

## Commentary

# Casting a wide cross-species transcriptomics net: convergent evolution of nickel hyperaccumulation

Nearly 800 plant species are able to accumulate certain metals or metalloids in their shoots to levels two- to three-orders of magnitude higher than normally found in other plants in the same habitat (Reeves *et al.*, 2018). This is an astounding trait since in plants, as well as in practically all other types of organisms, the normal physiological range of essential minerals such as zinc (Zn) – one of the hyperaccumulated metals – is rather narrow, with thresholds of deficiency and toxicity differing by only about a factor of 10 (Krämer, 2010). Perhaps even more amazing, hyperaccumulated elements include cadmium (Cd) and arsenic (As), environmental pollutants with no known biological function and highly toxic to practically all organisms. Thus, metal hyperaccumulating plants possess metal hypertolerance too, meaning they can thrive in the presence of soil metal concentrations that far exceed the capacity of most other plant species to cope with metal/metalloid overload. Molecular mechanisms of metal hyperaccumulation and hypertolerance have attracted considerable interest mainly for three reasons. First, metal hyperaccumulators are attractive models to understand the evolutionary processes underlying adaptation to harsh environments. Second, analysis of an extreme deviation can identify key nodes in the metal homeostasis network, which accomplishes the proper distribution of essential minerals to thousands of metal-requiring proteins and other target sites in plants. Third, the knowledge gained could potentially be applied to engineer biofortified crops enriched in micronutrients and to promote the use of hyperaccumulators for the phytoremediation of metal-contaminated sites or the phytoextraction (phytomining) of valuable metals. By far the most common hyperaccumulated metal is nickel (Ni), probably because Ni is highly abundant in the Earth's crust and Ni-rich serpentine soils therefore occur frequently. Still, the mechanistic understanding of Ni hyperaccumulation has been lagging behind Zn and Cd hyperaccumulation (Van der Pas & Ingle, 2019). In this issue of *New Phytologist*, García de la Torre *et al.* (2021; pp. 994–1006) report two important advances. The ability to hyperaccumulate metals or metalloids is found in dozens of families scattered across the phylogenetic tree, a pattern reminiscent of other complex adaptations including  $C_4$  photosynthesis and Crassulacean Acid

Metabolism (Edwards, 2019). Thus, metal hyperaccumulation most likely evolved many times independently, raising the fascinating biological question as to whether convergent evolution selected similar genetic changes that enable hyperaccumulation (Verbruggen *et al.*, 2013). García de la Torre *et al.* addressed this by performing transcriptome studies on pairs of related hyperaccumulator and nonhyperaccumulator species from a wide range of families. Their data suggest that, indeed, convergent mechanisms exist. Furthermore, for one of the candidate mechanisms apparently shared by phylogenetically distant Ni hyperaccumulators, they obtained direct evidence by genetic intervention, a first in Ni hyperaccumulation research.

---

*‘... for one of the candidate mechanisms apparently shared by phylogenetically distant Ni hyperaccumulators, they obtained direct evidence by genetic intervention, a first in Ni hyperaccumulation research.’*

---

### Cross-species transcriptome analyses

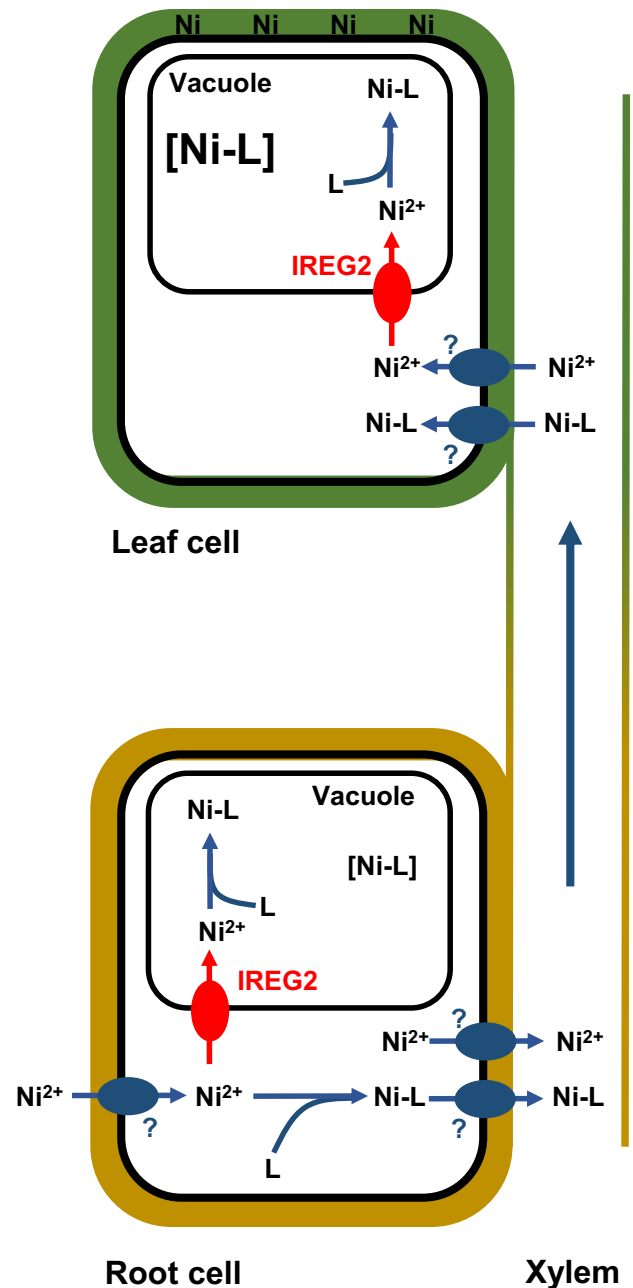
With the exception of two *Sedum* species, *Sedum alfredii* and *Sedum plumbizincicola*, most molecular research on metal hyperaccumulation has, to date, been performed in the two Brassicaceae models *Arabidopsis halleri* and *Noccaea caerulea*. A combination of comparative transcriptome studies, quantitative trait locus (QTL) analyses in progeny of crosses between hyperaccumulating and nonhyperaccumulating species or accessions, and targeted RNA interference experiments established a general scheme of Zn and Cd hyperaccumulation (Verbruggen *et al.*, 2009; Krämer, 2010; Corso & Torre, 2020). A key feature is the altered expression of several metal homeostasis genes encoding metal transporters or enzymes involved in the synthesis of metal ligands. Many of the identified changes, including the elevated expression of ZIP (*Zrt1/IRT1*-related proteins) transporter genes, HMA (*Heavy metal ATPase*) genes and NAS (*nicotianamine synthase*) genes, were found in both model systems. This suggests convergent evolution of hyperaccumulation at least within the Brassicaceae family. García de la Torre *et al.* cast a much wider net. They sampled leaves of Ni hyperaccumulators from five distinct families, growing on serpentine soils in France, Cuba and New Caledonia, the latter two representing biodiversity hotspots where many endemic Ni hyperaccumulating species are found. For comparison, related nonhyperaccumulator species or accessions were collected in the

This article is a Commentary on García de la Torre *et al.* (2021), pp. 994–1006.

same areas. RNA was sequenced and from the resulting reads, reference transcriptomes were generated and annotated. In order to allow functional comparison across lineages, clusters of orthologous groups (COGs) were inferred that contain orthologous genes differentially expressed between related hyperaccumulator and nonhyperaccumulator species in at least three different families. Altogether 26 such COGs were identified. The most robustly up-regulated COG among them corresponds to ferroportin (FPN)/IREG (iron-regulated) transporters. They are named after the only known cellular iron (Fe) export system in mammals, FPN. In humans, it is a key component of Fe homeostasis, controlling the release of absorbed Fe from enterocytes into the plasma (Coffey & Ganz, 2017). Other known FPN substrates include Zn, cobalt (Co) and Ni. FPN/IREG transporters had been implicated in Ni hyperaccumulation before as they were identified in intra-specific comparisons to be constitutively up-regulated in Ni hyperaccumulating accessions relative to nonhyperaccumulating accessions of *N. caerulescens* (Halimaa *et al.*, 2014) and *Senecio coronatus* (Meier *et al.*, 2018). García de la Torre *et al.* have now found strong evidence for a functional role of FPN/IREG genes across diverse families and thus for convergent evolution.

### The plant transformation challenge

Corroboration of a hypothesized gene function is most convincing when phenotypes of wild-type individuals can be compared to those of respective loss-of-function mutants. Whenever phenomena are studied that are not expressed by the model systems of molecular genetics, this calls for genetic interventions to obtain such mutants. A prerequisite for the use of RNA interference, artificial microRNAs or CRISPR-Cas is a successful plant transformation. The lack of reliable, highly efficient transformation protocols has, for a long time, hampered research on major crop plants and hyperaccumulators. The study by García de la Torre *et al.* now makes a significant step forward by developing a method to study gene function via knockdowns in a hyperaccumulator species. Thus far, few successful transformations of metal hyperaccumulating species have been reported (Liu *et al.*, 2017; Ahmadi *et al.*, 2018), none for *N. caerulescens*. Using *Rhizobium rhizogenes* root transformation, García de la Torre *et al.* obtained several lines of a Ni hyperaccumulating *N. caerulescens* accession carrying an amiRNA construct targeting the *IREG2* gene. This represents a major achievement, yet such lines are less informative than transformants with a stable knockdown of *IREG2* in leaves would be. Silencing of *IREG2* in roots caused a reduction of Ni accumulation. This is cautiously interpreted as evidence for IREG2-mediated vacuolar sequestration of Ni (Fig. 1), which is consistent with the demonstrated localization of IREG2 in the tonoplast and the transport activity for Ni ions shown by heterologous expression in yeast. Nickel accumulation in leaves, however, was not affected in the transgenic lines. Directly analysing the significance of elevated *IREG2* expression in leaves of diverse Ni hyperaccumulators, for example via targeted mutagenesis by CRISPR-Cas, will depend on further advances in plant transformation.



**Fig. 1** A hypothetical model of nickel (Ni) hyperaccumulation. Following uptake through unknown transporters possibly belonging to the ZIP family, Ni<sup>2+</sup> ions are chelated by low molecular weight ligands (L). IREG2 sequesters Ni in vacuoles of root and leaf cells. IREG2 expression is elevated in leaves of Ni hyperaccumulating species compared to related nonhyperaccumulator species. Root-expressed IREG2 possibly drives the uptake of Ni, explaining the strong correlation between *IREG2* transcript level and root Ni accumulation observed by García de la Torre *et al.* (2021; pp. 994–1006) in this issue of *New Phytologist*. Ligands are hypothesized to bind Ni not only in the cytosol but also in vacuoles (organic acids) and possibly the xylem. Cytosolic Ni-L complexes may reduce the rate of vacuolar sequestration in root cells. Several clusters of orthologous groups (COGs) up-regulated across a wide phylogenetic range of Ni hyperaccumulators suggest enhanced synthesis of specialized metabolites, functioning as (yet unidentified) low molecular weight Ni ligands. Other COGs indicate hyperaccumulator-specific cell wall modifications possibly related to Ni storage sites in the leaf apoplast. Nickel xylem loading and leaf uptake transporters are unknown.

## Convergent mechanisms besides FPN/IREG


The reference transcriptomes assembled by García de la Torre *et al.* represent a valuable resource for future studies. Some of those may be guided by other identified COGs containing genes differentially expressed in Ni hyperaccumulators. Several up-regulated COGs indicate a functional role of specialized metabolites such as flavonoids. An obvious hypothesis is that Ni hyperaccumulators synthesize higher concentrations of low molecular weight metal ligands. They are essential for different aspects of metal hyperaccumulation (Clemens, 2019; Van der Pas & Ingle, 2019). Following uptake into root cells, Ni ions have to be bound in the cytosol by molecules that are small enough to maintain symplastic mobility of Ni so that it can reach cells surrounding the xylem (Fig. 1). Inside the xylem, at least some of the Ni is assumed to be complexed with ligands too. Cytosolic ligands are then again needed in leaf cells. Inside vacuoles, Ni is probably associated with organic acids. Candidate Ni ligands, besides organic acids, include histidine and nicotianamine. However, available evidence suggests that their role is restricted to particular plant families (Van der Pas & Ingle, 2019). Thus, the existence of additional Ni ligands has to be postulated.

Another lead derived from the COG analysis points to specific cell wall modifications in Ni hyperaccumulators. Besides the vacuoles of leaf cells, the leaf apoplast is another storage site for the huge amounts of metal accumulated aboveground (Fig. 1), making alterations of the cation binding capacity of cell wall polymers a likely adaptation. Finally, the COG results highlight a possible driver of genetic variation selection acts upon. Tracing the molecular evolution of metal hyperaccumulation includes the identification of mechanisms underlying altered regulation of metal homeostasis and other genes. Copy number expansions and *cis*-regulatory mutations are known factors, exemplified by HMA4 and its crucial role in Zn and Cd hyperaccumulation (Hanikenne *et al.*, 2008). While comparable insights are missing for Ni hyperaccumulators, the identification of several COGs corresponding to class I retrotransposons suggests a possible reason for the propensity that some taxa may have to evolve metal hyperaccumulation. Ultimately, the degree of genetic convergence can only be determined by identifying the mutations and genomic changes causal for the higher expression of *FPN/IREG* genes in diverse Ni hyperaccumulators.

## Acknowledgements

Open access funding enabled and organized by Projekt DEAL.

## ORCID

Stephan Clemens  <https://orcid.org/0000-0003-0570-1060>

Stephan Clemens 

Department of Plant Physiology, University of Bayreuth,  
Bayreuth, D-95440, Germany  
(email: stephan.clemens@uni-bayreuth.de)

## References

- Ahmadi H, Corso M, Weber M, Verbruggen N, Clemens S. 2018. CAX1 suppresses Cd-induced generation of reactive oxygen species in *Arabidopsis halleri*. *Plant, Cell & Environment* 41: 2435–2448.
- Clemens S. 2019. Metal ligands in micronutrient acquisition and homeostasis. *Plant, Cell & Environment* 42: 2902–2912.
- Coffey R, Ganz T. 2017. Iron homeostasis: an anthropocentric perspective. *Journal of Biological Chemistry* 292: 12727–12734.
- Corso M, de la Torre VSG. 2020. Biomolecular approaches to understanding metal tolerance and hyperaccumulation in plants. *Metallomics* 12: 840–859.
- Edwards EJ. 2019. Evolutionary trajectories, accessibility and other metaphors: the case of  $C_4$  and CAM photosynthesis. *New Phytologist* 223: 1742–1755.
- García de la Torre VS, Majorel-Loulergue C, Rigail GJ, Alfonso-González D, Soubigou-Taconnat L, Pillon Y, Barreau L, Thomine S, Fogliani B, Burtet-Sarramegna V *et al.* 2021. Wide cross-species RNA-Seq comparison reveals convergent molecular mechanisms involved in nickel hyperaccumulation across dicotyledons. *New Phytologist* 229: 994–1006.
- Halimaa P, Lin Y-F, Ahonen V, Blande D, Clemens S, Gyenesei A, Häikiö E, Kärenlampi SO, Laiho A, Aarts M *et al.* 2014. Gene expression differences between *Noccaea caerulea* ecotypes help identifying candidate genes for metal phytoremediation. *Environmental Science & Technology* 48: 3344–3353.
- Hanikenne M, Talke IN, Haydon MJ, Lanz C, Nolte A, Motte P, Kroymann J, Weigel D, Krämer U. 2008. Evolution of metal hyperaccumulation required *cis*-regulatory changes and triplication of HMA4. *Nature* 453: 391–395.
- Krämer U. 2010. Metal hyperaccumulation in plants. *Annual Review of Plant Biology* 61: 517–534.
- Liu H, Zhao H, Wu L, Liu A, Zhao F-J, Xu W. 2017. Heavy metal ATPase 3 (HMA3) confers cadmium hypertolerance on the cadmium/zinc hyperaccumulator *Sedum plumbizincicola*. *New Phytologist* 215: 687–698.
- Meier SK, Adams N, Wolf M, Balkwill K, Muasya AM, Gehring CA, Bishop JM, Ingle RA. 2018. Comparative RNA-seq analysis of nickel hyperaccumulating and non-accumulating populations of *Senecio coronatus* (Asteraceae). *The Plant Journal* 95: 1023–1038.
- Reeves RD, Baker AJM, Jaffré T, Erskine PD, Echevarria G, van der Ent A. 2018. A global database for plants that hyperaccumulate metal and metalloids trace elements. *New Phytologist* 218: 407–411.
- Van der Pas L, Ingle RA. 2019. Towards an understanding of the molecular basis of nickel hyperaccumulation in plants. *Plants* 8: 11.
- Verbruggen N, Hanikenne M, Clemens S. 2013. A more complete picture of metal hyperaccumulation through next-generation sequencing technologies. *Frontiers in Plant Science* 4: 388.
- Verbruggen N, Hermans C, Schat H. 2009. Molecular mechanisms of metal hyperaccumulation in plants. *New Phytologist* 181: 759–776.

**Key words:** adaptation, convergent evolution, extremophiles, metal homeostasis, metal hyperaccumulation, transcriptome analysis.