Establishment and Application of Embryogenic Cultures for Conservation and Restoration of Multiple North American Ash Species

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The development of systems for mass clonal propagation systems for North American ash (*Fraxinus)* species would significantly aid ash germplasm conservation and restoration efforts in the face of the devastating losses of these trees to the exotic insect pest emerald ash borer (EAB; *Agrilus planipennis*) during the past two decades. We have initiated somatic embryogenic (SE) cultures of multiple ash species over the past several years and are applying them for clonal propagation of putatively resistant genotypes for EAB resistance testing, for conservation of ash germplasm via cryopreservation, and as targets for gene transfer for eventual testing of candidate EAB resistance genes. SE cultures have been initiated from open-pollinated and control-pollinated seeds of putatively EAB-resistant "lingering" green ash (*F. pennsylvanica*) and white ash (*F. americana*) trees provided by Ohio State University and US Forest Service Northern Experiment Station cooperators. Trees regenerated from these SE cultures will facilitate clonal testing for EAB resistance and, potentially, development of EAB-resistant ash varieties. We have optimized a cryopreservation protocol for the ash embryogenic cultures that will allow us to store the cultures while trees regenerated from the cultures are tested in the field. We have also initiated embryogenic cultures of multiple rare North American ash species, including Texas ash (*F. albicans*), Mexican ash (*F. berlandieriana*) and Carolina ash (*F. caroliniana*), and cryostored copies of the cultures to conserve germplasm of these species. With the recent appearance of EAB in Oregon in 2022, we have initiated a project with USFS Dorena Genetic Resource Center collaborators to develop SE cultures of Oregon ash (*F. latifolia*) and have already shown that this species can be clonally propagated via SE. Embryogenic ash cultures also make excellent target material for gene transfer via Agrobacterium-mediated transformation. Working with scientists at the University of Kentucky and the USFS, Southern Research Station, we have demonstrated that we can stably transform green ash and white ash embryogenic cultures to produce transgenic trees expressing marker genes. Work on testing RNAi technology as a means of conferring EAB resistance to ash trees is in progress. Overall, the research demonstrates the multiple contributions that SE technologies can potentially make to ash conservation and restoration.