IN PLANTA TRANSFORMATION OF NARROW-LEAFED LUPIN (LUPINUS ANGUSTIFOLIUS) SEEDLINGS

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In planta transformation of narrow-leafed lupin (*Lupinus angustifolius*) by vacuum infiltration with *Agrobacterium* was investigated. The apical area of young shoots was wounded by sonication prior to infiltration with *Agrobacterium* cells containing a reporter gene (35S *gus*) and a selectable marker gene (35S *bar*) for herbicide tolerance. Sonication time and infiltration time were optimized. Thirteen minutes of sonication with 10 minutes of infiltration with *Agrobacterium tumefaciens* AGL0 were the best conditions for delivery of *A. tumefaciens* cells. Longer times increased the number of fatalities of seedlings. Other important parameters, namely bacterial growth phase and the composition of infiltration solution, were investigated to increase stable transformation as determined by the number of blue spots in the tissue. Expression of the reporter gene was not stable over time. Seeds from 1720 treated plants were screened for expression of the *bar* gene by the application of 50 mg/l phosphinothicin (PPT). All the seedlings died in a manner similar to the controls. We then looked at the viability of *Agrobacterium* cells over time on the seedlings following infiltration to determine if the cells were dying before they could transfer the T-DNA to the seedlings. Firstly, we determined by *gus* gene expression on explants *in vitro* that T-DNA transfer began at Day 3 of the co-cultivation period, then increased rapidly to a maximum by Day 5. The viability of *A. tumefaciens* cells in planta was examined by counting the numbers of viable cells on the treated seedlings over time post-inoculation. Cells were isolated from seedlings and plated on medium containing lactose as a carbon source. The presence of 3-ketolactose around colonies indicated that they were *A. tumefaciens*. The assay showed that the cells had a very low rate of survival on the seedlings, and that by Day 4, when T-DNA transfer *in vitro* was approaching maximum efficiency, survival of *A. tumefaciens* cells on the plant was very low, about 10^3 times less than required for successful transformation of lupins via the routine *in vitro* route (CLIMA lupin transformation protocol). These findings may explain why *in planta* transformation has not yet been possible in lupin.