COTTON VARIETY AND BACTERIAL STRAIN INTERACTIONS DURING AGROBACTERIA-BASED GENETIC TRANSFORMATION

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Abstract

The influence of two different lines of *Agrobacterium tumefaciens* upon gene transfer, uptake, expression and integration in several cotton varieties has been examined. Both bacterial line and plant genotype contribute significantly to the success of cotton genetic transformation.

Introduction

Most commercially important cotton varieties are not currently direct candidates for biotechnological genetic manipulation due to the difficulty of producing uniformly transformed viable plants. The introduction of desired genes via Agrobacteria infection of tissue explants is largely limited to a few regenerable cotton varieties, while Agrobacteria infection and biolistic transformation of meristems is plagued by extremely low efficiencies of heritable genetic transformation. Here we explore the contribution of cotton genotype and bacterial line to the overall efficiency of gene transfer from Agrobacteria to cotton cells.

Methods

Cotton hypocotyl sections from several cotton varieties were scored for genetic transformation after co-cultivation with two commonly used laboratory strains of A. Tumefaciens: LBA4404 {Ooms, et. al. 1981} and EHA105 {Hood, el al. 1993}. Co-cultivation of the explants and Agrobacteria followed our standard cotton transformation protocol (Bayley et. al. 1992) with the exception that the explants were scored for transformation just prior to the time they would normally be transferred to liquid culture for induction of somatic embryogenesis. The reporter gene used to score for genetic transformation consisted of the ß-Glucuronidase (GUS) coding region (from the Escherichia coli *uidA* gene) modified to contain a plant-functional intron (Liu, et. al. 1992). The presence of a plant intron interrupting the coding region of the GUS gene prevents production of significant levels of ß-glucuronidase within bacteria. In plant cells the intron-containing GUS mRNA is processed to remove the intron sequence, allowing production of functional GUS enzyme. To insure high sensitivity during in vivo assays, the intron-GUS construct is driven by a strong plant-functional "super-promoter" (a hybrid of two T-DNA promoters) and incorporated into a T-DNA containing binary plasmid (Narasimhulu, et.al. 1996). Gus activity was detected by staining with 5-bromo-4-chloro-3-indoyl glucuronide (X-GLUC) which is converted to an intense blue chromophore by GUS activity.

Results

The results of these experiments (Figure 1) clearly indicated a wide range in the ability of different cotton varieties to successfully receive and express transgenic DNA introduced by Agrobacteria. The overall transformation efficiency (as measured by the percentage of surviving hypocotyl ends successfully transformed to GUS positive) varied from 0% (Tamcot/LBA4404) to 85.8% (Greg35XL/EHA105). Additionally, a consistent difference in the effectiveness of cotton genetic transformation was exhibited by the two *A. tumefaciens* lines tested, with the EHA105 line consistently displaying higher efficiency than the LBA4404 line

Based upon the explant data we attempted Agrobacterium-based transformation of partially embibed cotton embryo meristem. Using a protocol similar to that used with explants, it was possible to demonstrate both transient and stable transformation of tissue surrounding the tip of the developing seedling (data not shown). Transient expression of the introduced genes peaked early in the transformation process with significantly fewer plant cells exhibiting stable transgene expression. A small number of plantlets grown from treated embryos exhibited various localized patterns of stable transgene expression.

Discussion

The results of these experiments demonstrate that further characterization of different Agrobacteria strains is necessary for optimization of Agrobacteria-mediated genetic transformation of cotton (both explant and embryo/meristem). One approach for improving overall efficiency of the procedures tested is to seek a better matching of compatible Agrobacterial lines and cotton varieties. Additionally, evidence from other plant species suggests that the transformation phenotype is associated with a limited number of plant genes, opening the possibility of enhancing cotton genetic transformation through a better understanding (and potential manipulation) of the plant genes involved.

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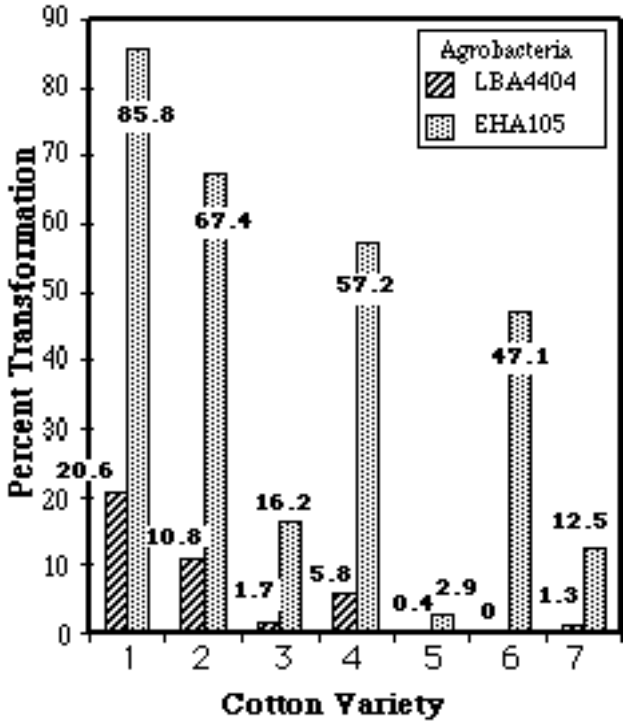

Figure 1. Graph showing transformation efficiencies for seven cotton varieties (**1**=Greg35XL, **2**=Greg65, **3**=HS26, **4**=Lankart57, **5**=Paymaster145 **6**=Tamcot, **7**=Coker312) co-cultivated with two lines of *A. Tumefaciens* (LBA4404-{backslash fill} and EHA105-{stipple fill}). The actual value for percent transformation is given above or within the appropriate bar.