Medi-SENS

Project title: Exploring Medicago truncatula natural genetic diversity to study nitrogen use efficiency during seedling growth under low temperature and nitrogen supply

Acronym: MeDi-SENS (Medicago Diversity, Seedling Establishment, Nitrogen use efficiency and Stress)

Project duration: 38 months – Start date: 1/11/2016 End date: 31/12/2019

Key-words: Medicago truncatula, natural genetic diversity, seedling establishment, heterotrophic growth, early autotrophic growth, low temperature, limitation of nitrogen supply, multistress, nitrate uptake, nitrogen remobilization, nitrogen use efficiency, GWAS

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Total cost of the project: 114 604 € (Do not include salaries others than those of the people hired for the project : PhD or post-doc)

Financial support required from « Objectif Végétal »: 114 604 €

Summary:

Rapid and homogenous seedling growth is crucial for successful crop establishment. Pre-emergence growth at the expense of seed reserves and transition to autotrophy are two steps highly susceptible to environmental cues. Earlier (in the season) and northwards sowings are already practiced for some species and proved to be a good strategy of crop management modification in a context of global climate change. Furthermore the development of sustainable agriculture aims at decreasing nitrate runoff that pollutes waterways and can lead to eutrophication by decreasing nitrate fertilization. Taking into account these conditions and subsequent changes of crop management mean that seedlings will encounter more frequently low temperatures during the establishment phase in a rhizosphere environment characterized by low levels of nitrogen availability. Low temperatures as well as nitrogen limitation are two stresses either individually or in combination that are susceptible to compromise successful seedling establishment by affecting shoot and root elongation and biomass production. It is worth to note that nitrate not only affects seedling establishment as a nutrient but also acts as a signal molecule that shapes root system architecture.

<u>Goals.</u> This project aims to explore the natural genetic diversity of *Medicago truncatula*, a model legume species, to highlight adaptive physiological behaviors for nitrogen use efficiency (NUE) and to identify Quantitative Trait Loci (QTL) associated to successful seedling establishment under the above described new sowing conditions combining low temperature and low nitrate fertilizer supply. To our knowledge, the genetic variability of physiological behaviors in response to limitation of nitrogen for early growth stages has never been explored as well as the combined impact of nitrogen supply and low temperature stress.

<u>Methodology.</u> 1) Seedling growth in the dark will be characterized using a core collection of 192 *M. truncatula* natural accessions in four environmental conditions (with and without nitrate in the growth medium, at both optimal and low temperatures). Hypocotyl and radicle traits (lengths, ramification and biomass) as well as nitrate and total nitrogen (N) contents will be measured. All these characterizations will allow estimating the involvement of exogenous nitrogen and of N reserve

mobilization in seedling growth as well as nitrate uptake for all accessions. Moreover, this strategy will allow highlighting potential groups with different behaviors in terms of NUE during heterotrophic growth under ample and limited nitrogen supply at both temperatures.

2) A genome-wide association study (GWAS) will be carried out to exploit linkage disequilibrium in the core collection, to study the genetic architecture of all the traits and to identify the strongest associated loci. This approach could also possibly allow highlighting some candidate genes that could be validated afterwards.

3) A small panel of genotypes selected to be representative of physiological behaviors will be then more finely analyzed. Organ elongation speed, relationships between genotype behaviors in the dark and under photoperiod will be investigated mimicking natural growing conditions. On this smaller panel special interest will be given also to identify, through ¹⁵N labeling experiments, respective contributions of N reserves and exogenous source of N (nitrate) to seedling growth under low temperature, and whether there is a link between seedling fitness and on one hand, root system architecture and on the other hand, endogenous *vs* exogenous source of N. This fine analysis of genotype behaviors should also allow identifying physiological traits for ideotype(s) that can fit seedling establishment in crops under low temperature and/or nitrogen supply.