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A note on the early transcriptional response in leaves and root of potato plants to cadmium exposure

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Abstract

Potato plants can accumulate a high amount of cadmium (Cd) in the tuber when grown in soils rich in Cd. The molecular mechanisms governing Cd accumulation in the potato plant are poorly understood. Here we performed an RNA-sequencing experiment to identify genes differentially expressed in the leaf and root of potato during early stages of Cd exposure. Results did not identify any significant transcriptional response in leaves under 1 or 5 mg kg^{-1} Cd after 72 h. However, in the roots we did identify 2,846 genes that were significantly differentially expressed after 72 h between plants grown in 5 mg kg^{-1} Cd and controls. These included genes involved in photosynthesis and autophagy being up-regulated, and genes involved in intracellular transport being down-regulated. This study is the first report on the transcriptome-wide response of potato to Cd stress, providing insight into the molecular mechanisms involved in the response.

Keywords

Cadmium • gene expression • potato • RNA-seq • transcriptome

Introduction

Cadmium (Cd) is a toxic metal for animals and humans if consumed in sufficient quantities (Clemens, 2006; Grant *et al*., 2008; Mengist *et al*., 2017, 2018a, 2018b). Cadmium has similar physicochemical properties to Zn, both being included in Group 12 of the periodic table. Cadmium is predominantly found in the earth's crust in association with zinc, lead and copper ores. Natural processes such as weathering and volcanic activities release Cd to the environment. However, these natural processes result in only trace levels of Cd being released (Clemens, 2006). The main sources of Cd in the environment are smelting, fossil fuel combustion, municipal waste incineration and disposal and agricultural practices including the application of sewage sludge and phosphate fertilisers, as Cd occurs naturally in rock phosphate (Clemens, 2006; Clemens *et al*., 2013; Roberts, 2014).

Cadmium is taken up by the basal roots, translocated to the shoot via the xylem and redistributed to the tuber via the phloem (Reid *et al*., 2003; Mengist *et al*., 2017). During the processes of "import and export", factors associated with uptake, transport and distribution can determine the final tuber Cd (Clemens, 2006; Clemens *et al*., 2013; Mengist *et al*., 2017, 2018b). Our previous studies revealed differences in Cd uptake and distribution among potato cultivars. Root Cd absorption, root-to-shoot translocation and distribution of Cd between organs were the major limiting factors for differential Cd accumulation in tuber of potato (Mengist *et al*., 2017, 2018b). Furthermore, the role of shoots and roots for Cd uptake and distribution was examined at two Cd concentrations (1 mg kg^{-1} and 5 mg kg^{-1} soil Cd). The pattern of Cd concentrations was different among organs in the two Cd treatments; at the low soil Cd level (1 mg kg^{-1} soil Cd) there were similar levels of Cd in shoots and roots. In contrast, the root sequestered much higher Cd than the shoot at higher Cd treatment (5 mg kg^{-1} soil Cd). Regardless of the soil Cd levels, tuber Cd concentrations were much smaller than root or shoot Cd concentrations (Mengist *et al*., 2018b).

The molecular mechanisms by which plants respond to changes in Cd stress are complex but of great importance and could be useful in developing strategies for elucidating the gene networks involved in plant responses to various kinds of stress (Oono *et al*., 2016). Cadmium is non-essential to plant cells and it is believed that plants do not have Cd-specific membrane transporters. Instead, Cd uptake and transport

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can be associated with other cation transporters, such as Zn^{2+} and Fe^{2+} transporters, because of the similar chemical properties exhibited by these cations (Sanita di Toppi & Gabbrielli, 1999; Clemens, 2006; Broadley *et al*., 2007; Sasaki *et al*., 2012; Brunetti *et al*., 2015). Elevated levels of Cd in the plant cells can be immobilised in the apoplasm (metal binding to the cell wall and intercellular spaces), chelation in the cytoplasm, compartmentalisation in the vacuole, loading to the xylem and translocation to the shoot and/or efflux back to the soil solution (Sanita di Toppi & Gabbrielli, 1999; Clemens *et al*., 2001; Broadley *et al*., 2007). Furthermore, ATP-binding cassette (ABC) transporters are involved in the transport of Cd complexes to the vacuoles (Song *et al*., 2010; Park *et al*., 2012; Brunetti *et al*., 2015).

Genome-wide profiling of the transcriptome is helpful to understand the functional elements of the genome, cells and tissues, and their roles in the development of resistance or tolerance to abiotic or biotic stresses (Wolf, 2013). RNA-seq analyses have been applied for Cd stress in different crops, including rice (Oono *et al*., 2016), tomato (Hou *et al*., 2017) and other *Solanum* species (Xu *et al*., 2012). However, to date, there has been no report regarding molecular mechanisms of Cd accumulation in potato. In this study, we aimed to identify differentially expressed genes and associated pathways under Cd stress using RNA-seq analysis from leaf and root organs of the potato cultivar, "Rooster", exposed to two different Cd concentrations (1 mg kg^{-1} and 5 mg kg^{-1} soil Cd) conditions.

Materials and methods

Experimental design and RNA extraction

Virus-free tubers of the variety Rooster were sprouted under constant diffused light conditions and planted in 10 L pots filled with a sand–soil mixture under controlled greenhouse conditions with 16 h light at 21°C. The plants were watered from above daily with 1 L nutrient solution as described in Dunbar *et al.* (2003). Briefly, the nutrient solution comprised (mM) 3.0 KNO_3, 1.0 $MgSO_4$, 0.5 NaCl, 2.5 $CaNO_3$, 0.5 K_2SO_4 and 0.5 KH_2PO_4. Micronutrients (in µM) were 20 B, 20 Mn, 10 Zn, 1.0 Cu and 0.5 Mo. Iron (25 mM) was added as a complex with diethylene triamine penta-acetic acid. The nutrient solution was adjusted to a pH of 5.5 with HCl. During the growth period, the plants were rotated every day to have uniform light exposure. At 45 d of planting, the plants were randomly assigned to one of three treatments. Plants in the first treatment did not receive any Cd and acted as the experimental control, plants in the second treatment received 1 mg kg^{-1} Cd for 72 h and plants in the third treatment received 5 mg kg^{-1} Cd for 72 h. Cd was applied in the form of $CdCl_2$ with the nutrient solution. Youngest leaves from the third row of the canopy were collected and flash frozen in liquid nitrogen and stored at –80°C until further processing. Similarly, the root samples were carefully washed with distilled water and kept at –80°C until further processing.

Total RNA extraction from leaf and root tissue was performed using Trizol reagent (Invitrogen, Waltham, MA, USA). The RNA concentration and purity was analysed by NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, Loughborough, UK). A260/A280 ratios were used to determine purity. RNA quality was also determined using the 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The RNA Nano 6000 chip was used and RNA integrity numbers (RINs) were calculated using manufacturer's software. All samples had an RIN greater than 8 and were used in subsequent analysis.

Library preparation and sequencing

Twenty-four samples (two organs, three treatments, each with four biological replicates) were submitted to LC Sciences (Houston, TX, USA) for complementary DNA (cDNA) library preparation and RNA sequencing. Twenty-four cDNA libraries were constructed using the TruSeq RNA sample Prep Kit (Illumina, San Diego, CA, USA) following manufacturer's instructions. The libraries were sequenced with an Illumina HiSeq 2000 sequencer, aiming to generate stranded 150 bp paired-end reads with a minimum of 30 million pairs per sample. The data have been deposited in European Bioinformatics Institute's ArrayExpress and has been assigned the accession number E-MTAB-7711.

Transcript quantification and differential expression

RNA-seq data were pseudoaligned to the reference transcriptome (The Potato Genome Sequencing Consortium, 2011), and transcript abundance determined using Kallisto (Bray *et al*., 2016). Differential gene expression was carried out using Sleuth (Pimentel *et al*., 2017) with four pairwise comparisons performed (leaf and root samples at both 1 and 5 mg kg^{-1} Cd for 72 h, each compared to controls). To identify the transcripts affected by Cd we ran a likelihood ratio test (LRT). A Wald test was also run to determine β estimates and only transcripts overlapping with the LRT analysis were retained. Gene ontology enrichment analysis was carried out using ShinyGO (Ge *et al*., 2020).

Results

Plant growth and transcript quantification

At the time of harvest, we did not see any symptoms of stress or differences between the treated and control samples. Root and leaf samples from both treated and control conditions

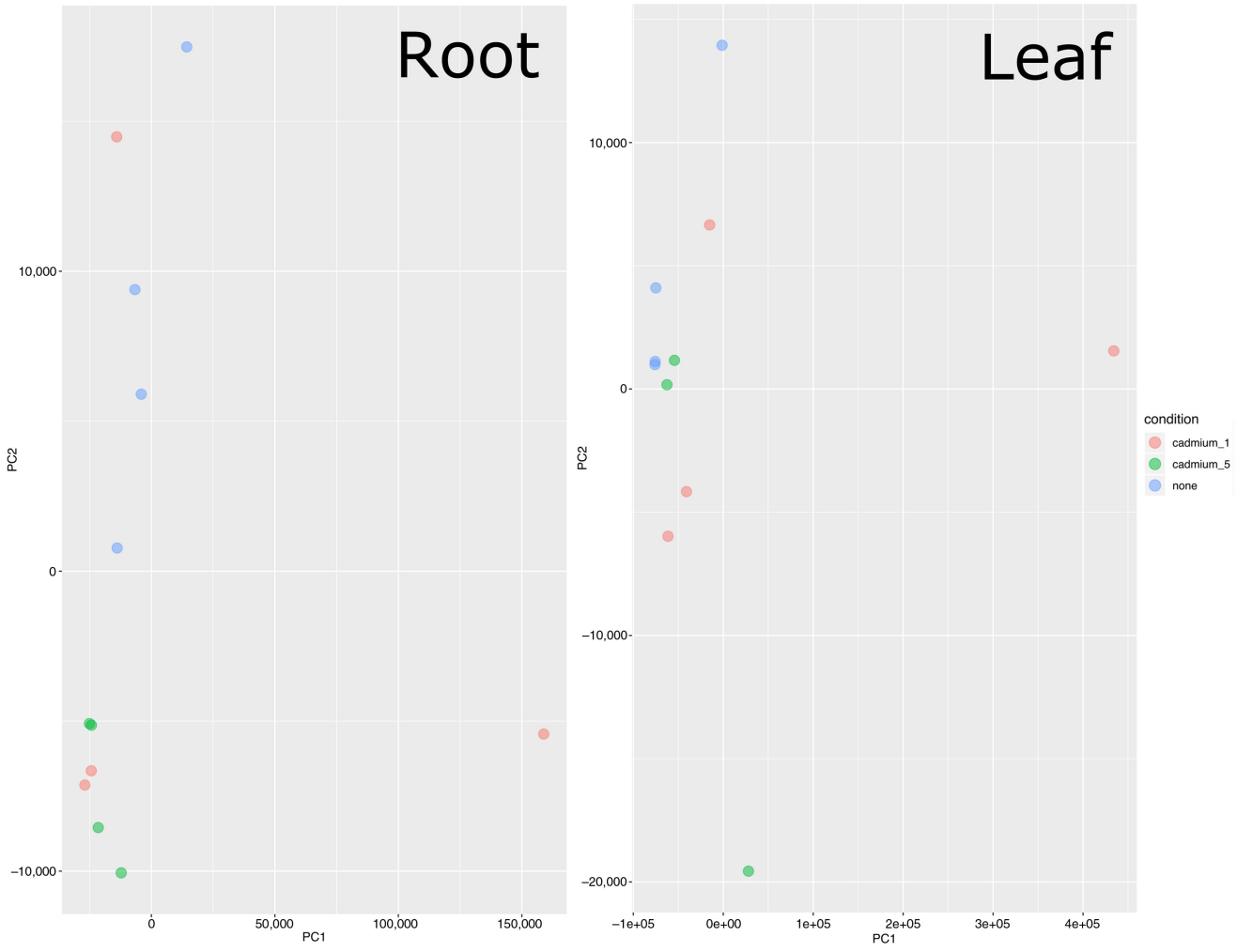

Figure 1. Principle component analysis of RNA-seq data. The first and second principle components of transcript abundance data in leaf and root of control, and 1 or 5 mg kg^{-1} Cd after 72 h. The outlier sample on PC1 in leaf is 1 mg kg^{-1} Cd, and the outlier sample on PC1 in root is 1 mg kg^{-1} Cd.

were collected and RNA extracted for library preparation and sequencing. A total of 23 strand-specific cDNA libraries (11 from leaves and 12 from roots) were constructed and sequenced on an Illumina HiSeq, generating paired-end reads ranging between 35.58 million pairs per sample and 70.87 million pairs per sample. A single leaf sample (5 mg kg^{-1} Cd) was removed from analysis due to a problem with the data file. Reads were aligned to the transcriptome with Kallisto and resulted in mapping rates between 71% and 78% with the exception of one leaf (1 mg kg^{-1} Cd) and one root (1 mg kg^{-1} Cd) sample (59% and 65%). Principal component analysis (PCA) on leaf and root samples did not show clear separation among treatments in leaf samples; however, there was separation on PC2 between control and 5 mg Cd in root samples (Figure 1). The single root and leaf samples that appear as outliers on the PCA are the two samples with the lower mapping rates identified above. A case could be made for removing these samples but they have been retained for the analysis presented here.

Differential gene expression under Cd treatment

In leaf we did not find any genes significantly differentially up- or down-regulated with our analysis approach under either 1 or 5 mg kg^{-1} Cd for 72 h. In root we also did not find any genes significantly differentially up- or down-regulated under 1 mg kg^{-1} Cd for 72 h; however, we did identify differentially expressed genes in root under 5 mg kg^{-1} Cd for 72 h. In total we identified 2,846 genes differentially expressed between control and 5 mg kg^{-1} Cd after 72 h (1,406 were down-regulated and 1,440 were up-regulated)

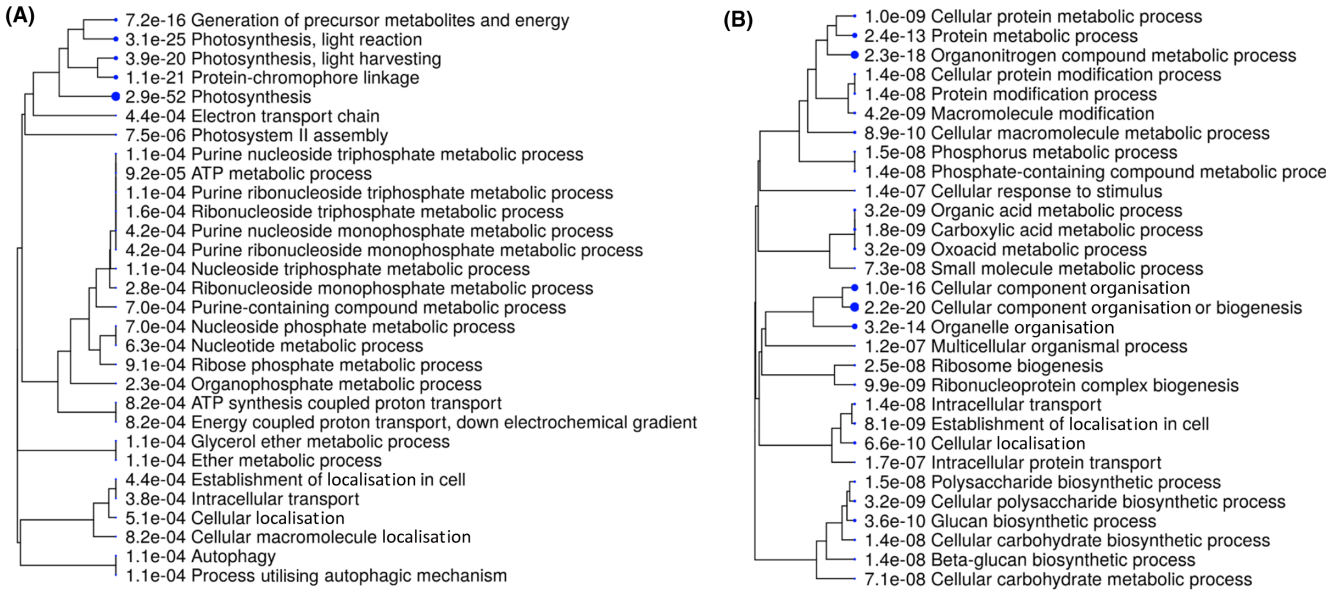

Figure 2. Gene ontology enrichment analysis. Hierarchical clustering of the top 30 significantly enriched categories in up-regulated (A) and down-regulated (B) lists of significantly differentially expressed transcripts in roots in response to 5 mg kg^{-1} Cd after 72 h. The size of blue dots alongside functional category corresponds to level of significance.

(https://doi.org/10.6084/m9.figshare.11114576.v1). Gene ontology enrichment analysis identified functional categories significantly enriched in both up- and down-regulated gene lists (Figure 2). In the up-regulated gene list, there were 38 genes mapped to the GO term *Response to chemical*, including a heavy-metal-associated domain containing protein (PGSC0003DMT400001367), and a collection of auxin responsive family proteins (deposit on Figshare prior to publication [private link to data for review https://figshare.com/s/e94cadf822173ad1a773]). Similarly, in the down-regulated gene list there were 53 genes mapped to the GO term *Response to chemical*, including peroxidase and glutathione reductase (https://doi.org/10.6084/m9.figshare.11114576.v1).

Discussion

To date, there have been no published studies investigating the molecular mechanisms of Cd tolerance in potato. This study used untargeted transcriptome profiling to examine molecular and cellular processes affected by early Cd exposure in leaves and roots of potato. We failed to identify any response in the transcriptome of leaves under 1 or 5 mg kg^{-1} Cd after 72 h. In line with these results, we did not identify any observable difference after 72 h between plants grown under Cd and controls. However, in the roots we did identify 2,846 genes that were significantly differentially expressed after 72 h between plants grown in 5 mg kg^{-1} Cd and controls.

The functional category *Photosynthesis* was significantly enriched (72 genes assigned) in the up-regulated genes, which includes ultraviolet-B-repressible proteins, oxygen-evolving enhancer protein, thioredoxin and photosystem II proteins among others. Previous research has identified that photosynthesis-related activities are modulated under Cd treatment (Tang *et al*., 2016; Chen *et al*., 2018), and it is already known that heavy metals such as Cd can damage chloroplasts and disrupt photosynthesis (Paunov *et al*., 2018). Furthermore, the use of the photosynthetic apparatus in the development of biosensors to monitor heavy metal contamination has been proposed (Rouillon *et al*., 2006). The functional category *Autophagy* was also significantly enriched (eight genes). Autophagy is a degradation and recycling pathway in plants, and it has been shown to be induced on exposure to heavy metals (Zheng *et al*., 2012). A study in tobacco seedlings recently demonstrated that 18 of 30 autophagy-related genes characterised were up-regulated in response to at least one heavy metal, including Cd (Zhou *et al*., 2015). Our results suggest that activation of autophagy also occurs in potato.

A Synaptotagmin gene (PGSC0003DMT400089685) showed the greatest down-regulation in roots under Cd. An *Arabidopsis* synaptotagmin gene (SYTA) was shown to regulate endocytosis and virus movement protein cell-to-cell transport (Lewis & Lazarowitz, 2010). Endocytosis provides

a major route for membrane proteins, lipids and extracellular molecules into the cell and is required for multiple cellular processes including nutrient uptake (Fan *et al*., 2015). Interestingly, Cd has been shown to disrupt endocytosis during pollen germination and tube growth in the conifer *Picea wilsonii* (Wang *et al*., 2014). Furthermore, a broad set of genes involved in directed movement of substances within a cell were down-regulated as indicated by the significant enrichment of the functional category *Intracellular transport* (34 genes assigned).

In conclusion, this study provides an insight into the early transcriptional response of potato to Cd. While no genes were significantly regulated in leaves, a large number were regulated in roots in the highest Cd exposure, with genes involved in photosynthesis and autophagy being up-regulated, and intracellular transport being down-regulated.

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