TRANSFORMATION OF TEXAS CULTIVARS

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Abstract

Although transformation of cotton has been accomplished, the method is extremely limited to a few genotypes which will regenerate in tissue culture. A system to transform any genotype of cotton has been developed in this laboratory using the shoot apex as the target tissue for *Agrobacterium*-mediated gene transfer. Shoot apices of aseptically germinated seeds are isolated and cultured on a Murashige and Skoog inorganic salt formulation with vitamins, sucrose, myo-inositol, and agar. The apices are cocultivated for 2-4 days with the *Agrobacterium* and then subcultured onto media to control the *Agrobacterium* growth and containing either hygromycin, kanamycin, or glufosinate as a selectable marker. In 3 to 8 weeks rooted plants can be recovered and placed in soil. Primary plants and progeny were screened either for hygromycin, kanamycin, or glufosinate resistance to leaf application. Southern analysis of progeny showed one to 4 inserts of the foreign gene.

Introduction

In the literature on cotton transformation using *Agrobacterium tumefaciens*, Coker 312 or Coker 201 were used (Firoozabady et al., 1987; Umbeck et al., 1987; and Perlak et al., 1990; Bayley et al., 1992). These systems relied on regeneration from hypocotyl or cotyledon explants from the cotton seedling. The limitation of this system has been the lack of success in regeneration of cotton plants from other genotypes including the commercially important cotton cultivars. In order to transfer the foreign genes from Coker cultivars, and extensive backcrossing program was required to move the desirable genes into commercial cotton cultivars. This can take 5-7 years. Clearly the priority in cotton biotechnology was to develop a cell culture system to regenerate any cotton cultivar. This priority applies to all other crop plants as well.

This laboratory developed a genotype independent system of transformation using the seedling shoot apex and *Agrobacterium* as the gene vector and was demonstrated effective on a dicot, petunia, and a monocot, rice (Ulian et al., 1989, 1994, 1996; Park et al., 1997). This paper describes preliminary experiments on cotton transformation funded by TXCOT using this system.

Discussion

Agrobacterium tumefaciens strains are classified as octopine, nopaline, succinamopine or L,L-succinamopine types. Opines are products produced by the tumors and are catabolized by the *Agrobacterium* strain. Virulence of the *Agrobacterium* is mediated by *vir* genes in the T-DNA, and the phenolic compounds produced by the wounded plant cells are important in the host pathogen recognition step for infection. The *Agrobacterium* strain will make a difference in regard to successful infection and transfer of the foreign gene into a plant (Smith and Hood, 1995). This research will examine three *Agrobacterium* strains, LBA4404, and octopine strain, Z7075, a nopaline strain, and EHA101, a L,L-succinamopine strain.

Two gene constructs will be examined. One containing the gene for resistance to hygromycin plus the insect resistance gene, Bt, and the other with the gene for glufosinate (herbicide) resistance plus the Bt gene. Additionally the Bt gene will be either the full version or a truncated version. These gene constructs in the three *Agrobacterium* strains were provided by Mycogen Plant Sciences. Earlier work with the kanamycin resistant gene with the GUS reporter gene will be presented. We will determine whether or not one selectable marker is more effective than the other, as well as, whether or not the length of the gene construct will have a significant impact.

An additional experimental variable was the inclusion of three cotton cultivars, Coker 312 as a positive control, Sphinx and CUBQHGRPIS to determine whether or not there were any differences in infection and gene transfer in regard to the *Agrobacterium* strain and gene constructs.

All of these factors as well as cocultivation variables have been significant in the success of foreign gene transfer using *Agrobacterium*. This paper describes preliminary results of these experiments which are ongoing. To date we have demonstrated transformation of CUBQHGRPIS progeny by Southern blots and phenotype expression of the selectable marker gene. Additionally a Sphinx primary plant was shown to express the insect resistance gene by insect feeding bioassay. Genotype independent transformation of commercial cotton cultivars was confirmed.

Summary

To date over 11,000 shoot apices of the three cotton cultivars have been cultured. If all the controls are taken out, about 4,900 apices were exposed to the *Agrobacterium* strains carrying the foreign genes. Some apices are still in culture. Seventy seven primary plants have survived selection, rooted and survived transfer to soil. Seeds have been collected from about half of these primary plants. Half of these seeds or over two thousand progeny seeds were germinated and leaf tested for damage to either kanamycin, glufosinate, or hygromycin. The seedlings which had no leaf damage to the antibiotic or herbicide were grown to

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flowering and F2 seeds have been collected. Southern blot analysis of the DNA from leaf tissue of some of these plants has been analyzed and were positive for the presence of the foreign gene.

A leaf from one Sphinx primary plant still in culture was removed along with a control in vitro leaf and examined using the insect feeding bioassay. The Sphinx leaf control was totally consumed within 3 days; however, the Sphinx leaf with the insect resistance gene showed little damage and killed the tobacco budworms.

A summary of the other results to date has shown:
74% of the surviving plants had the truncated version of the Bt gene,
efficiencies of transformation ranged from 2-4%,
there were differences among the cotton cultivars and the *Agrobacterium* strains.

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