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Genomics Data

Data in Brief

Transcriptomic profiling of Arabidopsis gene expression in response to varying micronutrient zinc supply

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ARTICLE INFO

ABSTRACT

Deficiency of the micronutrient zinc is a widespread condition in agricultural soils, causing a negative impact on crop quality and yield. Nevertheless, there is an insufficient knowledge on the regulatory and molecular mechanisms underlying the plant response to inadequate zinc nutrition [1]. This information should contribute to the development of plant-based solutions with improved nutrient-use-efficiency traits in crops. Previously, the transcription factors bZIP19 and bZIP23 were identified as essential regulators of the response to zinc deficiency in Arabidopsis thaliana [2]. A microarray experiment comparing gene expression between roots of wild-type and the mutant bzip19 bzip23, exposed to zinc deficiency, led to the identification of differentially expressed genes related with zinc homeostasis, namely its transport and plant internal translocation [2]. Here, we provide the detailed methodology, bioinformatics analysis and quality controls related to the microarray gene expression profiling published by Assunção and co-workers [2]. Most significantly, the present dataset comprises new experimental variables, including analysis of shoot tissue, and zinc sufficiency and excess supply. Thus, it expands from 8 to 42 microarrays hybridizations, which have been deposited at the Gene Expression Omnibus (GEO) under the accession number GSE77286. Overall, it provides a resource for research on the molecular basis and regulatory events of the plant response to zinc supply, emphasizing the importance of Arabidopsis bZIP19 and bZIP23 transcription factors.

Specifications

Organism/cell line/tissue	A. thaliana Col-0
Sex	N/A
Sequencer or array type	Affymetrix GeneChip Arabidopsis ATH1 Genome Array
Data format	Raw
Experimental factors	Arabidopsis T-DNA insertion double mutant bzip19 bzip23 vs. Wild-type control; shoot vs. root; deficient vs. sufficient vs. excess zinc supply
Experimental features	Transcriptomic profiling of Arabidopsis bzip19 bzip23 gene expression in response to varying micronutrient zinc supply
Consent	N/A
Sample source location	N/A

1. Direct link to deposited data

Deposited data can be found in the Gene Expression Omnibus (GEO) database: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE77286

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2. Materials and methods

2.1. Sample preparation

A. thaliana ecotype Columbia (Col-0), and an Arabidopsis T-DNA insertion double mutant (bzip19 bzip23) [2], were grown in a climate chamber with a long day photoperiod (16 h light at 22 °C, 8 h dark at 20 °C), at 120 μmol photon m^{-2} s^{-1}, and 50% relative humidity. Before germination, seeds were subjected to a 3-day stratification treatment in a cold room at 4 °C, in the dark, to promote uniform germination. Seeds were sown onto 0.55% (w/v) agar-filled microtubes, and grown in hydropony on a modified half-strength Hoagland's nutrient solution [3] containing 2 μM $ZnSO_4$ (Zn+). The hydroponic system consisted of 8-L-capacity containers ($46 \times 31 \times 8$ cm), with a non-translucent 3-mm-thick plastic lid containing holes for placement of agar-filled tubes. Three plants were grown in each container. The nutrient solution was replaced once in the first week, and twice a week in weeks thereafter. The two genotypes, i.e., wild-type (Col-0) and double mutant (bzip19 bzip23), were grown for three weeks with normal zinc supply (Zn+, 2 μM $ZnSO_4$) and then one week with low (Zn-, 0.05 μM $ZnSO_4$), normal (Zn+) or excess zinc
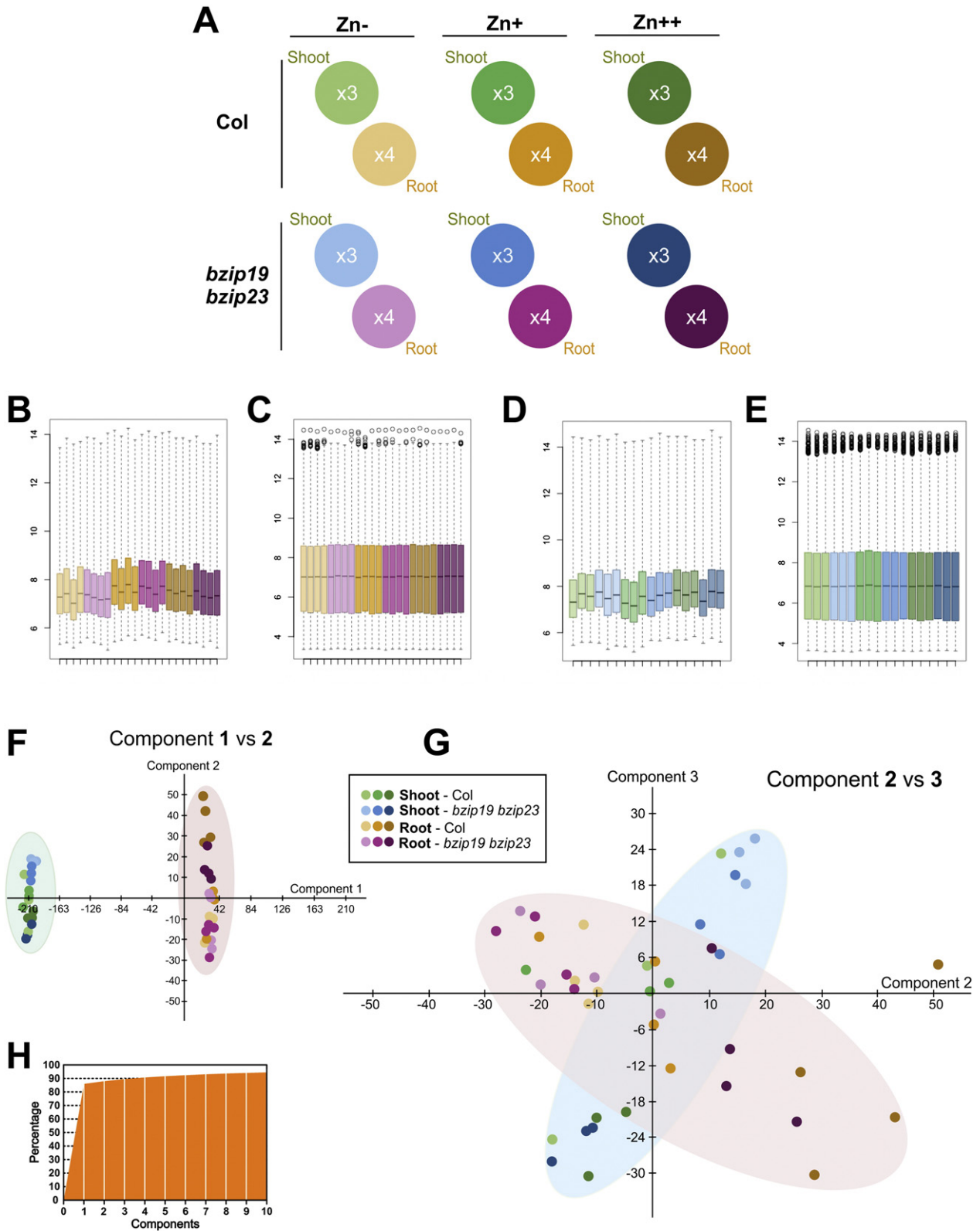
Fig. 1. Microarray analysis of the transcriptomic profile of the Arabidopsis T-DNA insertion double mutant *bzip19 bzip23*, in relation to the wild-type Col-0 ecotype, exposed to sufficient (Zn +), deficient (Zn-) or excess (Zn ++) zinc supply, in both shoots and roots. A. Experimental design summarizing the three study variables (zinc supply, genotype and tissue type), as well as the number of replicate hybridizations performed for each experimental condition. B–E. Boxplot analysis of expression (intensity) values for all genes in each hybridization. Root tissues prior to (B) or after (C) quantile normalization of intensity values. Shoot tissues prior to (D) or after (E) quantile normalization of intensity values. Box represents all values between percentile 25 and 75 and the central line in the box indicates the median. Interval represents minimum and maximum values excluding outliers. F,G. Principal component analysis (PCA), displaying variability of each microarray hybridization in components 1 and 2 (F) or 2 and 3 (G). H. Accumulative percentage of variability (Eigen values) explained by the first 10 components of the variance analysis.

supply (Zn + +, 25 μM ZnSO₄). For each genotype and treatment (Zn−/Zn+/Zn++), roots or shoots of six plants (two containers) were pooled. This pool corresponded to each single biological replicate. Plant tissue was snap frozen into liquid nitrogen and stored at −80 °C.

2.2. RNA extraction, labeling and hybridization

RNA was extracted with the RNeasy Plant Mini kit (Qiagen). For cDNA synthesis, 1 μg of total RNA was used as starting material. Targets were prepared with the one-cycle cDNA synthesis kit followed by biotin-labeling with the IVT labeling kit (GeneChip One-cycle target labeling and control reagents; Affymetrix), and hybridized to the ATH1 gene chip for 16 h, as recommended by the supplier (Gene expression analysis manual; Affymetrix). Four and three hybridizations were performed for independent biological replicates of the root and shoot material, respectively.

2.3. Data normalization and analysis

Raw data files were processed in Bioconductor/R using packages Affy [4] and Limma [5]. Background correction and quantile normalization were performed using the Robust Multichip Average (RMA) function. Principal component analysis (PCA) was conducted using Multi Experiment Viewer v4.0 (www.tm4.org/mev.html).

3. Results and discussion

Knowledge on of the molecular network modulating the response to zinc nutrition in the *Arabidopsis* plant model system is a timely subject, and will contribute to the development of plant-based solutions addressing nutrient use efficiency and adaptation to nutrient-limited or toxic soils [1,6–8]. Previously, the transcription factors bZIP19 and bZIP23 were identified as essential regulators of the response to micronutrient zinc deficiency in Arabidopsis [2]. Here, we describe a microarray dataset that provides a comparative analysis between Arabidopsis wild-type and the *bzip19 bzip23* double mutant, the latter displaying a hypersensitive response to zinc deficiency [2]. The experimental conditions include analysis of root and shoot tissues, and plant growth in varying zinc supply, i.e., deficient, normal and excess zinc (Fig. 1A). This dataset extends significantly on the previously reported transcriptomics study [2], by including shoot tissue analysis and the expression profile under sufficient and excess zinc supplementation. The quality of the dataset was demonstrated by the homogenous distribution of spot intensity values across replicates and even between traits (Zn levels), as depicted by boxplot graphs in roots (Fig. 1B,C) and shoots (Fig. 1D,E), where the effectiveness of the quantile normalization can be checked as well. Subsequently, we used PCA to analyze variance in the datasets for each of the 42 hybridizations, allowing us to infer on 1) quality of the datasets, 2) length of transcriptional rearrangements given the three working variables (zinc supply, genotype, tissue type) (Fig. 1F–H). Concerning the former, replicates within the 12 experimental conditions clearly clustered together across

PCA components 1–3, demonstrating the quality of hybridization and data normalization procedures, and ensuring the biological significance of future differential expression analysis on these datasets. Regarding the latter, results demonstrate that variance in component 1 clearly reflects root vs. shoot datasets (Fig. 1F), with at least 85% of gene expression variability being attributed to this variable (Fig. 1H). Even though components 2 and 3 still demonstrate the weight of the tissue type variable on transcriptional data, they also resolve datasets across a gradient of zinc supply, indicating that this is the second most significant variable affecting transcription variance (Fig. 1G). The impact of the *bzip19 bzip23* mutant genotype on transcription is therefore likely to be less extensive than remaining variables, which is foreseeable given that a reduce number of genes seems to be transcriptionally regulated by bZIP19/23 [2].

This dataset is a resource that can provide valuable information, generate extensive differential expression comparisons, or complement results of other experiments. Ultimately, it can contribute to a better understanding of the regulatory network modulating the response to zinc nutrition in Arabidopsis. In addition, it should provide further insight onto the particular roles that bZIP19 and bZIP23 transcription factors play in zinc homeostasis.

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