



PhD POSITION (3 years) – Starting November 1st 2020

Title: Biomolecular and ecophysiological insights to breed the ultimate tropical nickel phytomining crop

Supervisors: Prof. Guillaume Echevarria (University of Lorraine - INRAE), Dr. Antony van der Ent (University of Queensland) IRP SUCRE – **Joint supervision:** Prof. Mark Aarts (Wageningen University)

Key-words: *Genome sequencing, Transporters, Transcriptomics, Hyperaccumulation traits, Phyllanthus rufuschaneyi, Sabah, Biogeochemical cycling, breeding, agromining*

Candidate profile

The candidate has a foundation (MSc-level) in molecular biology and/or genetics and at least basic knowledge in agronomy. Additional skills are desirable (biogeochemistry, ecology). The candidate needs to speak English fluently and has good skills in English writing. Being able to work in different locations (France, Australia, Netherlands) is a prerequisite for the laboratory and fieldwork. Additional language skills (French or Dutch) are welcome. The field site is located in Sabah (Malaysia) in a remote area (Pahu village, Ranau). A high level of autonomy of the candidate is required to work with three advisors from three different institutions, and in the remote field site (only one or two times).

Conditions:

- **Gross Monthly Salary: 1950,- €** (Taxes and social security fees are deducted monthly)
- **Co-supervision by three different academic institutions**
- **Main location:** Nancy, Lorraine, France
- **Secondary location:** Wageningen, Netherlands

Context: The University of Lorraine is the 1st European University and 11th globally according to Shanghai 2020 Ranking in the field of Mining and Mineral Engineering. The University of Queensland is 12th globally. Together they have created an International Joint Lab dedicated in research on ‘Sourcing Unconventional Mineral Resources’ (SUCRE). Agromining (i.e. Cropping metal hyperaccumulator plants to recover high value metals) is among the key topics of SUCRE and has made the scientific expertise and reputation of The Laboratory of Excellence on Mineral Resources of University of Lorraine (LABEX Ressources21).

The first upscaling site for nickel agromining in tropical conditions is located in the state of Sabah (Malaysia) where the ideal candidate species has been discovered and studied over the past 5 years. It is now crucial before real upscale of this nickel crop is carried out, to understand the genetic control on the hyperaccumulation and growth traits of this species. This is the origin of this PhD project.

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Apply before September 15, 2020 (Please send CV and Letter of Motivation).

General Objectives: To determine the key biomolecular physiological mechanisms of Ni hyperaccumulation in *P. rufuschaneyi* to inform selection and breeding of an improved metal crop in the tropical biome.

The aims of this project would be achieved through a **programme** as follows:

1. To identify the genes and metal transporters involved with hyperaccumulation of Ni in *P. rufuschaneyi*, and to establish the effect of genetic variability on population dynamics;
2. To understand the physiological mechanisms that are responsible for Ni loading, Ni transport from root-to-shoot, and Ni re-distribution within tissues of *P. rufuschaneyi*;
3. To unravel key biogeochemical interactions in the native habitat (such as allelopathy, commensalism,...) deriving from the different genotypes.
4. To use all of the biomolecular and trait information collected to select and breed plants with the optimum characters for Ni agromining (in partnership with ECONICK who co-funds this project).

Research programme:

1. Screen individuals of the target species for morphological/physiological traits
2. Collect accessions of contrasting characteristics to provide a rich genotype pool for subsequent experiments
3. Detailed investigation of genetic and physiological and growth traits attributes including microsatellite markers, transcriptomics, photosynthetic activity, growth performance, nickel accumulation, yield
4. Statistically correlate genotypes and gene expression with ecological and biogeochemical behaviour of the individuals monitored

In Partnership with ECONICK

5. Selection and Breeding based on trait analysis (growth, Ni accumulation, efficiency of metal recovery from biomass evaluated by LRGP) – Field testing of different lines

Context / State of the Art:

In the frame of the collaboration between LabEx R21 (UL) and SMI-CMLR (UQ) which started in 2014 with the postdoc of Antony van der Ent at UL, two PhDs have been carried out in co-supervision by the two groups. The PhD of P.Nkrumah (2014–2018) allowed to determine the requirements for the agronomy of the wild species *Phyllanthus rufuschaneyi*, globally the most successful candidate species so far in tropical agromining (Nkrumah et al., 2016, 2019a,b,c). The PhD of R. Tisserand started in 2017 and is dedicated to the understanding of the role of hyperaccumulator plants (focussing on *P. rufuschaneyi*) in the biogeochemical cycling of nickel and possible consequences for long-term agromining operations. The consortium UL-UQ (IRP SUCRE) has also developed a methods (PhD of S. Lopez 2015-2018) to implement agromining as a strategy to recover nickel from mining of nickel laterite ores (contract with ERAMET-Weda Bay Nickel) and to initiate the environmental rehabilitation of the site after mining operations (Lopez et al., 2019a,b,c). As such, the consortium is the most advanced globally in implementing agromining in tropical biomes on natural ultramafic soils and mining wastes.

The currently proposed PhD project will concentrate on the fundamental aspects of the unravelling of the biomolecular mechanisms of nickel hyperaccumulation by *P. rufuschaneyi* – information that is key to developing improved metal crops. Apart from operational expenses that would be covered by Econick, a postdoc will be hired for three years to undertake several key steps prior to the PhD (developing methods for plant propagation, selection of the different genotypes to be included in the genetic screening) and to evaluate the best accessions through field testing.

Rufus Chaney and co-workers developed a breeding programme to select the best candidates for Ni agromining in the early 2000s by screening and selecting the best lines of *Alyssum murale* and *A.*

corsicum (Nkrumah et al., 2016). However, little is known about the genes involved in the different mechanisms of Ni hyperaccumulation, especially in tropical Ni hyperaccumulator plant species (Merlot et al., 2014). The only information available thus far points to overexpression of Fe transporters (IREG) in leaves and roots of a hyperaccumulator species compared to a close non-hyperaccumulating relative (Merlot et al., 2014). Transferring the IREG gene from hyperaccumulators to non-hyperaccumulators gives them resistance to high levels of Ni.

Phyllanthus rufuschaneyi is a rare endemic species of Sabah that only grows in ultramafic soils at low altitudes where Ni availability is very high (Bouman et al., 2018). This species is only known from three locations within a few kilometres from each other. Despite this small population size and ostensibly small genetic pool, the phenotype of the species is morphologically highly variable. A recent study on the biomass and Ni yield of 30 individuals revealed that plant growth in 12 months can vary from 200g to over 1000g per plant. Therefore, to be able to select performant cultivars for the commercially successful development of agromining, it is critical to understand the fundamental molecular mechanisms involved, and to harness these properties to develop the ultimate metal crop.

Details on the proposed research

In order to optimise the genotypes most suitable for agromining, tests can be made to study what improvements are possible. The first step to take is to determine the broad sense heritability (H_2) of traits. For calculation of the heritability, the phenotypic variance will need to be determined and distinguished in components, that is the genetic variance and the non-genetic or environmental variance. In order to get an estimate of the heritability of traits, we propose to identify 25 plants in the current field trial established by R. Tisserand's, *i.e.* 441 plants that were morphologically monitored for 2 years, which should represent the broad spectrum of total genetic variation within the species. Then, tall and short, green- and yellow-green-leaved, large and small-leaved, unbranched and branched, monoicous or dioecies accession will be selected. The next step will be to select out of these 50 genotypes, some 5–10 genotypes to test in small, replicated plots (3–4) for actual phytomining ability. From these plots again the heritability can be determined in order to select for the best 3–5 clones to do actual agromining trials. In parallel, after determining the initial heritability, a breeding strategy can be designed to improve the agromining properties and generate new genotypes. Such can be done by crossing combinations of two favourable, genotypes and screen the progeny. Once manual pollination is mastered, the breeding for improved agromining *P. rufuschaneyi* varieties. Econick, a start-up company from UL (LSE and LRGP) will participate in this phase (proposed Post-doc position in interaction with this PhD project).

The plant improvement and breeding will be coupled to detailed genetics, molecular biology and ecophysiological investigations. The *P. rufuschaneyi* accessions will be propagated vegetatively and phenotyped, including further analysis regarding gene expression and genetic co-segregation. Each accession will be genetically characterized using a genotyping-by-sequencing (GBS) method, *e.g.* DArTseq or alike, which is based on a low coverage, selective, next generation DNA sequencing, supplemented with low-coverage, non-selective short-read whole genome sequencing. The aim is to identify genes acting on molecular mechanisms involved in Ni hyperaccumulation in *P. rufuschaneyi*. In order to achieve this, the genotyping and physiological approach will be combined with a comparative transcriptomics approach based on RNA-seq technology, to establish transcriptomes of roots, young leaves, old leaves and flowers of *P. rufuschaneyi* and a closely-related, non-hyperaccumulating species of *Phyllanthus*. These will provide both genotype information as well as gene identity and expression information, which is crucial to initiate molecular genetic research on a so far novel species. Priority will be given to genes that are differentially expressed between the Ni-adapted *P. rufuschaneyi* and the non-adapted *Phyllanthus* reference species. This approach has worked well in the analysis of hyperaccumulation in the Brassicaceae, such as *Noccaea caerulescens*. We expect that these analyses will reveal genes involved in common mechanisms employed by these species to take up, translocate, sequester and tolerate high nickel concentrations. These candidates will be prioritized based on predicted function and further characterized regarding molecular function. The functional

characterization of some of the candidate genes will be initiated, which will include heterologous expression in model species, such as yeast and *Arabidopsis thaliana*, including complementation of mutants disturbed in metal homeostasis; intracellular localization studies; and patterns of expression using *in situ* hybridization. Genes with a confirmed role in nickel homeostasis will be used as genetic or expression markers to validate their importance. In addition, the list of candidate genes involved in nickel hyperaccumulation will be compared to the list of candidate orthologous genes in *N. caerulescens* to identify putative convergent mechanisms among species or in contrary mechanisms specific to *P. rufuschaneyi*.

Whole plant and tissue-level distribution of nickel and other elements will be mapped using a new micro- X-ray Fluorescence facility at UQ developed specifically for elemental imaging of living plant samples. Finally, novel synchrotron methods enable *in situ* determination of chemical speciation in the Ni uptake biopathway.

Finally, the interpretation of the genetics of the species will be carried out in the light of i) ecological characteristics and strategy of the species, ii) the biogeochemical role of the species in its native ultramafic environment, and of course, iii) the final characteristics and performance that can be obtain from the species in agromining systems.