

Year of CfP: 2008

Project No 0802-022 Completed

Project title: Adaptative responses of the N₂-fixing symbiotic interaction between *Medicago truncatula* and rhizobia to whole plant N limitation

Unit managing the project: LSTM (Laboratory of Tropical and Mediterranean Symbiosis) (CIRAD, INRA, IRD, Montpellier SupAgro, UMII)

Project leaders: Gisèle Laguerre (gisele.laguerre(a)supagro.inra.fr) and Marc Lepetit (BPMP, Plant Molecular Physiology and Biochemistry)

Research units from the Foundation's scientific network involved: BPMP

Sub-thematic axes: IPB-1 (Integrative Plant Biology 1: *Genetics and genomics, plant breeding, ecophysiology*), IPB-2 (Integrative Plant Biology 2: *Plant pests and diseases, integrated crop protection, population ecology*), STDI-1 (Socio-Technical Dynamics of Innovation 1: *Agri-environmental innovations, agri-ecosystems, resources management*)

Objectives:

In soil, the roots are constantly subjected to biotic and abiotic stresses that locally suppress the acquisition of N. Moreover, in legumes, roots formed nodules in the presence of heterogeneous and fluctuating bacterial populations consisting of several strains of rhizobia whose efficiency for symbiotic nitrogen fixation is variable. The continuation of plant growth in such situations depends on its ability to offset the deficit by increasing the acquisition capacity of the roots remained unstressed. Recent work has characterized these adaptive responses in *Medicago truncatula* (Ruffel et al., 2008, Plant Physiol 146: 2020-2035; Jeudy et al., 2010, New Phytol 185: 817-828). In the case of plants fed NO₃⁻, the plant compensates very quickly and very efficiently the N deficit, first by increasing the absorption capacity of roots remained supplied then preferentially stimulating their proliferation. There is also a compensatory response in N₂-fixing plants (in symbiosis with the model strain *Sinorhizobium meliloti* RCR2011 / local deficit caused by treatment Ar/O₂), but it is much less efficient and slower. It is not accompanied by any increase in the specific activity of existing fixing nodules (which seems to be always at its maximum) and only depends on the stimulation of nodule development in non-stressed roots remained supplied. Our objective was to determine in *M. truncatula* the impact of variability in bacterial efficiency to fix N₂ on the functional (nodular activity, acquisition of N in whole plant) and developmental responses (nodules and roots) exploiting the natural genetic variability exists among compatible rhizobia.

Studies will be conducted on the model species *M. truncatula* (*Mt*) and a unique collection of compatible rhizobial partners that has been constructed by the LSTM. The effect of genetic variability of rhizobia on the responses of the structures (nodule size, nodule number, root/nodule architecture) and the functions of the symbiotic organs (nodule activity, N intake) will be explored. This approach is the first step of a more ambitious project aiming to unravel bacterial mechanisms that contribute to the adaptation of plant to fluctuations of environmental factors.

Action carried-out and results obtained:

Screening of a collection of rhizobia (*S. meliloti* and *S. medicae*) was used to select strains with different levels of efficiency to fix N₂ in symbiosis with *M. truncatula* (efficiency lower or higher than that of RCR2011). The compensatory response of the plant has been characterized in plants nodulated with each of these strains in split-root devices. The effect of local suppression of N₂ fixation applied to half of the root system has been studied on the roots of the same plant remained fixing. For short-term responses (4 d), the suppressive treatment was to replace the air by a mixture of argon and oxygen. For long-term responses (30 d), nitrogen limitation has been obtained by nodulation of half of the root system with a strain of *S. meliloti* unable to fix N₂ (mutant fixJ of RCR2011).

The results show that the general pattern described above for the *M. truncatula* -RCR2011 symbiosis is conserved whatever the strain and its effectiveness for N₂ fixing. In our study, the responses observed at 30 days showed that the plant has fully compensated the local limitation of nitrogen uptake since biomass and nitrogen contents of plants subjected to treatment are equivalent to those of control plants regardless of the strain considered. For all strains the compensatory response to the localized nitrogen limitation depends only on the stimulation of nodule development and not on any increase of nodule specific activity on untreated (nitrogen fixing) roots. The observed responses are consistent with a systemic negative feedback exerted by the N assimilates of the plant on nodule development.

The magnitude of the adaptive response to long-term depends on the activity of N₂-fixing strain: indeed, more the strain is efficient, more the compensatory response is important. However, the response is not solely determined by the N status and N demand of the plant, but is also strongly dependent on the efficiency of the strain in interaction with the plant. Indeed, for poorly effective strains, although the overall level of fixation of N is insufficient to meet the N demand of the plant, nodule development reached a threshold. This suggests either a response limited by nutritional factors (limitation in C, for example) or a limitation by mechanisms linked to autoregulation of nodule number (AON) that seem to be partly determined by the nature of the symbiotic interaction. Studies with plants grown in an atmosphere enriched in CO₂ and / or using mutants affected in the AON (hypernodulant mutant) could help evaluate these two hypotheses.

Prospects for the future:

Various studies have shown that the host plant preferentially associated with certain bacterial genotypes from the genetic diversity available in the in the free-living soil populations. However, recent studies indicate that the population structure changes in the nodules during the vegetative cycle, varies according to changes in nodule development, and may vary with nutritional constraints experienced by the plant (Depret and Laguerre, 2008, *New Phytol* 179:224-235; Kiers et al., 2007, *Proc. R. Soc. B* 274:3119-3126). These results suggest that the various components of the rhizobial populations can be differentially mobilized by the symbiotic interaction in response to environmental conditions or plant development and thus contribute to the plasticity of plant adaptation to a changing environment for N supply. Therefore, our current work now aims to determine whether the plant, according to its nitrogen demand, is able to select the most effective bacteria for the formation of nodules and / or to favor the growth of existing nodules formed by the most effective strains. In the longer term, according to the results obtained, it is planned to develop a project to decipher the molecular basis of these adaptive responses.

Total Agropolis Fondation funding: € 8,840 (running costs)

Funding categorie(s): Agropolis Fondation small grants (support for small exploratory, risky and innovative projects)

Project duration: 1 October 2008 – 31 December 2009

Keywords: symbiosis – nitrogen – rhizobia – *Medicago truncatula* -