

EXPRESSION OF FOREIGN GENES IN TRANSGENIC YELLOW-POPLAR PLANTS

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Genetic improvement of yellow-poplar (*Liriodendron tulipifera*) by traditional breeding strategies has been a slow process because, like all forest trees, it has a long generation time. Genetic engineering by recombinant DNA technology has the potential to accelerate the introduction of new traits into forest trees. Certain considerations, however, should be taken into account when designing strategies to produce transgenic trees: (1) few tree species currently can be regenerated from protoplasts, (2) chimeras cannot be easily eliminated, as with herbaceous plants, by sexual transmission of the transformed genotype, and (3) large-scale production of transgenic trees will be accomplished most efficiently by somatic embryogenesis.

Transformation by microprojectile bombardment offers an approach to the production of transgenic plants from the growing number of tree species that can be regenerated from tissue culture. In this study, plasmid DNA was introduced by a particle gun into single cells and small cell clusters isolated from an embryogenic suspension culture of yellow-poplar. The plasmid pBI 121 carried marker genes encoding β -glucuronidase (GUS) and neomycin phosphotransferase (NPT II). Under constant antibiotic selection, transformed calli were isolated, subcultured into liquid medium, and regenerated into plants by somatic embryogenesis.

The number of copies of the GUS gene in independently transformed callus lines ranged from 3 to 30. An ELISA for NPT II and a fluorometric assay for GUS showed that the expression for both enzymes varied by less than four-fold among callus lines. A histochemical assay for GUS revealed a heterogeneous pattern of staining with the substrate X-gluc. However, expression of GUS and NPT II was detected by quantitative assays both in cell clusters that reacted positively (blue) and negatively (white) with X-gluc. Somatic embryos induced from transformed cell cultures were found to be uniformly GUS-positive by histochemical analysis. All transgenic plants sampled expressed the two marker genes in both root and shoot tissues. GUS activity was found to be higher in leaves than roots by fluorometric assays. Conversely, roots expressed higher levels of NPT II than leaves.