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Grosjean C., Huguet T.

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THE SPONTANEOUS NODULATION OF ALFALFA

C. Grosjean and T. Huguet[1]

ABSTRACT

The establishment of symbiosis between leguminous plants and the soil bacteria Rhizobia is investigated using the Nar or spontaneous nodulation phenomenon, i.e. ability of some plant to develop nodulations in the absence of Rhizobium. It is shown that the spontaneous nodule organogenesis is initialized in both Nar and non-Nar plants but more or less blocked further (at the nodule primordia stage), suggesting a regulation of the nodule morphogenesis after the induction of the primordium. Purified nodulation factors were shown to be implied in mitogenic phenomenon, increasing the nodulation phenomenon. Finally, the comparison between spontaneous and *Rhizobium*-induced nodulation suggests that the cortical divisions do not result from a bacterial activation but rather from a spontaneous reactivity of the cortex.

Key words: *Medicago sativa*, alflafa, spontaneous nodulation, NAR, *Rhizobium*

Interaction between leguminous plants and the soil bacteria Rhizobia results in the development of a newly formed plant organ, the root nodule, in which bacteria reduce atmospheric nitrogen into ammonia. The nodular organogenesis has been extensively studied by using legume and bacterial mutants, blocked at various steps of this developmental program, or by adding purified molecules, such as bacterial nodulation factors or phytohormones.

Another useful tool arose a few years ago, with the discovery of the Nodulation in absence of *Rhizobium* (also called Nar or spontaneous nodulation) in some genotypes of alfalfa (*Medicago Sativa* L.) (Truchet *et al.*, 1989). This phenomenon demonstrates that *Rhizobium* is not absolutely required for nodule organogenesis and raises many questions about the respective roles of the two partners. It also makes possible detailed analysis of the plant determinants involved in nodulation, avoiding any kind of exogenous activation.The ontogeny of the spontaneous nodulation is similar to the *Rhizobium*-induced nodulation, beginning with cell divisions in the inner cortex (Joshi *et al.*, 1991). The histological organisation of a fully developed spontaneous nodule is also characteristic of an indeterminate nodule, with an apical meristem, an outer cortex and an endodermis surrounding a central tissue and peripheral vascular bundles. Furthermore, the Nar phenomenon is inhibited by high concentrations of nitrogen in the culture medium and three early nodulins (i.e., nodule specific) genes (*ENOD2*, *ENOD12* and *ENOD40*) have the same pattern of histological expression in spontaneous and *Rhizobium*-induced nodules. These data of ontology, histology, gene expression and nitrate regulation are consistent with the definition of a nodule, the main difference being the absence of any bacteria or infection thread and the presence of numerous starch grains within the central tissue. This last point led Caetano-Anollés *et al.* (1993) to hypothesize that nodules initially functioned as carbon-storage organs, later evolving to adapt to the *Rhizobium* nitrogen fixation.

The spontaneous nodulation, observed in a small percentage (1-15%) of all the cultivars of *M. sativa* tested so far, was recently described on *Trifolium repens*, another allogamous, tetraploid legume species (Blauenfeldt *et al.*, 1994). In alfalfa, the character is conserved during clonal propagation and is transmitted through meiosis (Truchet *et al.*, 1989). A genetic model was proposed by Caetano-Anollès and Gresshoff (1992).

[1] Laboratoire de Biologie Moléculaire des Relations Plantes-Microorganismes, CNRS-INRA, BP 27, 31326 Castanet-Tolosan cedex, France. Tél. (33) 61 28 53 25 - Fax: (33) 61 28 50 61 E@mail: thuguet@toulouse.inra.fr

THE SPONTANEOUS INDUCTION OF NODULE PRIMORDIA

We further investigated the spontaneous nodule organogenesis, focusing on its first step, cell divisions in discrete foci of the inner cortex of the root, forming nodule primordia. In the root system of Nar plants, we observed, in addition to the spontaneous nodules, nodule primordia arrested in their development, suggesting that the overall number of nodules is controlled *via* a blockage of organogenesis at the primordium stage. Root systems of non-Nar plants were analysed as well, and we also observed, in some of them, spontaneous primordia in the inner cortex, arrested in their development. This implies that the spontaneous organogenesis did not carry on as far as the fully emerged nodule stage in these plants, suggesting a possible regulation of the nodule morphogenesis after the induction of the primordium. Therefore, we propose a new classification of the spontaneous nodular development, based on the most advanced stage observed in the root system grown in the absence of *Rhizobium*, and defined as follows: Nar, plants presenting at least one elongated, spontaneous Nodule; CDar, plants presenting at least one focus of cortical cell division but no elongated nodule; NCDar, plants displaying no visible cortical cell division focus.

EFFECTS OF THE ADDITION OF BACTERIAL NODULATION FACTORS

The purified nodulation factor of *R. meliloti* NodRmIV(Ac,S), when added to the growth medium at 10^{-10} M or 10^{-8} M, had a clear mitogenic effect, as already described (Truchet *et al.*, 1991): the number of plants with foci of nodular organogenesis increased dramatically. In non-Nar plants, the development did not go further than the bump or small emerging nodule stage, suggesting that the nodules induced by NodRm lack a persistent meristem. On the other hand, it was possible to observe fully emerged nodules with a persistent meristem when NodRm was applied to Nar plants.

These effects can be mimicked by the addition of the cytokinin benzylaminopurine at 10^{-9} M: the number of plants with cortical cell divisions increased but the development was blocked very early (at the primordium stage) in non-Nar plants, and carried on to the mature nodule stage in Nar plants only.

COMPARISON WITH THE *RHIZOBIUM*-INDUCED NODULATION

In order to know whether *Rhizobium* plays a part in the control of the total number of nodules per root (the so-called autoregulation), we compared the steps subject to regulation in presence or in absence of *Rhizobium*. When plants were inoculated, the major step of control was the induction of cortical cell divisions, as already published (Caetano-Anollés and Gresshoff, 1991): once primordia were initiated, few did not develop into nodules. On the other hand, we showed that in absence of *Rhizobium* the spontaneous development was often blocked before meristem formation, at the primordium stage. Why autoregulation applies at different developmental stages during the *Rhizobium*-induced and spontaneous nodulations remains to be addressed.

Furthermore, we observed that the presence of *Rhizobium* did not totally suppress the spontaneous nodular development: following inoculation, more primordia with an arrested development were observed in plants with a Nar genotype than in non-Nar plants, and we could not detect any infection thread above a large majority of them. This suggested that the cortical divisions were not the result of a bacterial activation but were rather due to a spontaneous reactivity of the cortex.

CONCLUSIONS

The Nar phenomenon proved itself an useful tool to precise what is due to the plant and what is due to the bacteria during the establishment of symbiosis. Unfortunately, it has been found only in tetraploid alfalfas so far, thus limiting any genetic analysis of the nodulation determinants. What seems promising is the analysis of the spontaneous nodulation in terms of coevolution between legumes and Rhizobia: were nodules ancestral carbon-storage organs, parasited by Rhizobia at first, before a complete partnership was established?

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