DEVELOPMENT OF A NEW GENETIC TRANSFORMATION SYSTEM FOR WHITE AND GREEN ASH USING EMBRYOGENIC CULTURES

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All North American ash species are under threat from the emerald ash borer (EAB; *Agrilus planipennis*), an exotic wood-boring beetle that has already destroyed millions of ash trees in the U.S. and Canada. One potential approach to managing this destructive insect is to develop a host-induced gene silencing strategy employing transgenic ash that express RNA interference (RNAi) constructs targeting EAB-specific genes. Exogenous double-stranded RNA triggers the RNAi pathway and can silence key genes, disrupting protein function and causing insect mortality. An important prerequisite for advancing this technology is a reliable transformation/regeneration system for ash. We tested embryogenic cultures of white ash (*Fraxinus americana*) and green ash (*Fraxinus pennsylvanica*) as target material for Agrobacterium-mediated gene transfer with marker genes. In a series of experiments, cells of different suspension-grown white ash and green ash embryogenic cultures were collected and inoculated with *A. tumefaciens* strain AGL-1 carrying the pFHI-GUSi expression vector, which, in addition to the intron-GUS reporter gene, also includes the nptII selectable marker gene. After inoculation, cells were maintained on semi-solid medium that contained 35 mg/l geneticin and 300 mg/l timentin to select for transformed cells and eradicate Agrobacterium. Once putative transgenic events were identified, they were assayed at every developmental stage from callus to plantlets using the fluorometric assay for β-glucuronidase to confirm the presence of the transgene. When plants were obtained, DNA was isolated from leaf tissue and PCR was used to confirm the insertion of the intron-GUSi gene. To date, two genotypes each of white ash and green ash have been transformed and multiple events from both species have been successfully acclimated to soil.