SHOOT APEX TRANSFORMATION OF COTTON USING *AGROBACTERIUM*

Gould J, Zhou Y, Shen Y, Magallanes-Cedeno, Lou J,
Forest Science Department, Texas A & M University & TAES,
College Station, TX

Abstract

Aside from the narrow range of genotype-specificity of creating transformed plants through somatic embryogenesis, plants regenerated in culture through tissue dedifferentiation, harbor silent genetic mutations which result in lowered yields. A method based on inoculation of isolated seedling shoots with the biological vector *Agrobacterium tumefaciens*, overcomes these problems (Gould & Smith, 1988; Gould et al., 1991a; Gould et al, 1991b; US Pat. 5,164,310). This method was used to generate putative transgenic plants of *Gossypium hirsutum*: Acala SJ-2, Tamcot HQ95, Tamcot Sphinx, Stovepipe, CA 3076, CA 3085, and 91D-92 which were fertile and produced R_1, R_2, and R_3 progeny carrying the transferred genes.

Introduction

In this method, hormonal manipulation of plant development *in vitro* is kept to a minimum to permit the native drive of the apex to control plant regeneration, making the procedure genotype-independent. No other regeneration protocol is necessary. Plants generate directly from inoculated shoots on a simple MS based medium that can be used to regenerate many different cotton varieties and diverse plant species (Gould et al., 1991a; Gould et al., 1991b). In addition, the genetic mutations recognized as 'somaclonal variation', induced in callus-based and somatic embryogenesis tissue cultures (Hirochika, 1993), are rare in plants regenerated from shoots, shoot apices (most commercial nursery stock), and plants that have been freed of virus through apical meristem culture. Shoots from seedlings were inoculated with *Agrobacterium tumefaciens* EHA101, EHA105 or LB4404.

Results

The 'Super Binary Vector' TOK233 in LB4404 from Japan Tobacco (Hiei et al., 1994) produced lower transformation & recovery rates compared with *A. tumefaciens* EHA101 & EHA105 containing standard binary vectors. It is also our experience that isolation & inoculation of more than 50 shoots at a time lowers recovery rate. Assays for GUS activity using a fluorogenic substrate (IGG; Fleming et al., 1996), were more rapid yet still consistent with results using X-Gluc and with PCR amplification/blot hybridization. Southern (DNA) analyses of genomic DNA isolated from newly regenerated plants, and from progeny of older regenerated plants, show the transferred genes present in: regenerated plants (R_0), and in progeny (R_1 & R_2) of 2 older families: G1 & G9 (Acala SJ2). A recently regenerated plant, inoculated with TDC, tryptophan decarboxylase, which was shown to be effective in reducing whitefly reproduction by 95% (Thomas et al., 1995; Courtesy of C. Nessler), contained high levels of tryptamine, indicating expression of the transferred gene.

Rates. Our success in recovering plants from inoculated shoots is 25%. Of these plants, the percent that are GUS+ range from 25-48%, depending on the strain of *Agrobacterium* used in the inoculation. EHA101 and EHA105 produced approximately 50% and 40% GUS+ plants respectively, while the Super Binary Vector in LBA4404 produced 28%, or half as much (Table 1). Using EHA101& EHA105, overall transformation efficiency is estimated to be 8-12% (25%x40%=10%; and 25%x50%=12.5%).

Progeny & Inheritance. DNA isolated from the R_2 progeny of two original regenerated plants (G1, G9) was screened using PCR amplification for transferred *uid*A (beta-glucuronidase, GUS) and *nptII* (kanamycin resistance). Five plants were identified and the DNA from these plants subjected to PCR amplification for transferred *uid*A and *nptII* genes. Amplified fragments were separated by gel electrophoresis, blotted to a membrane and was hybridized with the probe for the specific gene amplified. Plants in the third generation group (R_3) are GUS+ (using IGG). These results strongly suggest stable genomic integration of transferred genes and germline inheritance. Southern (DNA) and other methods of analysis of the R_3 are in progress. This research is supported the Texas State Support Committee through Cotton Incorporated.

References

Fleming AJ, Manzara T, Gruissem Kuhlemeier C. 1996. Fluorescent imaging of GUS activity and T-PCR analysis of gene expression in the shoot apical meristem. The Plant Jour. 10:745-754.

Gould J, Smith R. 1988. Shoot tip culture as a potential transformation system. Proceedings: Beltwide Cotton Production Conferences, National Cotton Council, Memphis TN.

Gould, J.H., S. Banister, M. Fahima, O. Hasegawa and R. H. Smith. 1991a. Regeneration of *Gossypium hirsutum* and *G. barbadense* from the shoot apex. 1991. Plant Cell Rept. 10:12-16.

Gould, J.H., M. Devey, O. Hasegawa, E. Ulian, G. Peterson, R.H. Smith. 1991b. Transformation of *Zea Mays*

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 1:453-454 (1997)
National Cotton Council, Memphis TN

L., using *Agrobacterium tumefaciens* and the shoot apex. Plant Physiol. 95:426-434.

Hiei Y., S. Ohta, T. Komari, T. Kumashiro. 1994. Efficient transformation of rice (Oryza sativa L.) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA. The Plant Journal 6: 271-275.

Hirochika H. 1993. Activation of tobacco retrotransposons during tissue culture. EMBO J. 12:2521-2528.

Jefferson R.A. 1987. Assaying chimeric genes in plants: the GUS gene fusion system. Plant Mol Biol Rep. 5:387-405.

Thomas JC, Adams DG, Nessler C, Brown J, Bohnert H. 1995. Tryptophan decarboxylase, tryptamine and reproduction of the whitefly. Plant Physiol. 109:717-720.

Table 1. Gus positive regenerated (r_0) cotton

Tag #	*Variety (Plasmid)*	*GUS activity*	*PCR/ SOUTHERN*	
(5)	Stovepipe (Act-Gus)	+/+	+/+	
(9)	Stovepipe (TOK233)	+/+		
(12)	Stovepipe (TOK233)	+/+		
(14)	Stovepipe (Act-Gus)	+/+	+	
(26)	Stovepipe (pTOK233)	+/+	+	
(27)	Stovepipe (pTOK233)	+/+		
(28)	Stovepipe (Act-Gus)	+/+		
(29)	Stovepipe (Act-Gus)	+/+		
(32)	Stovepipe (Act-Gus)	+/+	+	
(33)	CA 3076 (pGus3)	+/+		
(53)	Stovepipe (Act-Gus)	+/+	+	
(74)	CA 3084 (pGus3)	+/+	+/+	+
(86)	CA3076 (pGus3)	+/+	+/+/+	+
(88)	CA 3084 (pGus3)	+/+	+/+	

Specific Rates:

LB4404 (pTOK233) = 4+/14 *inoculated* = 28% *of recovered plants*
EHA105 (pAct-GUS) = 6+/15 *inoculated* = 40% " " "
EHA101(pGUS3) = 4+/8 *inoculated* = 50% " " "