

**PROGRESS TOWARDS GENOTYPE
INDEPENDENT TRANSFORMATION**
Linda K. Koonce, Molecular Biology
Jane Dever, Plant Breeding
Teresa Burns, Tissue Culture
Norma Trolinder, Plant Physiology, BioTex
Lubbock, TX

Abstract

Regeneration and transformation of cotton is genotype dependent. A review of past accomplishments in regeneration and transformation has led to questions and hypothesis of the most promising techniques. Genetic studies have indicated that a blocker gene system controls the regeneration trait. Although several cultivars other than Cokers have now been regenerated, regeneration remains genotype dependent. New methods of transformation that negate the need for regeneration via somatic embryogenesis have been surveyed to determine which seems most likely to provide satisfactory efficiency of transformation.

Recovery of split shoot tips (meristems) after “whiskers” treatment, after *Agrobacterium* inoculation and under control conditions was optimized. Essentially 100% of untreated split shoot tips can be recovered but only 80 % can be recovered with when treated with *Agrobacterium* alone and a lower percentage when treated with whiskers alone. Only 50% of meristems can be recovered when treated with *Agrobacterium* and whiskers. Maximum recovery after occurred when shoot tips were isolated 6 hours after radical emergence and when treatment was administered 36 hours after culture. In order to further improve efficiency of the ultimate system a selection system for transformation events in the meristem must be available. Normally shoot tips grow away from the selective source and escape. A time, dosage, and transfer system was developed that will allow kanamycin for selection of transformed germline tissue.

Expression of the GUS marker gene was achieved in germline tissue after inoculation with *Agrobacterium*. However, frequency of transformation events in this tissue is extremely low. Further work is necessary to optimize transformation frequency in meristematic tissues with all of the methods screened.