Comparative Analysis of the Infection Process in *Casuarina glauca* and *Discaria trinervis*

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Two basic patterns of nodule initiation are found among actinorhizal plants. The first one involves initial entry by the microsymbiont as an intra-cellular penetration through curled root hair; this mode of infection is observed in *Casuarina glauca*. The second pattern involves an initial phase of inter-cellular colonisation of the root tissue, with neither root-hair involvement nor intracellular penetration until the nodule primordium stage. Three of the eight host families are nodulated via the root-hair infection pathway, whereas the other five actinorhizal families initiate nodules via intercellular colonization. The actinorhizal shrub *Discaria trinervis* that grows in Patagonia belongs to this second category.

Molecular analysis of the infection process in *C. glauca* has led to the identification of several marker genes that are expressed during the early stages of the symbiotic interaction, when root hairs become infected by the actinomycetal hyphae. These include the subtilase gene *Cg*12, the auxin influx-carrier gene *CgAUX*1 and *CgDMI*3, a calcium-calmodulin dependent kinase gene. Besides, the *MtENOD*11 promoter from the realy nodulin gene of *Medicago truncatula* was also found to drive reporter gene expression in root hairs of *C. glauca* infected by *Frankia CcI*3.

In order to compare the intracellular and intercellular infection pathways in the two actiorhizal plants *C. glauca* and *D. trinervis*, a procedure for rapid functional analysis of symbiotic genes in *Discaria* was tested using the biological vector *Agrobacterium rhizogenes*. Two different strains of *A. rhizogenes*, A4RS and ARqua1, carrying a 35S-GFP construct were used on *Discaria.* Transgenic roots expressing the reporter gene GFP were obtained for both strains with transformation efficiencies up to 80%. Roots obtained with ARqua1 were more similar to wild-type roots and were easier to nodulate compared to A4RS.

Genetic transformation of *Discaria* was then achieved with the constructs MtENOD11-GUS, Cg12-GUS and CgAUX1-GUS. Preliminary data indicate that CgAUX1-GUS was found to retain its non-symbiotic pattern of expression but did not respond to *Frankia* infection. On the opposite, MtENOD11-GUS exhibited a similar pattern of expression during the symbiotic process in *D. trinervis* as compared to the expression observed in roots of *M. truncatula* upon infection by Rhizobium.

These data pave the way for the introduction of gene constructs that will contribute to decipher the early steps of the association with *Frankia*. This work was financially supported by the Argentinian-French bilateral Project ECOS Sud No. A07B02.

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