	3	2.3	4	3.0	5	3.8

Note: Hormone-related KEGG pathways have a gray background.

Table 2 The top 20 GO molecular function based on number of common DEGs

Gene Ontology Molecular Function	DEG_item
protein binding	88
ATP binding	52
DNA binding	45
zinc ion binding	33
nucleic acid binding	31
oxidoreductase activity	28
protein kinase activity	24
metal ion binding	21
structural constituent of ribosome	20
RNA binding	18
transcription factor activity, sequence-specific DNA binding	17
calcium ion binding	14
transmembrane transporter activity	14
catalytic activity	13
hydrolase activity, hydrolyzing O-glycosyl compounds	12
GTP binding	10
electron carrier activity	9
GTPase activity	9
heme binding	9
nucleotide binding	8

Figures

Figure 1

Cd stress analysis of *T. hispida*. The leaves Cd (A) and H₂O₂ (B) concentrations in *T. hispida* at control or under 150 µmol L⁻¹ CdCl₂ treated for 24 h, 48 h or 72 h. (C) DAB staining of *T. hispida* leaves under control or after cadmium stress. Each treatment contained three separate repeats with at least 60 seedlings. Control: under normal conditions. * (P < 0.05) indicate signification difference compared with control.

Figure 2

DEGs of *T. hispida* under cadmium treatments. (A) Gene number analysis of differentially expressed genes (DEGs). in the treated transcriptomes compared with the control. (B) Venn diagrams showing unique and shared DEGs between the cadmium treated transcriptomes compared with the control.

Figure 3.

Figure 3

GO terms and KEGG pathways involved in ROS and hormones production in *T. hispida* under cadmium treatment. (A) GO analysis of biological process terms, (B) molecular function terms and (C) KEGG pathways involved in hormones between the cadmium treated transcriptomes compared with the control. The x-axis in (A, B) indicates the percentage of DEG snumbers vs background gene numbers in each GO term. The x-axis in (C) indicates the percentage of DEGs numbers vs background gene numbers in each KEGG pathway.

Figure 4

Gene Temporal Expression Pattern analysis. Expressive trend pattern analysis of DEGs. (A) Heat map analysis of DEGs. FPKM values of DEGs were used to make heat map analysis. (B) Expressive trend pattern analysis of DEGs. The value of ratios take the base 2 logarithm were used to analysis each gene`s expression trend in every group. For each gene, ratios = FPKM of the gene in sample/FPKM value of the gene in control.

Figure 5

Cadmium tolerance analysis of ThUGT gene. (A) qRT-PCR analysis (B) staining analysis and (C) physiological indicators determination of ThUGT transgenic *T. hispida* under 100 μmol L⁻¹ CdCl₂ stress. The above seedlings were treated with 100 μmol L⁻¹ CdCl₂ or control for 24 h. Each treatment contained three separate repeats with at least 45 seedlings. Con: *T. hispida* plants transformed with empty pROKII. ThUTG: *T. hispida* plants transformed with ThUTG. Control: normal conditions. * (P < 0.05) indicate signification difference compared with control.

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