

Antibiotics Effects on the Elimination of *Agrobacterium tumefaciens* from Loblolly Pine (*Pinus taeda* L.) Zygotic Embryo Explants and on Transgenic Plant Regeneration

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Poster Abstract

Three antibiotics were evaluated for their effects on the elimination of *Agrobacterium tumefaciens* during the genetic transformation of loblolly pine (*Pinus taeda* L.) using mature zygotic embryos as targets. *Agrobacterium tumefaciens* strains, EHA105, GV3101, and LBA 4404, all harboring the plasmid pCAMBIA1301, which carries the selectable marker gene, hygromycin phosphotransferase (hpt) controlled by the cauliflower mosaic virus 35S promoter and terminator, and the uidA reporter gene (GUS) driven by the cauliflower mosaic virus 35S promoter and the terminator of nopaline synthase gene, were used in this study. Exposure to 350 mg/l carbenicillin, claforan, and timentin respectively for up to 6 weeks did not eliminate the *Agrobacteria*, while antibiotics at 500 mg/l eradicated them from the co-cultivated zygotic embryos. All three antibiotics increased callus growth and shoot regeneration at 350 mg/l and 500 mg/l each, but reduced callus growth and shoot regeneration at 650 mg/l when compared with controls. Putative transgenic calli were selected for continued proliferation and differentiation on 4.5 mg/l hygromycin-containing medium. Transformed calli and transgenic plants produced on a selection medium containing 4.5mg/l hygromycin were confirmed by GUS histochemical assays, by polymerase chain reaction (PCR), and by Southern blot analysis. These results are useful for future studies on optimizing genetic transformation procedures in loblolly pine.

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