High Efficiency Transformation of Loblolly Pine *(Pin us taeda* L.) Using Green Fluorescent Protein as a Vital Screenable Marker

Wei Tang and Ronald J. Newton [1]

Poster Abstract

An engineered green fluorescent protein (m-gfp5-ER) gene under the control of the 35S Cauliflower Mosaic Virus promoter was used to develop a facile and rapid loblolly pine *(Pious taeda* L.) transformation system via *Agrobacterium tumefaciens-mediated* transformation of mature zygotic embryos. Green fluorescent protein has been introduced into three different loblolly pine families that are considered recalcitrant to transformation. The m-gfp5-ER gene produced bright-green fluorescence easily detectable and screenable in loblolly pine tissue 3-30 days after explants were co-cultivated with *Agrobacterium*. A high-level of GFP expression was detected in transgenic cells, tissues, and plants, and was localized in specific cells derived from cotyledons, hypocotyls, and radicles of mature zygotic embryos. Furthermore, in vitro and in vivo monitoring of GFP expression permitted a rapid and easy discrimination of transgenic shoots, and drastically reduced the quantity of tissue to be handled and the time required for the recovery of transformed plants. Integration of the m-gfp5-ER was confirmed by polymerase chain reaction (PCR), by Southern and northern blot analysis, and by junction DNA sequence analysis. Molecular analysis of *Agrobacterium* T-DNA loci in transgenic loblolly pine demonstrated that most of transgenic plants were derived from single transformation events. GFP-expressing shoots were also observed in loblolly pine explants co-cultivated with *Agrobacterium* but cultured in a medium without the selective agent kanamycin. This provides the opportunity to regenerate transgenic plants without using selectable-marker antibiotic-resistance genes, which will enhance the commercialization of transgenic plants.

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[1] Department of Biology, East Carolina University, Greenville, North Carolina 27858.