

Comparative Genomics of Zn Homeostasis in *Arabidopsis thaliana* and *Thlaspi caerulescens*

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INTRODUCTION

Thlaspi caerulescens is a natural Zn, Cd and Ni hyperaccumulator, closely related to *Arabidopsis thaliana*, and a model plant species to study metal hyperaccumulation and to identify Zn homeostasis genes based on their differential regulation in both species. We addressed the molecular genetic regulation of hyperaccumulation using genetic tools available for *Arabidopsis*.

METHODS

To identify genes involved in the response to differences in Zn supply, we examined transcription of *Arabidopsis* and *T. caerulescens* genes in roots of plants grown under deficient, sufficient and excess Zn, using Agilent 3 *Arabidopsis* long oligonucleotide microarrays. *Arabidopsis* T-DNA insertion K.O. and overexpression mutants were examined for phenotypic alterations related to metal tolerance and metal accumulation. Promoters of identified metal homeostasis genes were cloned and their sequence compared.

RESULTS AND DISCUSSION

Arabidopsis and *T. caerulescens* responded differently when exposed to increasing Zn concentrations in terms of the Zn content in their roots and leaves (Fig 1). While *Arabidopsis* kept the leaf Zn content low even at high Zn exposure, *T. caerulescens* hyperaccumulated Zn in leaves already at low concentrations. For Fe in leaves, there was not much difference between both species.

A total of 608 significant Zn-responsive genes with at least a 3-fold difference in expression were detected in *Arabidopsis* and 352 genes in *T. caerulescens*. We found over 2200 genes significantly higher expressed (> 5-fold) in *T. caerulescens* compared to *Arabidopsis* in response to Zn exposure. Several Zn homeostasis genes were constitutively expressed in *T. caerulescens* compared to *Arabidopsis*. Interestingly, exposure of *Arabidopsis* to excess Zn induced the expression of genes typically involved in Fe uptake, suggesting an effect on Fe homeostasis not seen in *T. caerulescens*. Further confirmation of Fe-homeostasis related genes was obtained by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) analysis. It showed clearly that *IRT1*, the major Fe uptake transporter in plants, is much lower expressed in *T. caerulescens* than in *Arabidopsis*, and it also showed very different expression profiles for Nicotianamine Synthase (*NAS*) genes in both species.

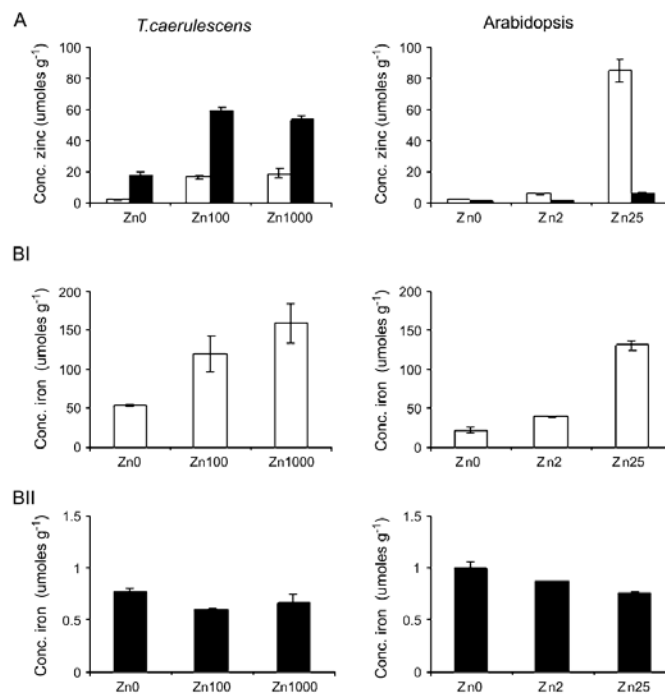


Fig 1: Zinc (A) and Fe (BI: roots; BII: leaves) content of *T. caerulescens* and *Arabidopsis* roots (white bars) and leaves (black bars) of plants grown for 3 weeks in 0.5 x Hoagland's solution before 1-week exposure to deficient, sufficient and excess Zn (0/0 μ M, 100/2 or 1000/25 μ M ZnSO₄, respectively) (van de Mortel et al. 2006).

Currently we are studying the function relevance of the differential expression of ZIP-like Zn transporters *ZNT1*, 2, 5 and 6; the vacuolar *ZTP1* cation diffusion facilitator; the four nicotianamine synthases (*NASI*, 2, 3, 4) and several transcriptional regulators. The overexpression of *NAS* genes induced metal tolerance, especially to Ni, but did not alter accumulation. The 35S::*ZTP1* transgenic plants showed more tolerance to excess Zn than a wild type. Heterologous promoter-GUS gene fusions were used to understand the difference in Zn responsive regulation between both species.

CONCLUSIONS

Thlaspi caerulescens is a useful model species to understand plant Zn homeostasis. In a comparative genomics analysis with *Arabidopsis*, both species are instrumental for the identification and functional analysis of genes relevant to the process.

REFERENCES

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