MICROPROJECTILE MEDIATED STABLE TRANSFORMATION IN *PINUS TAEDA* L. (LOBLOLLY PINE)

A.-M. Stomp, D. Robertson and J. Parsons
Forestry Dept., North Carolina State University
Raleigh, NC

Methods for stable gene transfer and expression are critical for the advancement of molecular biology in forest tree species. We report the first stable expression of neomycin phosphotransferase in meristematic tissue of loblolly pine. Approximately 4000 loblolly pine cotyledons were bombarded with tungsten micropro jectiles carrying pRT99 plasmid DNA, using the DuPont PD-1000 biolistic particle delivery system optimized for transient expression (GUS) with this tissue. Plasmid pRT99 carries neomycin phosphotransferase (NPT II) and beta-glucuronidase (GUS), each under the control of a CaMV35s promoter. Cotyledons were cultured for shoot production following the procedures of Mott and Amerson (1982), with the medium containing either 10 or 20 mg/L kanamycin sulfate. Cotyledons were subcultured every two weeks. Gene expression was followed by growth and meristematic development on kanamycin and by GUS histochemical staining. After 3 months in culture, approximately 200 cotyledons remained alive and had proliferated meristematic tissue and 14 shoots. Meristematic tissue has been analyzed for GUS activity, the presence of NPT II protein (by ELISA), and has been found to contain NPT II protein, but assays of GUS expression levels are inconclusive. We are in the process of analyzing DNA from this material and of rooting shoots which continue to grow on kanamycin.

Mott, RL and HV Amerson (1982) N. C. Agric. Res. Ser. Tech. Bull. #271. 1-14.