Data in Brief

Transcriptome of barley under three different heavy metal stress reaction

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ABSTRACT

In the present study, we used Illumina sequencing technology (HiSeq 2000) to sequence the transcriptome of barley (*Hordeum vulgare* L., cv. Morex) under three different heavy metal stress conditions: copper, zinc and cadmium. For each of those metals, the concentration causing a 50% inhibitory effect for root growth (EC_{50}) was determined. We sequenced the total RNA of both roots and shoots from barley with and without heavy metal treatments in three replicates. Raw reads of the transcriptome project have been deposited in NCBI's BioProject accession number PRJNA382490. The obtained transcriptomic data will be useful for further studies focusing on heavy metal tolerance and comparative transcriptome analysis in barley.

Specifications

Organism/cell line/tissue	*Hordeum vulgare* L., cv. Morex/roots and shoots
Sex	N/A
Sequencer or array type	Illumina HiSeq 2000
Data format	Raw data: FASTQ file
Experimental factors	Copper, zinc and cadmium treatment
Experimental features	Roots and shoots from *Hordeum vulgare* treated with copper, zinc and cadmium were collected for RNA isolation and sequencing.
Consent	N/A
Sample source location	Seeds of *H. vulgare* L., cv. Morex (Accession number: BCC 906) originating from the United States of America were provided by the Genebank IPK Gatersleben of the Leibniz Institute of Plant Genetics and Crop Plant Research, Germany.

1. Direct link to deposited data

https://www.ncbi.nlm.nih.gov/bioproject/PRJNA382490.
https://www.ncbi.nlm.nih.gov/sra/SRP104085.

2. Introduction

Metals are naturally occurring elements present in soil, rock, air, water and living organisms. Some of them are required by plants in trace amounts. Heavy metals such as copper or zinc are important micronutrients and they are necessary for normal plant growth and development. Copper (Cu) plays a role in electron-transfer reactions because of its ability to cycle between an oxidized state Cu(II) and reduced state Cu(I) [1]. Zinc (Zn) is the second most abundant metal in plants. Due to its physico-chemical properties, zinc is a cofactor for many enzymes applied in metabolism of nucleic acids, proteins, carbohydrates and lipids [2]. On the other hand, there are heavy metals like cadmium (Cd), mercury (Hg) or lead (Pb) which have no known function as nutrients. However, essential as well as nonessential metals can be toxic to plants in elevated concentrations. Toxic effect of heavy metals lies in their chemical similarity with other elements. Displacing essential elements in proteins and enzymes disrupts proper function of these biomolecules. Heavy metal excess in cell causes oxidative stress due to formation of free radicals and reactive oxygen species [3,4].

Heavy metals are important environmental pollutants. They enter the environment through natural geochemical cycles, microbial activity and human activities like mining, energy production or agriculture. Heavy metals are dispersed in soils and sediments, dissolved in groundwater and surface water and also diffused in the air and their accumulation are detrimental for humans [5]. There is an urgent need for transcriptome datasets of heavy metal affected plants to screen for relevant genes regulating toxic effect of metal excess [6].

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Table 1

Half maximal effective concentration of cadmium, copper and zinc on barley root growth. Root elongation was calculated from the average of ten treated plants and ten non-treated plants. EC$_{50}$ of cadmium, copper and zinc were determined as 80 μM, 50 μM and 570 μM respectively.

Cadmium concentration (μM)	Root elongation (%)	Copper concentration (μM)	Root elongation (%)	Zinc concentration (μM)	Root elongation (%)
0.01	127	0.01	110	200	136
0.1	112	0.1	105	300	148
1	100	1	106	400	104
10	83	10	119	500	79
50	64	20	120	510	78
60	61	30	76	530	75
70	56	40	55	550	79
80	51	50	52	570	50
90	37	60	29	590	41
100	34	70	2	600	39
1000	4	100	2	700	29
		1000	1		

Here we report the transcriptome sequencing of barley (*Hordeum vulgare*) that was growing under heavy metal stress condition, as well as the establishment of the concentration of copper, zinc and cadmium that causes a decrease in root elongation of 50% compare to none-treated plants.

3. Experimental design, materials and methods

3.1. Plant material

Barley seeds (*Hordeum vulgare* L., cv. Morex; Accession number: BCC 906) were obtained from the genebank IPK Gatersleben of the Leibniz Institute of Plant Genetics and Crop Plant Research, Germany.

3.2. Toxicity assay

In order to determine the half maximal effective concentration (EC$_{50}$) on the growth of the roots for each of the heavy metal: copper, zinc and cadmium, an assay was conducted. The seeds were grown on a series of media in which only the amount of tested heavy metal varied (Table 1).

The pH of the basal nutrient solution (see Table 2 for composition) was adjusted with 1 M of potassium hydroxide (KOH) to 5.5–6.0 (Podar, 2013). Barley seeds were germinated at 20 °C in the dark on filter paper moistened with deionized water. After 48 h the primary root length was measured for ten seeds which were then transferred to polypropylene pots containing 1 L of test medium, which was changed every 24 h to maintain the composition. Test media were aerated throughout the exposure time. Relative humidity was 50%, and day/night temperatures were 20 °C/16 °C during 16 h/8 h photoperiod.

Table 2

Basal nutrient solution composition.

Compound	Formula	Amount
Monopotassium phosphate	KH_2PO_4	0.4 mM
Potassium sulfate	K_2SO_4	0.4 mM
Heptahydrate magnesium sulfate	$MgSO_4 \cdots 7H_2O$	0.6 mM
Ammonium nitrate	NH_4NO_3	1 mM
Tetrahydrate calcium nitrate	$Ca(NO_3)_2 \cdots 4H_2O$	2 mM
Ferric sodium ethylenediaminetetraacetic acid	FeNaEDTA	75 μM
Tetrahydrate manganese(II) chloride	$MnCl_2 \cdots 4H_2O$	7 μM
Zinc chloride	$ZnCl_2$	3 μM
Pentahydrate copper(II) sulfate	$CuSO_4 \cdots 5H_2O$	0.8 μM
Boric acid	H_3BO_3	1.6 μM
Dihydrate sodium molybdate	$Na_2MoO_4 \cdots 2H_2O$	0.83 μM

The length of the primary roots was measured after five days of exposure and root elongation (RE) was calculated as follow:

$$RE = \frac{REt}{REc} \times 100$$

where REt is the elongation of root length in the test medium and REc is the elongation of root length in the control. EC$_{50}$ of cadmium, copper and zinc were determined as 80 μM, 50 μM and 570 μM respectively. Roots from non-treated plants and roots under EC$_{50}$ concentration were rinsed once in 0.5 M EDTA (pH 8.0) and twice in deionized water. Further they were immediately frozen in liquid nitrogen and stored in −80 °C until RNA extraction.

3.3. RNA isolation, library preparation and RNA sequencing

Three plants grown in heavy metals concentrations that correspond to established EC$_{50}$ values and three control plants were used for each of the three treatments. Total RNA was extracted separately from roots and shoots of each plant using NucleoSpin RNA Plant kit (Macherey-Nagel) after their storage in liquid nitrogen. The amount and integrity of the extracted RNA was determined by NanoDrop 1000 spectrophotometer (Thermo Scientific) and visually after electrophoresis on a 1.2% agarose gel containing ethidium bromide. In total 36 RNA samples (2 μg each) were provided to Genomics Core Facility Center (EMBL Heidelberg) for construction of cDNA libraries with poly(A) + selection and sequencing. Sequencing libraries were prepared using a TruSeq RNA Sample preparation Kit v2 (Illumina, San Diego, CA, USA), according to manufacturer's protocol. Sequencing libraries were

Table 3

Sequencing results for cadmium, copper and zinc experiments. Number of reads is the sum of three replicates of sequencing.

Experiment	Treatment	Tissue	Number of reads
Copper	Control	Shoots	21,539,100
Copper	Control	Roots	15,779,712
Copper	Treated	Shoots	10,739,460
Copper	Treated	Roots	12,812,092
Zinc	Control	Shoots	10,939,912
Zinc	Control	Roots	11,651,650
Zinc	Treated	Shoots	12,624,212
Zinc	Treated	Roots	12,631,846
Cadmium	Control	Shoots	10,483,926
Cadmium	Control	Roots	10,245,240
Cadmium	Treated	Shoots	12,137,818
Cadmium	Treated	Roots	13,186,234

pooled in equimolar concentration and sequenced on an Illumina HiSeq 2000, producing 2×50-nucleotide paired-end reads. In total about 155 million reads were sequenced (Table 3). All RNA-Seq generated for this project have been deposited in the National Center for Biotechnology Information (NCBI) under the BioProject accession number PRJNA382490.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgement

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