Nickel Hyperaccumulation in *Thlaspi goesingense*

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Aim

By comparing the Ni hyperaccumulator *Thlaspi goesingense* Hálácsy / Brassicaceae (Fig 1) and the non-accumulator *Thlaspi arvense* L., ***we aim to dissect the underlying biochemical and molecular mechanisms involved in Ni hyperaccumulation.***

Introduction

Hyperaccumulator species provide a unique model for the study of metal physiology in higher plants. A better understanding of these processes should stimulate the development of **phytoremediation** - the use of plants to remove pollutants, including toxic metals, from the environment.

• Using various physiological methods we have been able to demonstrate that the Ni hyperaccumulation phenotype in *T. goesingense* is determined by Ni resistance and not enhanced Ni transport to shoots (Fig 2, 3) (Krämer et al., 1997). Vacuolar compartmentalization of Ni, as a Ni-citrate complex, appears to play a central role in this Ni tolerance mechanism (Fig 4, 5) (Krämer et al., in preparation).

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• Recent evidence also suggests that free histidine, a good Ni chelate, is involved in the mechanism of Ni hyperaccumulation in the genus *Alyssum* / Brassicaceae (Krämer et al., 1996). We are investigating whether in *T. goesingense* activity of key enzymes involved in histidine biosynthesis are altered in response to Ni, focusing on the enzymes involved in the 1st, 5th and the final steps of histidine biosynthesis (Fig 6, 7).

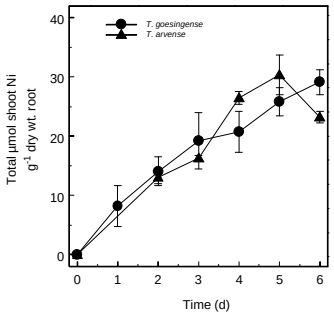


Fig 2. Ni uptake into shoots of *T. goesingense* and *T. arvense*.
Nickel uptake is expressed as the amount of shoot Ni taken up per unit root dry biomass. Statistical analysis by two-way ANOVA indicated that there was no significant difference in the total amounts of Ni transported to the shoot per unit root dry biomass between the two species.

Fig 1. *Thlaspi goesingense* growing in its native ultramafic habitat near Redschlag/Austria.
Nickel concentrations in the leaves of *T. goesingense*, as analyzed on site by X-ray fluorometry, were 93.3 ± 16.8 µmol Ni g^{-1} dry biomass (SE; *n*=6), more than 3 times higher than the Ni concentrations measured in the soil surrounding the roots of these plants of 24.5 ± 2.4 µmol Ni g-1 (SE; *n*=6), and 20 times higher than in leaves of non-accumulator species growing near by on the same site.

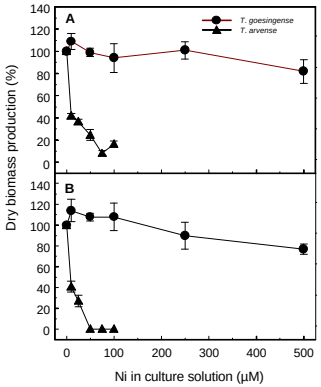


Fig 3. Ni tolerance of *T. goesingense* and *T. arvense*
Tolerance was expressed as a percentage of dry biomass production of shoots (A) and roots (B) relative to the respective controls grown in the absence of Ni. Statistical analysis by one-way ANOVA, indicated that biomass production was not significantly reduced at any Ni concentration in *T. goesingense*, but was significantly reduced at the lowest Ni concentration of 10 µM in *T. arvense* ($P < 0.001$).

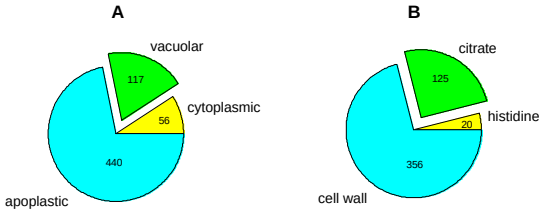


Fig 4. Cellular distribution and speciation of Ni in *T. goesingense* shoots. (**A**). *T. goesingense* were exposed to 10 µM Ni for 7 days. Shoots were harvested and the tissue fractionated by isolating protoplasts and intact vacuoles. Ni concentrations in each compartment are given as nmol Ni g^{-1} fresh biomass. (**B**). The coordination environment of shoot Ni was determined in vivo using X-ray absorption spectroscopy. Concentration of each Ni species given as nmol Ni g^{-1} fresh biomass.

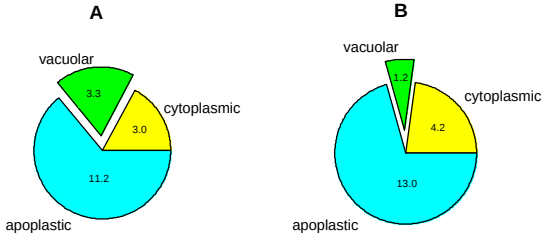
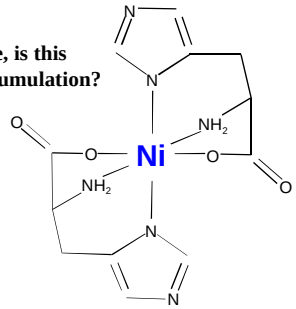


Fig 5. Comparison between the cellular localization of Ni in *T. goesingense* and *T. arvense* after 1d exposure to 1 µM Ni. Cellular distribution of Ni in *T. goesingense* (**A**) and *T. arvense* (**B**). Ni concentration given as nmol Ni g^{-1} fresh biomass. *T. goesingense* vacuoles contained 2.75 time more Ni than vacuoles from *T. arvense*, even though the total cellular Ni concentrations were equal in both species.

Ni chelation by histidine, is this involved in Ni hyperaccumulation?



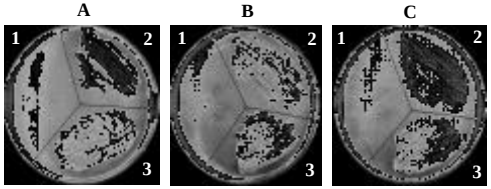


Fig 6. Three key histidine biosynthetic genes cloned from *T. goesingense* by functional complementation in *E. coli*. Three *T. goesingense* cDNA's was isolated by screening a λ-triplex cDNA library for complementation of *HisG*⁻ (**A**), *HisB*⁻ (**B**) and *HisD*⁻ (**C**) mutations in *E. coli*. The 3 clones represent ATP phosphoribosyl transferase (THG1, accession no. AF003347), imidazolglycerol phosphate dehydratase (THB1, accession no. AF023140), and histidinol dehydrogenase (THD1, accession no. AF023141). Each plate contains the following; (1) Appropriate mutant unable to grow in the absence of histidine; (2) Mutant transformed with complementing *T. goesingense* cDNA; (3) Wild-type TOPP10F` *E. coli* strain.

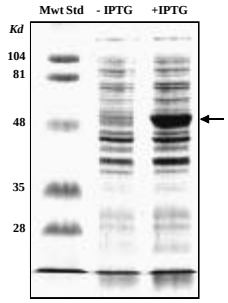


Fig 7. SDS-PAGE gel of total protein from *E. coli* transformed with the pET-30 expression vector containing the THG1 gene. Cells were induced at 22ºC with 0.4 mM IPTG for 3 h, resulting in expression of the THG1 gene and accumulation of the gene product (see arrow).

Conclusions and Future Work

• Ni hyperaccumulation from hydroponics is governed by Ni tolerance.

• Vacuolar accumulation of Ni plays an important role in Ni tolerance

• Histidine biosynthetic genes will be used to probe the role of free histidine in Ni hyperaccumulation

• The cDNA library will be screened in yeast and E. coli for cDNA's involved in Ni tolerance.

References

Krämer U, Cotter-Howells JD, Charnock JM, Baker AJM, Smith JAC (1996) Free histidine as a metal chelator in plants that accumulate nickel. Nature **379**: 635-638.

Krämer U, Smith RD, Wenzel WW, Raskin I, Salt DE (1997) The role of metal transport and tolerance in nickel hyperaccumulation by *Thlaspi goesingense* Hálácsy. Plant Physiol **115**: 1641-1650.