PROPAGATION OF "LINGERING ASH" GENOTYPES VIA SOMATIC EMBRYOGENESIS FOR EMERALD ASH BORER RESISTANCE TESTING

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Ash (*Fraxinus* sp.) is an economically and ecologically significant genus of hardwood forest tree. Unfortunately, North America's native ash resource is currently threatened by the rapid spread of emerald ash borer (EAB; *Agrilus planipennis*), an exotic wood-boring beetle, which is causing widespread damage and mortality of ash trees in North America. Our research employs germplasm gathered from green ash *(Fraxinus pennsylvanica*) and white ash (*Fraxinus americana*) trees to optimize a somatic embryogenesis *in vitro* propagation system, ultimately, in order to generate EAB-resistant planting stock to aid ash restoration efforts. Building upon preliminary green ash somatic embryogenesis work (Li et al. 2014), our work focuses on producing embryogenic cultures and somatic seedlings of "lingering" white ash. "Lingering" ash trees are healthy trees which are intermingled with dead ash trees where EAB infestation has caused almost complete mortality of mature ash trees in a particular area. The fact that these lingering ash trees have survived infestation may indicate genetically-based resistance or tolerance to EAB. By cloning lingering ash trees through somatic embryogenesis, it may be possible to propagate trees that are naturally resistant to EAB, eliminating the need to use gene transfer techniques to produce transgenic trees that carry resistance to EAB. A culture initiation experiment using seed and zygotic embryo explants from lingering ash parents in Michigan showed that seed collection date and explant type (naked zygotic embryo versus whole seed) significantly affected embryogenesis induction, while, 2,4-D concentration (2 or 4 mg/L) and explant length did not, as long as the zygotic embryo was over 4.5 mm long. Induction ranged as high as 37.5% for some genotypes and dates. White ash embryogenic cultures produced somatic embryos that were used in subsequent germination experiments, which tested the effects of activated charcoal and gibberellic acid (GA3) on germination. The treatment combining 0.5 g/L activated charcoal and 10 mg/ L GA3 produced the most vigorous somatic seedlings in the shortest time. Several of these somatic seedlings have survived transfer to *ex vitro* conditions and are growing in the greenhouse. By successfully inducing embryogenesis from explants gathered from lingering ash trees, our results show a promising step toward clonal propagation of potentially EAB-resistant trees. Once optimized, this system could eventually be scaled-up for mass clonal propagation of EAB-resistant planting stock to aid forest restoration in areas affected by EAB.

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