Development of Embryogenic Cultures for Conservation and Restoration of Redbay, Swamp Bay and Sassafras

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Since the effects of the vascular disease laurel wilt on redbay (*Persea borbonia*) trees were first described in 2002, it has spread rapidly throughout redbay and swamp bay (*P. palustris*) populations in the southeastern U.S., with very high mortality rates. The disease, caused by the redbay ambrosia beetle (*Xyleborus glabratus*) and its fungal symbiont (*Harringtonia lauricola*), has also rapidly increased mortality of sassafras (*Sassafras albidum*) trees in the region. One approach to potential restoration of these species is to employ natural genetic tolerance to the ambrosia beetle and/or fungal pathogen, which may already exist in populations of the affected species. Researchers at the University of Florida have identified redbay genotypes that appear to be tolerant of the pathogen. We are developing somatic embryogenic (SE) culture systems for all three species for eventual use for mass propagation of laurel wilt-tolerant genotypes. Immature fruit were collected by University of Florida cooperators from multiple redbay clones in their collection, and from swamp bay trees on the UGA campus in July and August 2020 and 2021. Immature fruit were collected from four sassafras source trees growing in a USFS test planting in Athens, GA in June 2022. Immature embryos were dissected from the fruit and cultured with associated endosperm on woody plant medium (WPM) containing either 2 mg/L 2,4-D or 0.2 mg/L picloram. Most redbay and swamp bay explants either made small amounts of non-morphogenic callus or turned black in culture within several weeks. Repetitively embryogenic cultures were only recovered from one redbay explant and one swamp bay explant cultured in 2021. Four repetitively embryogenic cultures were established from sassafras explants representing two source trees. All the embryogenic cultures were induced on medium with 0.2 mg/L picloram. Repetitive somatic embryo production has been maintained in the redbay and swamp bay cultures for almost two years and in the sassafras cultures for one year by monthly transfer to fresh medium. The cultures also have produced developing somatic embryos with cotyledons on the same medium. Transfer of sassafras somatic embryos to liquid basal medium lacking picloram promoted production of large, spherical somatic embryos resembling mature zygotic embryos following ten weeks in suspension culture in the dark. Some of these embryos produced roots and short shoots following transfer from suspension to semisolid basal medium with activated charcoal and incubation in the light. Cold pre-germination treatments are now being tested to see if they will enhance sassafras somatic embryo germination. With further development, we believe that SE technologies will aid in the conservation and restoration of North American Lauraceae species affected by laurel wilt.