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Adaptation of Cotton Shoot Apex Culture to Agrobacterium-Mediated Transformation

JEAN H. GOULD[1,*] and MARIA MAGALLANES-CEDENO[2]
[1]*Forest Science Department, Texas A & M University, College Station TX 77843, USA*
[2]*Biochemistry Department, University of Nevada, Reno NV, USA*

Abstract. A protocol is presented for rapid genotype-independent transformation and regeneration of cotton (*Gossypium* spp.) from shoots isolated from germinating seedlings. Isolated shoots are inoculated with a super-virulent strain of *Agrobacterium tumefaciens*, subjected to a mild antibiotic selection, and directly regenerated as shoots *in vitro*. Shoots do not dedifferentiate and mutation rates are low. Rooted shoots can be obtained within 6–10 weeks of isolation and inoculation depending on the cotton cultivar.

Contents: This manuscript contains: Introduction, Materials and Methods (with detailed protocol), Discussion, twenty references, and three illustrations.

Illustrations (line drawings of shoot isolation procedure):

Figure 1. The seedling shoot is embedded in the stem between the cotyledons.
Figure 2. Remove one cotyledon by pushing down until it snaps off. This exposes the shoot apex. The seedling shoot apex, or epicotyl, is removed from the seedling and cultured in MS + Kin for 3–5 days.
Figure 3. A lateral section of the cotton shoot is removed to expose the meristems in the apical region. This area is then inoculated with *Agrobacterium* and recultured.

Key words: *Agrobacterium*, cotton, *Gossypium*, shoot apex, transformation

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[*]Author for correspondence. e-mail: jean@rsgis4.tamu.edu
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