INSERTION MUTAGENESIS IN *M. TRUNCATULA*

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We have been studying the symbiosis between *Sinorhizobium meliloti* and the leguminous model plant *Medicago truncatula*, with the major aim at better understanding the molecular basis of nodule development.

As a contribution to the development of *M. truncatula* as a model plant, we have improved embryo regeneration and transformation of *M. truncatula* lines R108-1 and Jemalong J5.

In order to facilitate the identification of genes and gene functions implicated in nodule development we have initiated an insertion mutagenesis program in *M. truncatula* using different transposons or T-DNAs as tags. Plants containing a T-DNA insert that can generate *in vivo uidA*-gene fusions (Bouchez et al. 1993, C. R. Acad. Sc. Paris 316, 1188-93) were screened for GUS activity in roots and nodules. From 200 transgenic plants analysed, 24 expressed GUS activity in roots and nodules in different tissues, indicating that we have tagged different gene promoters. Among those, a gene homologous to the early nodulin MtN3 was identified. T-DNA insertion in another gene coding for a protein of unknown function in eukaryotes yielded homozygous mutant plants that were dwarf. The mutation in *M. truncatula* was complemented by the homologous arabidopsis gene and phenocopied (dwarf plants) by applying RNAi to this gene in arabidopsis. This plant represents the first T-DNA tagged mutant in *M. truncatula*.

In parallel, we have also tested the transposition capacity of the retrotransposon *Tnt1* of tobacco in *M. truncatula*. *Tnt1* transposed mainly during the early steps of the *in vitro* regeneration of *M. truncatula* plants. We have regenerated 200 independent transgenic plants carrying on average 15 copies of *Tnt1* and these elements are stable in their progeny. By retrocloning and PCR walk, we showed that *Tnt1* inserts preferentially in genes and could thus be an efficient tool for mutagenesis in *M. truncatula*.