MODIFICATION TO IN PLANTA TRANSFORMATION

(This protocol was placed on the electronic Arabidopsis bulletin board; July 1992.)

Chris Somerville's lab.

* This non-tissue culture method to get transformed Arabidopsis was first described by Chang et al. at the Arabidopsis meeting in Vienna. Ljerka Kunst and George Haughn invested some time to get the method to work in their respective laboratories. Then Ljerka introduced it to Chris Somerville's Lab where it has now become the method of choice for Arabidopsis transformation. In general, we are performing the procedure as described in Chang's poster abstract from the meeting in Vienna, but the percentage of transformed seeds is not as high as reported. We grow and treat the plants in the following way:

1. PROCEDURE

(i) Seed 10-12 Arabidopsis plants (Columbia, Landsberg and RLD have been tested) in a 6in. pot containing Arabidopsis soil and grow them under continuous light, or long day photoperiod.

(ii) When the plants start to bolt (flowering shoot <2 cm), we cut the flowering shoot and all visible axillary buds with a forceps, or a razor blade. On the wounded area we apply a droplet of overnight culture of Agrobacterium tumefaciens (strain C58 or GV3101 containing the desired vector).

(iii) When the plants make secondary flowering shoots (< 2 cm), remove them again and infect as above. After the second infection, plants are grown to maturity to produce seeds.

(iv) All the seeds from one pot are bulk harvested, or collected in three batches from each pot.

(v) Transformed seeds are selected on MS plates containing the appropriate antibiotic (Kanamycin, 50 ugml-1; Hygromycin, 30 ugml-1, Chlorsulfuron, 0.05 ngml-1). 2000 sterilised seeds are spread regularly per 150 mm petri dish (10,000 seeds in case of Chlorsulfuron selection).

(vi) After 10 days to three weeks transformed plants are easily visible. If you have a good seed set (>1g seeds per pot) you can expect an average of one transformant per pot.