I realize that many labs have tried the in planta transformation protocols developed in my lab, but only a few have succeeded. (This technique has not yet been successful in the Dean lab.) The protocol certainly works, but one has to know what one is doing and has to try many plants at least until you get the feeling. The following is the protocol I distributed at the Arabidopsis training workshop in Japan (Nov.26,1991), which was organized by Dr. Okada at NIBB, Okazaki.

1. BACKGROUND
   * Agrobacterium genetically colonises wounded plant cells by transferring a defined segment (T-DNA) of its Ti plasmid into the plant nuclear genome. Although this natural gene-transfer mechanism has been widely adopted in plant transformation, the possibility of generating transformed progeny by Agrobacterium infection in planta has not been successfully exploited. Here we show stably transformed progenies of a crucifer plant, Arabidopsis, are efficiently obtained by simple incision of primary shoots at their bases and by subsequent Agrobacterium inoculation at the wound site. Adventitious shoots arising from the infected wound site were transformed at a high frequency and produced transformed progenies later.

2. SEED GERMINATION AND PLANT GROWTH
   (i) Soak seeds on the filters wetted in 10mM KNO₃, 10mM NaPO₄, pH 7.0 and place at 4°C. in the dark for 48 h.
   * Seeds may be directly sown on soil without imbibition and/or cold treatment, but the germination will not be uniform.
   (ii) Sow cold-treated seeds on compounded soil (e.g., Metromix 200) or vermiculite soaked with Hogland's solution and cover the pots with saran wrap.
   (iii) Transfer germinated seedlings to a controlled growth chamber (16h light/8h dark).
   (iv) For healthy growth, water and fertilise sufficiently and protect from fungal or insects attack.
   * For efficient transformation, plant materials must be healthy.
   * Fertilise using Hogland's solution or a commercial mixture (e.g., Hyponex).

3. WOUNDING AND AGROBACTERIA INFECTION
   (i) When the primary inflorescences have grown to 2-3 cm in height, remove them by severing the basal part with a scalpel or scissors and remove auxiliary buds as completely as possible with fine forceps. This step is most critical. You want to remove as many preformed shoot meristems as possible, and to make the reorganization of shoot meristems from infected and transformed cells.
   * The timing of infection is important.
(ii) Sometimes it helps to make deep wound into the remaining stem with a needle or toothpick.

(iii) Inoculate wounds with Agrobacteria from overnight culture grown in YEP media. * Overnight culture Agrobacteria are concentrated to 1/5 volume by centrifugation at 600 rpm for 5 min, followed by resuspension in the remaining medium.

(iv) Place inoculated plants in the shade or low light intensity for 2-3 d at 28°C.

(v) Transfer plants (pots) to standard growth conditions, until secondary inflorescences emerge (about 10-14 d)

(vi) One or more inflorescences should develop from the wound site. Remove the early emerging inflorescences and reinoculate Agrobacteria onto the wound sites by repeating the procedure described above.

(vii) The efficiency of transformation may be tested by an assay of leaves on the newly formed shoots.
* We used pBI121, a binary vector containing the NPT II and GUS genes within the T-DNA region.

(viii) The later emerging inflorescences are maintained through maturity for seed generation.