## Reg. No: 2022/47/B/NZ2/01835; Principal Investigator: dr Anna Ihnatowicz

The aim of this study is to better understand the genetic basis and molecular mechanisms underlying plant responses to defined and combined environmental stresses, namely bacterial infection and iron (Fe) deficiency. Fe is crucial micronutrient for every kind of living organism. It plays essential role in metabolic processes such as DNA synthesis, respiration, photosynthesis, and is cofactor of many enzymes. Role of Fe homeostasis in immunity and its relation with infections was shown across all kingdoms of life - different types of pathogens are likely to compete with their hosts for the acquisition of Fe. Also the relationship between plant resistance to pathogens and Fe availability is well documented. Mechanisms of Fe homeostasis in plants, pathogens, and beneficial microbes play key roles in plant-microbe interactions. Fe deficiency is also a widespread agricultural problem that reduces plant growth and crop yields, particularly in alkaline soils. One-third of the world's agricultural area is composed of calcareous soils. The high pH associated to such soils leads to the precipitation of Fe that is therefore not available anymore and generate severe growth perturbation of plants.

Plants and microorganisms have evolved a set of active strategies for Fe uptake from the soil that are based on acidification, chelation and reduction processes. One of such important processes determining the interaction of plants with the soil environment is root exudation. It is a process during which plant roots secrete into rhizosphere the bioactive molecules, called root exudates, that can change the soil microbiome. These compounds belong to various chemical classes including a class of secondary metabolites called coumarins that are produced by plants in response to environmental stimuli. Coumarins that are secreted to the rhizosphere by roots are involved in several processes determining plant interactions with the soil environment, both with biotic and abiotic factors. It was shown recently by several groups, including our research team, that the secretion of coumarins is essential for Fe acquisition under Fe-deficient conditions in a model plant *Arabidopsis thaliana*. Interestingly, the important role of coumarins in plant immunity and their release into the rhizosphere upon pathogen infection was discovered. Exuded coumarins were shown to reshape root microbiome in Fe-starved plants and possibly protect plants from pathogenic fungi. However, the precise mechanisms underlying these processes remain also largely unknown.

All this taken together shed light on the strong relationship between Fe homeostasis, coumarin accumulation and plant immunity. To study this important point, we will analyze variability in the response of Arabidopsis natural populations (accessions) to infection with Dickeya spp. Plant pathogenic bacteria belonging to Dickeya spp. cause soft rot disease devastating potato and many other crops. Therefore, to investigate the effect of plant Fe status on success of microbial infection, we decided to use *Dickeya* spp./Arabidopsis pathosystem. For this purpose, a large set of 100 Arabidopsis accessions from the HapMap collection, will be grown in various hydroponic cultures. Firstly, plants will be grown in Fe-deficient hydroponics. Another scenario include Fedeficient plants grown hydroponically that additionally will be subjected to selected plant pathogenic bacteria from the genus *Dickeya* spp. The next plant culture will be carried out in hydroponics in optimal Fe conditions and subjected to infection with the same bacterial strain. Hydroponics is a method of growing plants in a water based, nutrient rich solution, here mimicking Fe-deficient soil. An advantage of using Fe-hydroponics is that one has control over the culture medium and the nutrient solution, making such system a useful approach to investigate Fe-deficiency. Hundreds of plant samples will be collected and coumarins/Fe accumulation will be quantified using ionomic and metabolomic approaches. Subsequently, we will perform genome-wide association studies (GWAS) aiming to identify associations between single nucleotide polymorphisms (SNPs) and distinct phenotypic traits. GWAS was pioneered in human genetics more than ten years ago. Nowadays it is routinely applied to identify underlying genetic variation for many traits including important agronomic and nutritional traits in plants.

Further detailed analysis of candidate genes associated with the variation in Fe-deficiency responses and variation in plant susceptibility to *Dickeya* spp. infection under Fe-limiting conditions, will reveal a so far undescribed components of the coumarin synthesis pathway linked to "the battle for iron". Fe-deficiency is one of a major constraint for plant growth that causes severe losses of crop yields and quality. Considering that dietary deficiencies of Fe are a substantial global public health problem that affect up to two billion people worldwide, the study of plant responses to Fe-deficiencies are becoming even more important in nowadays.