ECLENUS

Project title: **Exploring new ways to control Legume nitrate utilization and signaling** / Explorer de nouvelles voies pour contrôler la signalisation et l'utilisation du nitrate

Acronym: **ECLENUS**

Project duration: 19 months - Start date: 01/10/2019 End date: 30/04/2021

Key-words: sustainable agriculture, agronomic context, *Medicago truncatula*, nitrate signal, primary root growth, early legume establishment, reactive oxygen species, differential gene expression, metabolomics, fluxomics

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Summary:

Context

Legumes play an important role in human and livestock diet due to their high protein content in seeds and aerial parts (which can be used as forage). Legumes also have a key role in cropping ecosystems by fixing atmospheric N_2 thereby contributing to nitrogen enrichment in soils and thus to sustainable agriculture. Primary root growth is crucial for successful seedling establishment and depends on nutrient availability such as soil nitrate concentration, which is rather variable. Legumes do require nitrate during early seedling development since atmospheric N_2 fixation is not functional yet. That said, nitrate is not only a nutrient but also a signal involved in the regulation of primary root growth. We have recently shown that contrary to model plants like Arabidopsis where moderate nitrate concentration stimulates primary and lateral root growth, in legumes nitrate inhibits primary root growth at early developmental stages (model plant *Medicago truncatula*). **This represents a significant change in paradigm for nitrogen nutrition**: First, since soil nitrate concentration varies spatially in the field and can reach high values locally, this may explain why legume cultivation leads to considerable heterogeneity in seedling establishment; Second, since nitrate itself inhibits its own utilisation, this should in principle lead to a vicious circle whereby nitrate leaching and aquifer pollution is favoured over its consumption by legumes.

Goal:

The project **ECLENUS** aims to open avenues to manipulate nitrate signaling so as to improve seedling homogeneity and ability to capture soil nitrate. Solving this question is crucially important because legume seedlings optimized for nitrate signaling and utilisation would benefit to both the environment and yield. To answer these questions, the project will take advantage of nitrate-insensitive genotypes that have been found recently, including a knock-out mutant unable to generate reactive oxygen species (ROS) via the RBOHF pathway. That is, we will elaborate on our recent discovery on the essential role of ROS in mediating nitrate signaling in legumes. **Our objectives will thus be to directly assess nitrate capture and metabolic utilization and its potential modulation by ROS using genetic and physiological tools. That way, we will tackle the hypothesis that the down-regulation of ROS-mediated nitrate signaling can be a simple technique to improve nitrate utilization during legume cultivation.**

Methodology

To do so, we will carry out metabolite integrative analyses (metabolomics) and protein (enzyme) analyses (proteomics), to explore the nitrate assimilation pathway in the primary root of the three genotypes (wild-type, nitrate-insensitive *npf6.8* and *rbohf*) grown without or with nitrate, H_2O_2, a ROS, or a H_2O_2 scavenger. Furthermore, we will take advantage of isotopic methods, that is, use ^{15}N-labelled nitrate to trace the fate of assimilated nitrate to main metabolites and measure the nitrogen use efficiency (NUE), which is key parameter for assess the efficacy of plant nutrition. The present project *(i)* aligns with the position of world leader of the Region Pays de la Loire in isotope biochemistry, *(ii)* involves Prof. Guillaume Tcherkez, recently appointed locally as a fellow *Connect Talent*, and *(iii)* utilizes new instruments (IRMS, GC-MS) that come along with his appointment.

Abbreviations used

GC: gaz chromatography ; IR: isotope ratio ; MS: mass spectrometry/spectrometer; NUE: nitrogen use efficiency; ROS: reactive oxygen species