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Identification of *Arabidopsis* Genes that are Necessary for *Agrobacterium*- mediated Plant Transformation

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Objectives

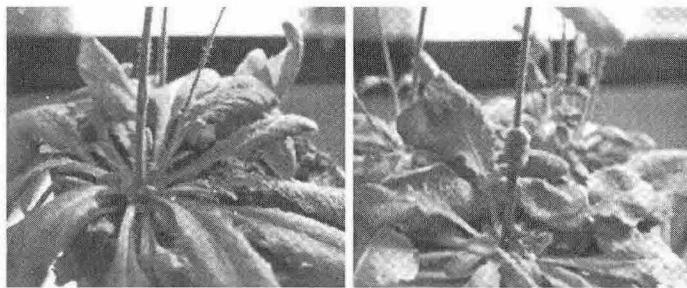
Agrobacterium tumefaciens-mediated plant transformation is complex molecular cellular reactions in which many bacterial and host plant factors interact cooperatively. We have worked on investigation of the plant genes necessary for *Agrobacterium*-mediated transformation not only to make significant advances in our understanding of how *Agrobacterium* transform plant efficiently, but also to develop new methods to transform agronomically important plants that are recalcitrant to *Agrobacterium tumefaciens*-mediated transformation so far.

Materials and Methods

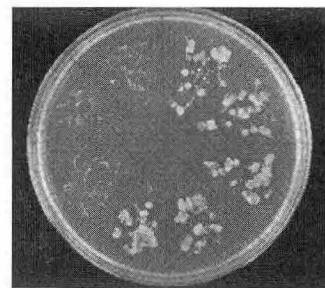
1. Materials: T-DNA insertion mutant pool of *Arabidopsis thaliana*
2. Methods: Screening T-DNA insertion mutants for resistance in response to *Agrobacterium* infection using an *in vitro* root inoculation and flower bolt inoculation. Plasmid rescue and complementation test of mutation phenotype.

Results and Discussion

To identify plant factors and determine their functions in the *Agrobacterium*-mediated plant transformation, we first screened T-DNA insertion mutant lines of *Arabidopsis thaliana* for resistance in response to *Agrobacterium* infection using an *in vitro* root inoculation assay. We identified two mutant lines (T-618, T-1059) and isolated genes disrupted in mutants. A BLAST search showed that the T-618 and T-1059 mutants have T-DNA insertions in the VDAC gene that is essential for apoptosis in animal and the gene encoding F-box containing protein that is likely to involve in protein degradation via SCF complex-mediated ubiquitination, respectively. To prove unequivocally that the resistance phenotype of mutants is due to disruption of these genes, we are performing complementation test of mutation phenotype. Our Preliminary results showed that T-618 mutant was complemented with wild-type VDAC gene, indicating VDAC is required for successful *Agrobacterium*-mediated plant transformation. Currently we are investigating the function of VDAC in *Agrobacterium*-mediated plant transformation.



Flower bolt inoculation assay



In-vitro root inoculation assay

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