

A variety of regulatory mechanisms are involved in the nitrogen-dependent modulation of the nodule organogenesis program in legume roots

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Combined nitrogen (N) sources are known to strongly affect initiation, development and functioning of Nitrogen-Fixing-Nodules whose formation is triggered by lipochitin-oligosaccharide signals secreted in the rhizosphere by the Rhizobium partner. The rapid effects of N supply on nodule initiation have been mainly described when N sources are present at the moment of Rhizobium inoculation or purified Nod Factors addition. We recently reported that high ammonium nitrate growth conditions might also strongly affect the nodulation competence of *Lotus japonicus* plants, prior to the Rhizobium inoculation. This is a long-term effect, which suggests a change of the general nutritional status as the signal controlling the reduced nodulation capacities. The mechanisms underlying these inhibitory pathways are apparently different and the identification of the molecular actors involved may provide new insights into the linkage between N environmental changes and root organogenesis programs.

Plants have evolved an amazing capacity to respond quickly and in a very effective way to the environment commitments by modulating their root and shoot developmental processes. The symbiosis is an excellent example of how the plants adapt to the changing environment, as nodulation does not occur when fixed nitrogen is readily available in the soil (Fig. 1). The mechanisms underlying this environmental control are not elucidated yet although the first observations on the inhibitory

effect of different N sources were reported long time ago. The effect of nitrate has been extensively studied and the inhibitory action on nodule initiation seems to act locally,^{1,2} whereas both a systemic and local nitrate action was reported for nodules growth and N fixation activity.^{3,4} However, it must be taken into account that different N sources might have different effects on nodule organogenesis. We previously reported discrimination between nitrate and ammonium effects on the Nod factor dependent transduction pathway.⁵ Both N sources affect early stages of the cascade of events leading to nodule formation but their actions were positioned in different points on the transduction pathway. In particular, ammonium action takes place earlier than nitrate by affecting even root hair deformation events.⁵

The analysis reported in Omrane et al.⁶ indicates that N supply can also modulate the early steps leading to nodule initiation by predisposing *Lotus japonicus* plants to a successful/unsuccessful interaction with Rhizobium, prior to the inoculation. The inhibitory effect of 10 mM NH_4NO_3 on the legumes nodulation competence is maintained for at least six days after transfer in permissive, low N concentration, suggesting the existence of a negative feedback that is controlled by the general nutritional status of the plant. Our preliminary data obtained with a split-root experimental system where two root sides are exposed to different N regimes seem to confirm this hypothesis. Of course a systemic effect controlled by the general nutritional status of the plant should not change with different N sources such as

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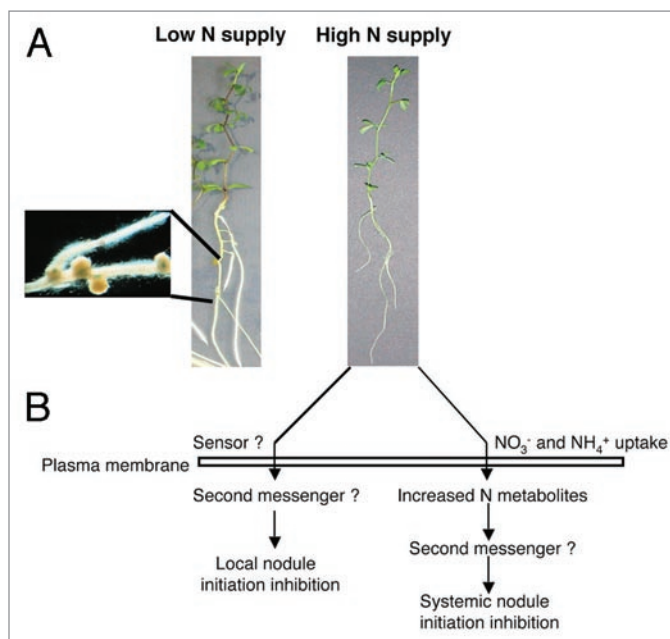


Figure 1. (A) *Lotus japonicus* plants inoculated with *Mesorhizobium loti* in a N-free condition (left) and in the presence of 10 mM ammonium nitrate (right). (B) Scheme of the dual N dependent regulation of the nodulation program.

ammonium and nitrate. Nevertheless, plants might reveal N sources preferentiality and this could affect the external concentration required for the systemic inhibitory effect to occur.

The mechanisms and factors involved in both local and systemic N supply inhibitions as well as the potential targets of their action on the pathways leading to nodule initiation are almost completely unknown (Fig. 1). Some insights into the elucidation of these signaling pathways could come from the information obtained on the effects of nitrate on root architecture in *Arabidopsis thaliana*.⁷ Both local and systemic nitrate effects on the secondary root elongation and early stages of development have been described.^{8,9} The effect of local nitrate supply was not observed in the *axr4* auxin-resistant mutant, suggesting an overlap between the nitrate and auxin response pathways,⁹ whereas the systemic effect was modulated by the nitrate accumulation in the shoot.^{9,10} In the latter case the long-distance signal could be a shoot-derived auxin signal that is modulated by the nitrate status of the shoot.¹¹ Although legume nodules and lateral roots are structurally and developmentally distinct, several analogies can be identified. For example, actinorhizal nodules and nodules formed on the roots of *Parasponia* are considered to be modified

lateral roots;¹² both non-legume nodules and lateral roots are characterized by a single vascular bundle that traverses the nodule. Furthermore, peanut nodules share with lateral roots the pericyclic site origin and structures elicited by *Rhizobium meliloti* mutants appearing to resemble roots more closely than nodules were described.¹³ A physiological relationship also seems to be present between lateral roots and nodules as nodulated clover seedlings have fewer lateral roots than un-inoculated plants.¹² The involvement of plant hormones as a secondary signal for nodule morphogenesis has been extensively discussed^{12,14-16} and a cytokinin receptor with a crucial role in the Nod factor transduction pathway has been recently identified.¹⁷⁻¹⁹ Gresshoff in 1993,²⁰ proposed the so-called auxin-burst-control (ABC) hypothesis to explain nitrate inhibition and other aspects of nodule initiation in legumes (e.g., autoregulation of the nodules number). The hypothesis suggests that Nod-factor perception alters axial and radial auxin transport, allowing the initiation of cortical cell divisions through a shift in the local auxin-to-cytokinin ratio. Such changes alter pericycle and epidermis responses, leading to nodule formation. Once an increased amount of nodule initiation has occurred, the shoot responds through an increase in translocation of

auxins, leading to an auxin burst, which in turns is inhibitory for further nodule initiation. The ABC hypothesis predicts that nitrate increases the auxin sensitivity of root cortical cells and thus in the presence of nitrate, cortical cells are strongly prevented from sensing the Nod-factor-related auxin decrease. Experimental data reported that a 45% increase of IAA content was actually observed in inoculated soybean plants grown 1 mM nitrate, whereas no apparent increase in root auxin content was observed in the presence of high nitrate.²¹

The transcriptomic approach described in Omrane et al.⁶ potentially permitted the identification of molecular actors of the regulatory circuit linking the accumulation of N metabolites in the roots to inhibition of nodule formation (Fig. 1). The analysis of the regulated sequences might be also performed at the light of the recent findings revealing an association between plant nutrients and regulation mediated by miRNAs.²²⁻²⁴ A recent paper describes in *Arabidopsis* the involvement of microRNA167 (miRNA167) and one of its targets, Auxin Responsive Factor 8 (*ARF8*) in the early arrest of lateral root development under high rates of N supply.²⁵

In conclusion, legumes represent an excellent experimental system to investigate different organogenesis programs

modeling general root architecture and nodulation. These processes are both strongly modulated in response to external N supply and possible overlapping can be found between regulatory mechanisms and factors involved in the response to different N sources and concentrations.

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