IONIS

Project title: Impact of exogenous nitrogen on early seedling establishment of legumes in the context of sustainable agriculture Acronym: IoNiS (Impact Of exogenous Nitrogen on early Seedling establishment of legumes)

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Summary:

Context: Legumes play an important role in human and livestock alimentation due to their high level of proteins in seeds and aerial parts used as forage. Legumes by fixing atmospheric N_2 also take part to a significant ecosystemic service in cropping systems contributing to nitrogen enrichment of soils and thereby to sustainable agriculture. While many studies on legume biology are focusing on symbiotic nitrogen fixation processes, our team aims to characterize the early post-germinative processes leading to successful legume establishment taking into account the impact of exogenous nitrate or abiotic stresses. Primary root growth is crucial for successful seedling establishment. It depends on nutrient availability and notably on the level of nitrate content in the soil which varies greatly. This is also true for legumes during early seedling development, when the fixation of atmospheric N_2 is not in place. However, nitrate is not only a nutrient but also a signal involved in the regulation of primary root growth. At early stage, we showed in *Medicago truncatula*, a model legume, that nitrate can inhibit root growth.

Goal: The goal of this project is to decipher the nitrate signaling pathway that leads to a reduction of primary root growth in *M. truncatula* by the restriction of cell elongation. We have recently found that the nitrate transporter MtNPF6.8 acts as a nitrate sensor involved in the response to nitrate, mutants deficient in MtNPF6.8 (*npf6.8*) being insensitive to nitrate. How the signal, perceived by the transporter, is transduced is still not known. However, the fact that abscisic acid (ABA), a vegetal hormone, restored the inhibitory effect of nitrate in *npf6.8* mutants suggests that ABA is involved in the signal transduction. Reactive oxygen species (ROS) are also likely to play a role in the mediation of the nitrate signal because they govern root growth through cell division and elongation. Thus, nitrate should modify the ROS balance in roots. Because both ABA and ROS are known to control gene expression, nitrate signal may also alter gene expression through these two mediators. We want to determine how nitrate modifies the ROS balance and identify the genes mediating the nitrate signal. A special interest will be given to genes encoding redoxins or transcription factors possibly redox regulated, all being good candidates to mediate the signal downstream as well as to other target genes to gain clues on the mechanism underlying the reduction of root growth through the function of the genes identified. We want to develop within the IRHS unit a novel competence for the integration of the data at the two levels of gene expression and unravel post-transcriptional regulation events.

Methodology: We will mainly use two genotypes contrasting for nitrate sensitivity, a wild type genotype sensitive to nitrate (R108) and the *npf6.8* mutant in the same genetic background, which is deficient in a nitrate sensor and has lost the nitrate sensitivity. The impact of nitrate on ABA or ROS accumulation in different parts of the root will be assessed. The incidence of a modification of ABA signaling or ROS content on root growth, will also be determined through a pharmacological approach or a reverse genetic approach using mutants affected in ABA signaling or ROS production. In parallel, the impact of exogenous nitrate or ABA on gene expression in the wild type and in the *npf6.8* mutant lines will be determined by both transcriptomic and proteomic studies. We will work in synergy with experts in transcriptomics and bioinformatics (present locally) and proteomics (Paris Saclay).