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DISSERTATION SUMMARY

Development of a general strategy to obtain stable transgenic plants in non-embryogenic lines using the *Agrobacterium tumefaciens* plant transformation method

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The aim of our group is to identify, isolate and analyse plant genes required for symbiotic nitrogen fixation. We used the map based cloning strategy to achieve these goals. After genetic mapping of a Nod⁻ mutation in tetraploid alfalfa, our group carried out the chromosome walking and sequenced the BAC clones covering the NOD region. In this way we could identify a candidate gene coding for a receptor-like protein kinase which carried mutation in the Nod⁻ alfalfa plant and also in four mutant alleles of *Medicago truncatula* and two mutant alleles of *Pisum sativum* in which the mutation was mapped to the same region (dmi2 and sym19 mutants).

My responsability in our group was to carry out the plant transformation experiments including (A) the complementation of the Nod⁻ plants and (B) *the in vivo* characterization of the gene and the protein.

(A) We performed the complementation of *Medicago sativa* and *Medicago truncatula* Nod⁻ plants via *A. tumefaciens* and *A. rhizogenes* transformation method respectively, with the wild type NORK (Nodule Receptor Kinase) gene.

Since the Nod⁻ alfalfa line is not embryogenic we made a cross with the highly embryogenic Regen S line and in the F1 population we identified two highly embryogenic individuals. For the complementation we used two approaches: (1) identification and transformation of embryogenic Nod⁻ plants in the F2 population obtained after self-pollination of the embryogenic F1s and (2) after transformation the F1 individuals, self-pollination of transformants and identification of individuals that are homozygous for the mutant allele and carry the transgene.

(1) We identified 15 Nod⁻ individuals and found one embryogenic Nod⁻ plant that we transformed with the NORK gene in order to rescue the mutant phenotype. These transgenic plants are developing now and will be analysed for the presence of transgene and for the complementation. (the embryo formation period was prolonged to nine months in the embryogenic test).

(2) We transformed the F1 individuals via *A. tumefaciens* using five different constructs carrying the NORK gene and we obtained transgenic plants with three of them. The transgenic plants were self-pollinated and the individuals of the F2 populations were genotyped for a PCR-based marker closely linked to the NORK gene. Eight individuals of the 622 plants tested were homozygous for the mutant allele. Seven of these plants developed nodules on their roots. Southern blot analysis was performed to confirm that the mutant NORK allele was homozygous in these plants. It was proved that 7 individuals of the 8 candidates were homozygous for the mutant allele and six carried the transgene which complemented the mutant phenotype in all of the cases. In this way we could develop a general strategy to obtain stable transgenic plants in non-embryogenic lines using the *Agrobaterium tumefaciens* plant transformation method. We also transformed Nod⁻ *M. truncatula* (R38 and TR25) plants via *Agrobacterium rhizogenes* (Arqua strain) using pCAMBIA plant transformation vector carrying the NORK gene. Four plants out of ten carrying transgenic roots were complemented (they have root nodules with bacteroids after infection with *Sinorhizobium meliloti*).

(B) The *in vivo* characterization of the NORK gene and the protein: (a) the temporal regulation of the gene (promoter-GUS fusions). We obtained transgenic *M. truncatula* plants (by *A. rhizogenes* plant transformation) carrying NORK promoter fused to the GUS reporter gene. According to these experiments we can conclude that NORK promoter is a constitutive one, being expressed in the root except the root tip, while to the 35S promoter, used as a control, is expressed in all the tissues of the root. Microscopic studies are necessary to specify in which tissues and cells the NORK gene is expressed. (b) the effect of the overexpression of the gene (35S promoter versus own promoter-NORK cDNA). We obtained transgenic *M. truncatula* plants carrying the NORK cDNA driven by 35S promoter and its own promoter (NORK promoter) but we could not achieve complementation with these constructs.

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