

Transcriptomic (RNA-seq) analysis of genes responsive to cadmium stress in *Theobroma cacao*

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INTRODUCTION

Dietary exposure to the heavy metal Cadmium (Cd) can lead to several health problems. At significant levels, Cd has been detected in cacao beans (*T. Cacao*) grown in South American countries, including Colombia. Despite this, the mechanisms involved in the uptake, transport, and accumulation of Cd in cacao are not known. Excess exposure to Cd is highly toxic to plants, as it affects photosynthesis, growth, and the REDOX state of the plant. Studies reported differentially expressed genes (DEG) involved in metabolic pathways including photosynthesis, phenylalanine metabolism, ribosomes, flavonoids, cell wall biosynthesis, and membrane transporters, amongst others. These studies also reported differential expression of metal ion transporters. Primarily, upregulation of transporters of Zn and Fe such as HMA2 genes, ZIP family, NRAMP, and YSL. In this work, we explore how expression changes of functionally annotated genes follow changes in metal accumulation in cacao leaves and roots when exposed to Cd, and how these can be used to identify genes and mechanisms by which this happens in cacao.

MATERIALS AND METHODS

A Cd low accumulator cacao genotype, TSH 660, and a high accumulator, PA 121, were grown in a hydroponic platform and exposed to 10 ppm Cd in the hydroponic media to investigate Cd uptake mechanisms. Accumulation levels in leaves and roots were measured as well as the effect on gene expression using transcriptome sequencing.

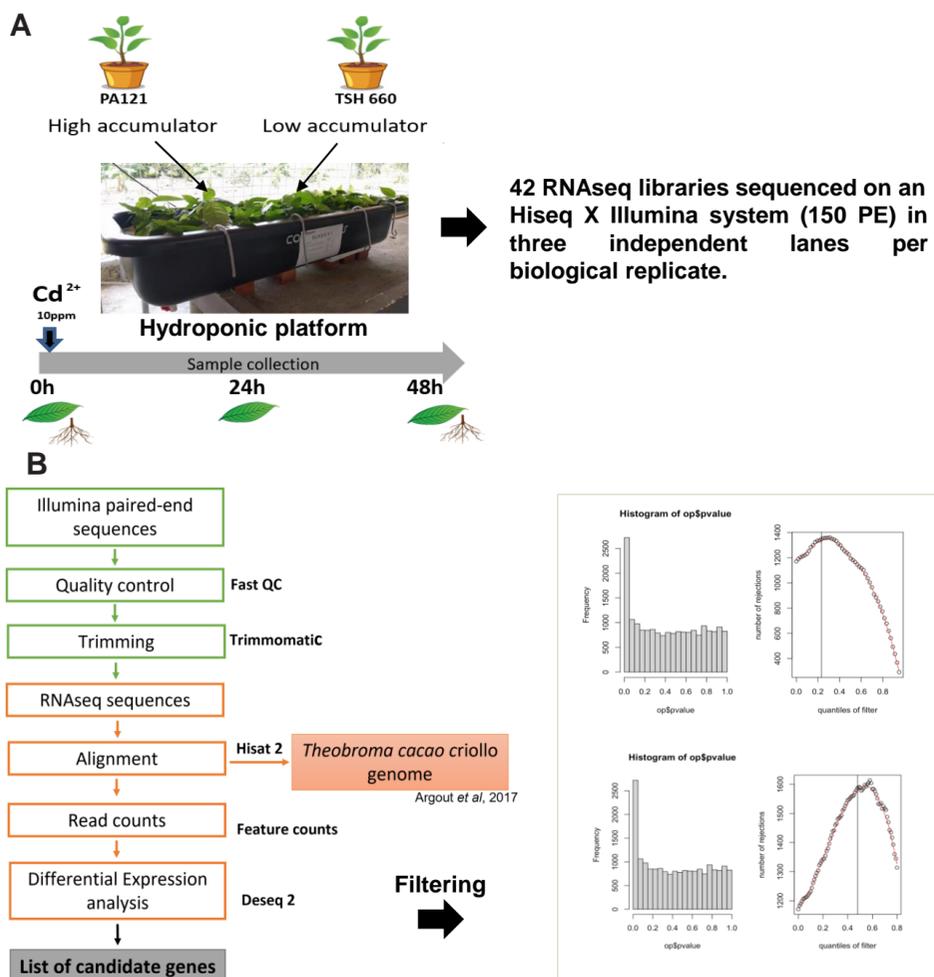


Figure 1. Transcriptomic analysis of genes responsive to cadmium stress in cacao. **A.** Experimental design hydroponic platform. **B.** Bioinformatic workflow to RNAseq assay.

RESULTS

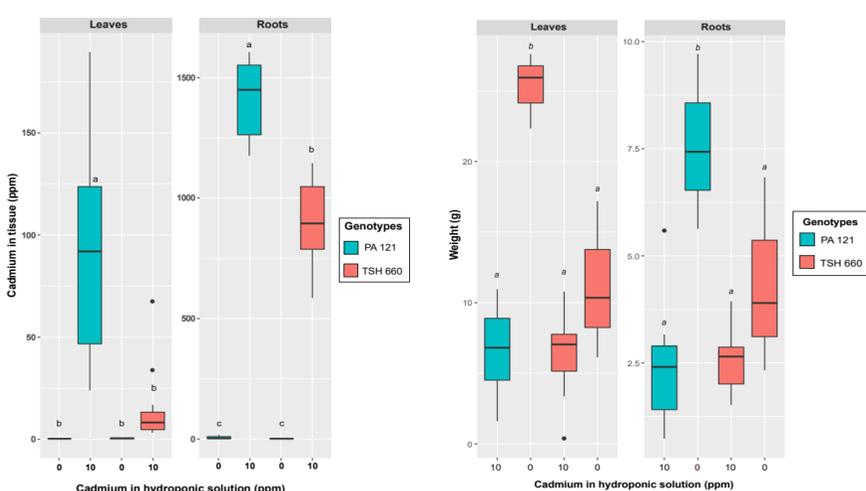


Figure 2. Phenotype data of plants grown in a hydroponic platform with 10 ppm of Cadmium. **A.** Cadmium concentration in both tissues. **B.** Weight of leaves and roots after cadmium treatment.

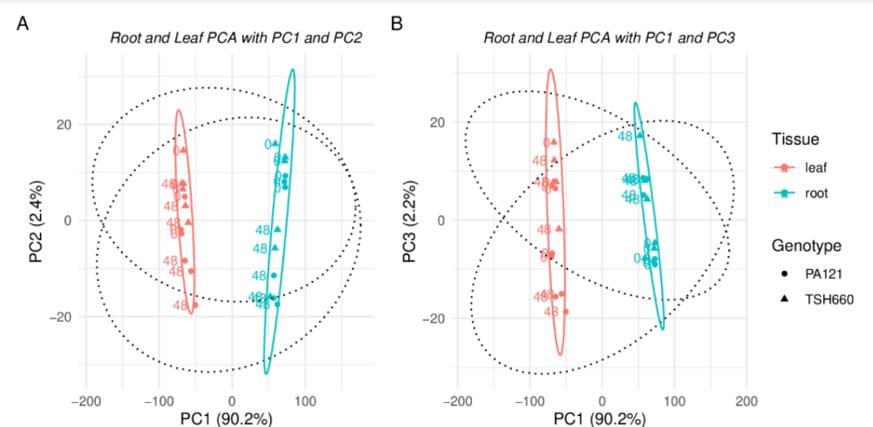


Figure 3. Principal Component Analysis of the transcriptome of two cacao genotypes in response to Cd treatment. The colors of the data point indicate tissue type, TSH660▲ and PA121●.

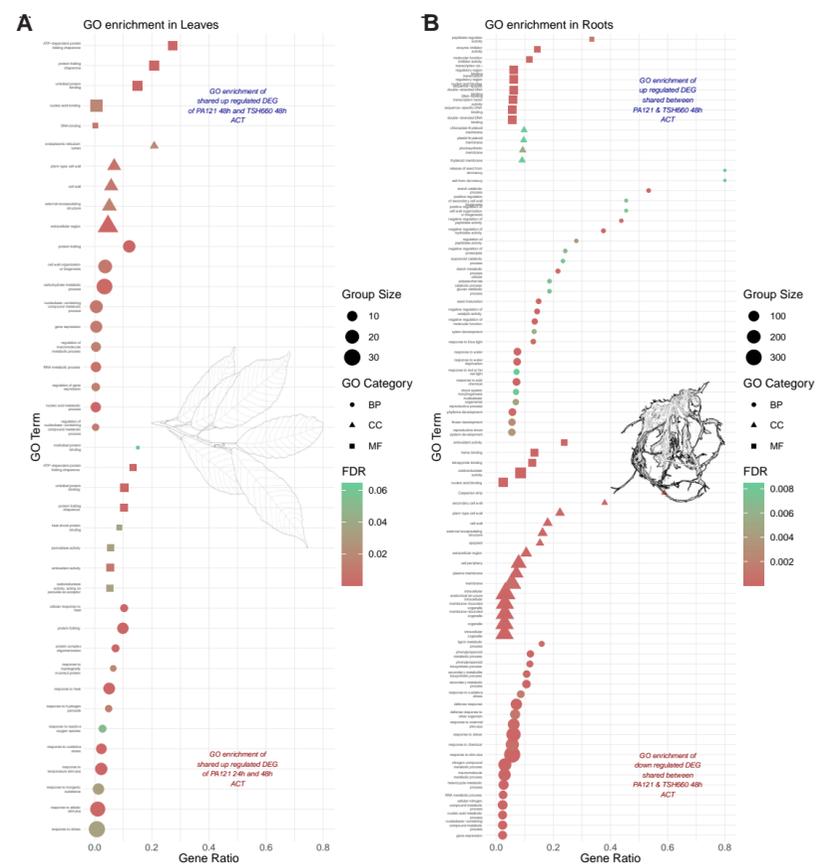


Figure 4. Enriched GO terms in DEG shared between genotypes. **A.** Leaves **B.** Roots

CONCLUSIONS

We identified a contrasting response between two genotypes in a hydroponic platform of cadmium uptake at the phenotypic level. The weight tissue of PA 121 was significantly different between treatments in both tissues after cadmium treatment. The GO terms showed in the leaves a higher number of expressed differential genes related to the stress response, abiotic stimulus, and carbohydrate metabolic process in both genotypes. Functions such as ion transport, cell wall biosynthesis, and lignification were strongly associated with Cd treatment. The genes found in this study could be an essential tool for the genetic improvement of the species to generate new varieties with low cadmium absorption, which will allow in the future to reduce costs in cocoa production and guarantee a cadmium-free cocoa that does not affect human health.

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