



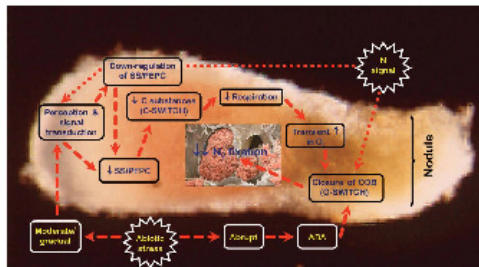
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**Physiological regulation of symbiotic N<sub>2</sub> fixation in  
the model legume**

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## 1 INTRODUCTION

### 1.1 *Medicago truncatula* – A model for legume research

Legumes are usually defined by their typical flower structure and the ability of many of them to form root nodules in presence of symbiotic bacteria named rhizobia. With more than 18,000 species, legumes are found from the arctic circle to the tropics and include many crops of agronomic importance for grain production, pasture and forestry (Graham and Vance, 2003). The ability of more than 88% of legumes to obtain nitrogen from the air through root nodules was probably a major determinant in this evolutionary, ecological and economical success (de Faria *et al.*, 1989). Interestingly, the study of symbiotic associations with rhizobia as well as with arbuscular mycorrhizal (AM) fungi also drove the development of two model legumes: *Medicago truncatula* Gaertner and *Lotus japonicus* (Regel) K. Larsen. While *M. truncatula* is an annual Medic from the Trifolieae tribe and a close relative of alfalfa and clovers, *L. japonicus* belongs to the Loteae and is more distant from cultivated cool season legumes than *M. truncatula*. This phylogenetic distance to economically important crops is critical in the choice of *M. truncatula* by many researchers and support by numerous funding agencies. The use of both model legumes allows unique comparative genomic studies within the legume family as well as the comparison between two patterns of root nodule development: indeterminate with a persistent nodule meristem in the case of *M. truncatula* and determinate in *L. japonicus*. Unfortunately, these two models belong to the same cool season legumes (Galegoid clade), whereas soybean and common bean are tropical season legumes (Phaseolid clade). Soybean is therefore proposed as a third model legume for

both its own economic weight and the phylogenetic proximity to other important crops (Jackson *et al.*, 2006).

Commonly known as 'barrel medic' because of the shape of its seed pods (Figure 1), *M. truncatula* is a plant of Mediterranean origin, which is well adapted to semiarid conditions, and is a winter-growing annual (Barker *et al.*, 1990). It is a near relative of alfalfa (*Medicago sativa*) — the world's economically most important forage legume.

*Medicago truncatula* L. Gaertner has many attributes that make it a valuable model species for genomics and genetics (Ellwood *et al.*, 2006; Rose *et al.*, 2008). Key characteristics that make *M. truncatula* a valuable model system for legume genomics and genetics include small diploid genome (500 Mb), rapid generation time, self-fertilization, highly abundant seed production, numerous ecotypes, readily transformed with *Agrobacterium tumefaciens* and *A. rhizogenes*, efficient nodulation with *Sinorhizobium meliloti*, ready colonization by *Glomus* spp., a range of susceptibility to root pathogens including nematodes, extensive EST sequencing from a number of organs at various developmental stages, high-density genetic linkage map of *M. truncatula*'s eight linkage groups, robust evidence for macro- and microsynteny with other legume species, extensive array of mutants, and agricultural importance. The acceptance of *M. truncatula* as a model legume is evidenced by the National Science Foundation's recent award of \$10.8 million for full genome sequencing (Ivashuta *et al.*, 2004).

*Medicago truncatula* is native to the Mediterranean basin and is found in a wide range of habitats. It is therefore not surprising to find a high level of variation among and within natural populations (Bonnin *et al.*, 1996). *M. truncatula* is used as a fodder crop in

ley-farming systems in Australia, and a large and diverse collection is housed at the South Australian Research and Development Institute (SARDI) (Ellwood *et al.*, 2006).

Like many higher plants, *M. truncatula* forms symbiotic associations with a wide array of arbuscular mycorrhizal (AM) fungi. As a legume, *M. truncatula* is also able to develop root nodules with *Sinorhizobium meliloti*, which is one of the best-characterized *Rhizobium* species at the genetic level (Galibert *et al.*, 2001). Cultivation-independent techniques have been used to sample the diversity of microbes associated with *M. truncatula* roots at various developmental stages and they reveal an extremely dynamic genetic structure of its rhizosphere (Mougel *et al.*, 2006).

The *Medicago* community has therefore identified many ecotypes and developed a wide range of mutants and transgenic lines. A current goal of the International *M. truncatula* steering committee is to address the need for a stock center able to maintain, amplify and distribute these lines to an ever-growing community.

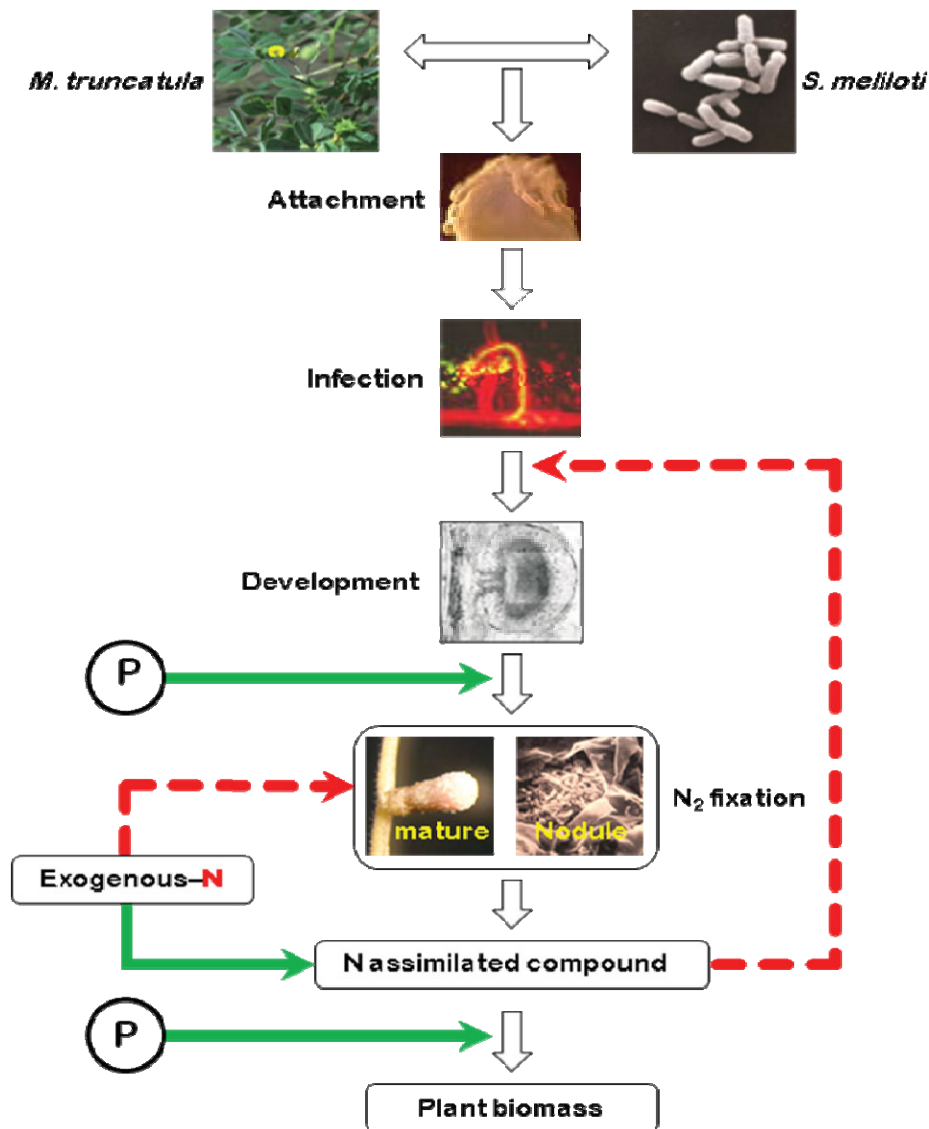


**Figure 1.** *Medicago truncatula* is a model legume for genome sequencing and functional genomics. This photo depicts seeds, leaves, flowers and pods (adapted from May and Dixon, 2004).

## 1.2 Symbiotic N<sub>2</sub> fixation

*Medicago truncatula*, like most legumes, establishes a symbiotic association with a compatible strain of *Sinorhizobium*. The (*Sino*)*rhizobium*–legume symbiosis is a well-organized system involving many steps (Figure 2): signal exchange and recognition of the symbiotic partners; attachment of the rhizobia to the plant root hairs; root hair deformation; invasion of the root hair by rhizobia; infection thread formation; nodule initiation; bacteroid development; and formation of N<sub>2</sub>-fixing nodules (e.g., Mylona *et al.*, 1995). Nodules of various leguminous species are grouped into two main types; determinate and indeterminate (Hansen, 1994). In determinate nodules [e.g., soybean (*Glycine max* L. Merr.), common bean (*Phaseolus vulgaris* L.)], cell division over a short period of time and the nodules are usually spherical. In contrast, indeterminate nodules [e.g., pea (*Pisum sativum* L.), alfalfa (*Medicago sativa* L.)] possess a meristem which gives rise to differentiated cells that may become infected with rhizobia. Due to the continued cell division indeterminate nodules are generally cylindrical in shape (Hansen, 1994). From a physiological point of view, *M. truncatula* nodules have not been studied extensively but based on the shape, they have indeterminate type.

Once symbiosis is established the host plant provides carbon substrate as a source of energy; and the bacteria reduce atmospheric N<sub>2</sub> to ammonia, which is exported to the plant tissues for protein synthesis (Paul and Clark, 1996). The effectiveness and efficiency of the symbiotic system is dependent markedly on the mutual compatibility of both partners (Keyser and Li, 1992).



**Figure 2.** Symbiotic formation and control points for regulation of infection and nodulation of *Medicago truncatula* × *Sinorhizobium meliloti*. Dashed red lines with arrow indicate inhibition. Green full lines with arrow indicate stimulation.

The effectiveness is considered a polymorphic trait where specific interactions between the host plant and symbiotic bacteria contribute to the success of the interaction. Despite the selection of certain rhizobial strains, which thought to be effective, inoculation does not always lead to increased N<sub>2</sub> fixation. When the symbiotic