Microprojectile-Mediated Genetic Transformation of Embryogenic Sweetgum Cultures

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Poster Abstract

With its rapid biomass accumulation, sweetgum (*Liquidambar styraciflua*) may be particularly suitable for phytoremediation purposes, especially if it can be engineered with genes that enhance tolerance and/or accumulation of heavy metals. Sweetgum has been transformed using *Agrobacterium tumefaciens*-mediated transformation of shoot explants or nodule cultures, followed by adventitious shoot regeneration, and by microprojectile bombardment of nodule cultures. We tested microprojectile bombardment of embryogenic cultures as an alternative means to produce transgenic sweetgum trees, with the long-term goal of engineering the species with genes conferring heavy metal resistance. Embryogenic cultures were obtained by culturing immature seeds on an induction/maintenance medium (IMM) with 2,4-D and BA. Cultures proliferated as proembryogenic masses (PEMs) with monthly transfer to fresh medium and formed suspensions following transfer to liquid IMM. Bombardment parameters were optimized using transient GUS expression. Different osmotic conditioning and selection agents and concentrations were tested to determine suitable levels of these agents for sweetgum PEMS. PEMs representing 4 different genotypes were size-fractionated and bombarded with pBI426, which contains a translational fusion between the GUS and the NPTII coding region, under the control of a double 35S promoter. Following selection on proliferation medium supplemented with 50 mg/l of kanamycin, 8 kanamycin-resistant sweetgum lines were recovered, all from one of the bombarded genotypes. While all lines remaining kanamycin-resistant were GUS- and PCR-positive, and Southern analysis indicated stable integration of the transgenes, neither the transclones nor the untransformed control line from which they were derived were capable of producing somatic embryos, having apparently lost this potential over time. Sweetgum transclones cryostored for several months maintained GUS expression.

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