GENETIC TRANSFORMATION OF ACALA AND COKER COTTONS WITH ANTIFUNGAL CONSTRUCTS BY *AGROBACTERIUM* AND PARTICLE BOMBARDMENT

K. Rajasekaran, J.W. Cary, A.R. Lax, and T.E. Cleveland
USDA, ARS, SRRC
New Orleans, LA
D.M. Anderson Phytogen, J.G. Boswell Cotton Seed Breeding Company
Corcoran, CA
C. Chlan
University of Southwestern Louisiana
Lafeyette, LA

Abstract

We have initiated transformation experiments using several antifungal gene constructs to inhibit the growth of *Aspergillus flavus* in cotton. A synthetic antifungal peptide D4E1 has been shown to inhibit the germination of *A. flavus* conidia and is fairly resistant to degradation by proteases. Gene constructs encoding D4E1 and other antifungal proteins/peptides are being utilized in our transformation experiments.

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Two different genetic transformation methods, *Agrobacterium* and particle bombardment, were optimized on several Coker and Acala cotton (*Gossypium hirsutum* L.) varieties using GUS and NPT II marker genes. Seedling explants such as immature hypocotyl and cotyledon segments of both Coker and Acala cotton varieties were more readily transformed by *Agrobacterium tumefaciens* and A. *rhizogenes* than shoot tip meristems and embryogenic cell suspension cultures. Several hundred transformed plants carrying the GUS and NPT II genes were regenerated from *A. tumefaciens* treated seedling explants and embryogenic cell suspension cultures. Southern analyses of randomly selected transformants revealed presence of one copy of the introduced gene (GUS or NPT II).

Seedling explants, shoot tip meristems and embryogenic cell suspension cultures of both Coker and Acala varieties were also transformed by particle bombardment. Transformation frequencies in experiments involving seedling explants and shoot meristems were very low primarily due to problems in identifying and selecting for the transformed cells or sectors. Particle bombardment of cotton shoot meristems often resulted in only epidermal transformation indicating lack of penetration of the DNA coated gold particles propelled by the PDS-1000 helium biolistic device. However, 3% of the meristem bombarded plants showed stable epidermal expression, including expression in the fiber, of the introduced GUS gene for more than four years of vegetative multiplication. On the other hand, embryogenic cell suspension cultures of these varieties were transformed at very high frequencies using the biolistic technique. The transgene copy number in biolistically transformed plants from cell suspension cultures ranged from one to four. Plants regenerated from transformed embryogenic cell suspension cultures exhibited high incidence of male and/or female sterility. This problem can be overcome to some extent by using freshly initiated cell suspension cultures.

The advantages and disadvantages of each transformation method using different explants from different genotypes should be taken into consideration in studies aimed at cotton transformation.

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