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## Nickel hyperaccumulation in *Thlaspi caerulescens*: a rare micro-evolutionary event

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2009

### **document version**

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### **citation for published version (APA)**

Richau, K. H. (2009). *Nickel hyperaccumulation in Thlaspi caerulescens: a rare micro-evolutionary event*.

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**Summary****Nickel hyperaccumulation in *Thlaspi caerulescens*: a rare micro-evolutionary event**

Heavy metal hyperaccumulation is a comparatively rare trait in the plant kingdom. Although progress has been made, the physiological and molecular basis, as well as the ecological function of the phenomenon is far from being well understood yet. Enhanced capacities for root uptake, root to shoot translocation and sequestration of metals in leaves are the major characteristics of hyperaccumulators. *Thlaspi caerulescens*, a Zn/ Cd/ Ni hyperaccumulator, offers excellent opportunities to study the genetics and physiological mechanisms underlying these traits, since it exhibits a distinct intraspecific variability in metal accumulation, translocation and tolerance. Furthermore, the high degree of DNA sequence identity (89% in coding sequences) between *T. caerulescens* and the plant genetics model, *Arabidopsis thaliana*, presents good opportunities to utilize the molecular tools and genomic information available for *A. thaliana*.

The present work was undertaken to obtain a better understanding of the heavy metal hyperaccumulation and tolerance traits and their interrelationships. We compared plants from two contrasting *T. caerulescens* accessions, one from the serpentine area of Monte Prinzera (MP) in northern Italy and a Belgian calamine accession, La Calamine (LC). A single MP x LC cross was used to study the genetic correlation between Ni and Zn accumulation and between Ni accumulation and Ni tolerance. Therefore, parental accessions as well as F<sub>3</sub> and F<sub>4</sub> progeny of the interaccession cross were phenotyped. The phenotypic distributions for Zn and Ni accumulation of the parental populations were non-overlapping, with MP having higher foliar metal

concentrations than LC. Ni tolerance was also higher in MP, but the parental distributions here were overlapping. The F<sub>3</sub> and F<sub>4</sub> progeny exhibited a clear segregation for the Ni and Zn accumulation trait as well as for Ni tolerance. Variance and covariance analysis of the F<sub>3</sub> progeny demonstrated significant heritability values ( $h^2$ ) for Ni and Zn foliar accumulation (0.70 and 0.59, respectively) and Ni tolerance (0.47), as well as a significant positive genetic correlation between the foliar accumulation of Ni and Zn ( $r_A^2 = 0.77$ ). However, Ni tolerance and Ni accumulation were uncorrelated. Regressing the F<sub>4</sub> family means on the F<sub>3</sub> parent values yielded similar estimates for the heritabilities of Ni and Zn accumulation in the leaves (0.66 and 0.55, respectively)

Even though in previous studies histidine has been implicated to play an important role in Ni hyperaccumulation, its precise function remained elusive. Therefore, we investigated the role for histidine in plant-internal metal transport, both at the levels of root to shoot translocation and tonoplast transport, between calamine, serpentine and non-metallicolous accessions of *T. caerulescens* and the non-hyperaccumulating non-metallophyte congeneric species, *T. arvensis*. We compared (1) root and shoot histidine concentrations in plants grown with and without Ni in the nutrient solution (2) Ni tonoplast transport in energized root- and shoot-derived tonoplast vesicles, with Ni supplied as free Ni, Ni-citrate or Ni-histidine, (3) the effect of exogenous histidine supply on Ni xylem loading, along with, (4) the distribution of Ni over root segments and root tissues. Within the present study we show accumulation of Ni in mature root cortical cells of *T. arvensis* and a high-Ni-accumulating *T. caerulescens* accession, but not in low-accumulating *T. caerulescens* accessions. Compared to *T. arvensis*, the concentration of free histidine in *T. caerulescens* was 10-fold enhanced in roots, but only slightly higher in leaves, regardless of Ni exposure. Ni uptake in MgATP-

energized root- and shoot-derived tonoplast vesicles was almost completely blocked in *T. caerulescens*, but uninhibited in *T. arvense*, when Ni was supplied as a 1:1 Ni-His complex. Exogenous histidine supply enhanced Ni xylem loading in *T. caerulescens* but not in *T. arvense*. Therefore we conclude that the high rate of root to shoot translocation of Ni in *T. caerulescens* as compared to *T. arvense* depend on the combination of two distinctive characters, i.e. a greatly enhanced root histidine concentration and a strongly decreased ability to accumulate histidine-bound Ni in root cell vacuoles.

We also carried out some transcriptome comparisons between *T. caerulescens* accessions and F<sub>4</sub> lines with contrasting tolerance and accumulation characteristics derived from the LC x MP cross, with the aim to find candidate genes responsible for the intraspecific differences in Zn and Ni accumulation and Ni tolerance. To this end we used the full genome Arabidopsis Agilent3 array. In general, only a few genes appeared to be more than 3-fold differentially expressed, among which not a single metal homeostasis-related gene. It seems that the high number of metal homeostatic genes that are differentially expressed in cross-species comparisons between hyperaccumulators and non-hyperaccumulators, are expressed at similar levels in the LC and MP *T. caerulescens* accessions, in spite of the strongly different phenotypes for Ni and Zn accumulation. These differences are possibly due to post-translational regulation or structural alterations of metal processing proteins. Alternatively, it is conceivable that fairly subtle differences in transcript levels may have rather drastic phenotypic effects.