SONICATION EFFECTS AND TRANSIENT GENE EXPRESSION FOLLOWING AGROBACTERIUM TRANSFORMATION

B.-M. Lee, C. Zapata, S. H. Park, K. M. El-Zik, and R. H. Smith
Department of Soil & Crop Sciences
Texas A&M University
College Station, TX

Abstract

The efficiency of *Agrobacterium*-mediated transformation of cotton is low. In an attempt to enhance infection and transformation of the cotton seedling shoot apex using *Agrobacterium tumefaciens* LBA4404 as the vector, sonication treatment to enhance wounding and transformation efficiency was investigated. The *pat* gene which encodes resistance to the herbicide, glufosinate, was used as a selectable marker. Survival of the shoot apices on 2.5 mg/l of glufosinate ammonium, ppt, a level which kills all apices not expressing resistance, was used to indicate efficacy of treatment. Sonication treatment can enhance wounding of the tissue by creating fissures or channels throughout the targeted tissue. Initial sonication treatments of 0, 5, 10, 15, 30, and 60 seconds at 60 watts, 47 KHz, show an increase in shoot apex survival between 5 to 15 seconds sonication treatment over the control (o second) shoot apices.

Introduction

Smith et al. (1997) first reported transformation of cotton, *Gossypium hirsutum* cv CUBQHRPIS using the seedling shoot apex cocultivated with *Agrobacterium tumefaciens*. The transformation efficiency was eight plants from 1010 apices cultured or about 0.8%. There are many factors which can be evaluated to increase the efficiency of *Agrobacterium*-mediated transformation of plant tissue (Smith and Hood, 1995). Some of these factors include temperature of cocultivation, *Agrobacterium* strain used, use of *vir* inciting compounds like plant extracts and acetosyringone, supervirulent plasmids, different gene promoters and constructs.

The first publication using sonication for transformation was that of Joersbo and Brunstedt (1990). They demonstrated that mild sonication of sugar beet (*Beta vulgaris* L.) and tobacco (*Nicotiana tabacum* L.) protoplasts facilitated the uptake of plasmid DNA with some loss in viability of the protoplasts. Transient expression of the foreign DNA was increased by 7-15 fold over expression following electroporation. No long-term deleterious effects of sonication were observed. Zhang et al. (1991) described using ultrasound for direct gene transfer into tobacco leaf segments with a frequency of 22% transgene expression, and transgene expression was observed in progeny. More recently, Trick and Finer (1997) demonstrated that sonication resulted in small and uniform fissures and channels throughout the targeted tissue which may allow *Agrobacterium* easier access to the plant cell several layers within the tissue. They sonicated cotyledons from *Glycine max* Jack, embryonic cultures of *Aesculus glabra*, leaf tissue of *Vigna unguiculata*, seedlings of *Picea glauca* and *Triticum aestivum* and immature embryos of *Zea mays* and cocultivated with *Agrobacterium*. The method is referred to as SAAT or sonication-assisted *Agrobacterium*-mediated transformation. They saw a 100 to 1,400-fold increase in transient expression of b-glucuronidase in these tissues. This report describes preliminary research using sonication to enhance wounding of the cotton shoot apex for *Agrobacterium*-mediated gene transfer.

Discussion

Table 1 shows cotton shoot apex survival on selection media (2.5 mg/l ppt) without sonication treatment after two months in vitro. The survival percentage ranges from 0.8 to 16%. The two lower rates of survival, 1.7 and 0.8% were obtained with just the *pat* gene and either a truncated or full version of the *Bt* gene. The truncated version of the *Bt* gene results in a higher survival rate of the shoot apex. The higher levels of survival, 8 and 16% were obtained using the supervirulent plasmid, pAD1289, containing the mutant *vir*GN54D gene for constitutive expression of the *vir* genes.

Table 2 shows the results of cotton shoot apex survival on selection medium after different time exposure to sonication after one month in vitro. Additionally, plasmids with and without the supervirulent plasmid were used. The 0 sonication treatments show an increase in survival with the supervirulent plasmid from 8.6 to 13.5%, respectively. It appears that sonication from 5 to 15 seconds does have a positive effect on shoot apex survival. However since the shoot tips have only been in culture on selection for one month, it is anticipated that more shoots will be killed over the second month.

Summary

Cotton shoot apex survival on the selection medium is an indication of transient expression of the gene for herbicide resistance. It does not indicate that stable integration of the T-DNA has occurred. Survival of the shoot tips is an early indicator of the benefit of various parameters tested to enhance efficiency of T-DNA transfer from the *Agrobacterium* to the shoot tissue. It does appear that the supervirulent plasmid is having a positive effect of T-DNA transfer and expression. Additionally, there is a preliminary indication that extra wounding by sonication has a positive effect on T-DNA transfer.

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Table 1. Sphinx cotton shoot tip survival on selection medium (2.5 mg/l ppt) indicating transient expression of the *pat* gene. *Agrobacterium tumefaciens* LBA4404 was the vector.

Plasmid	# shoot tips isolated	# shoot tips surviving *	% survival
A	158	3	1.7
B	50	4	8.0
C	130	1	0.8
D	267	43	16.1
Control	110	0	0

* After 2 months

A = *pat* + Truncated *Bt*

B = *pat* + Truncated *Bt* + Supervirulence

C = *pat* + Full length *Bt*

D = *pat* + Full length *Bt* + Supervirulence

Table 2. Sphinx cotton shoot tips surviving on selection medium (2.5 mg/l ppt) indicating transient expression the *pat* gene. Shoot tips were sonicated for various time intervals.

	# shoot tips for each	# shoot tips surviving (%)*					
		Sonication time (sec)					
Plasmid	trt.	0	5	10	15	30	60
C	152	13(8.6)	21(13.8)	18(11.8)	15(9.7)	11(7.2)	8(5.3)
D	80	11(13.8)	20(25.0)	26(32.5)	15(18.8)	17(21.3)	15(18.8)
Total	232	24(10.3)	41(17.7)	44(19.0)	30(12.9)	28(12.1)	23(9.9)

* After 1 month