APPLICATION OF SOMATIC EMBROGENESIS FOR MASS PROPAGATION OF HYBRID HEMLOCKS

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The eastern North American hemlock species, eastern hemlock (*Tsuga canadensis*) and Carolina hemlock (*Tsuga caroliniana*) were important components of eastern forest ecosystems, but over the past few decades, they have been devastated by hemlock woolly adelgid (HWA). Interest in hybridizing these hemlocks with Asian hemlock species, Chinese hemlock (*T. chinensis*), Northern Japanese hemlock (*T. diversifolia*), and Southern Japanese hemlock (*T. sieboldii*), has recently increased, due to the Asian species' resistance to HWA. Breeders have successfully crossed Carolina hemlock with each of the Asian species, but testing and eventual scaled-up production of promising hybrids could be greatly enhanced by combining hybrid breeding efforts with *in vitro* propagation and cryopreservation. In this study, embryogenic cultures of hybrid hemlocks were initiated by culturing immature zygotic embryos derived from interspecific crosses on a modified Litvay medium containing 2,4-dichlorophenoxyacetic acid and 6-benzylaminopurine. Abscisic acid (ABA) and activated carbon were tested at different levels in embryo development medium for their effects on somatic embryo development and maturation, and we found that 56 µM ABA and 2 g/L activated charcoal produced the highest numbers of cotyledonary-stage somatic embryos. Cotyledonary somatic embryos were given a pre-germination desiccation using a slow-drying process and then used in experiments to test the effect of light quality on somatic embryo germination and conversion, which employed LEDs of different wavelengths in comparison to the fluorescent light control. Preliminary results indicated that hemlock somatic embryos incubated under fluorescent light developed red hypocotyls, while hypocotyls of those incubated under red light supplied by LEDs remained green. Different light qualities also differentially affected other aspects of hemlock somatic embryo germination. We also demonstrated the potential to conserve germplasm of these hybrids via cryopreservation of the embryogenic cultures. This is first report describing an *in vitro* propagation and cryopreservation system for hybrid hemlocks via somatic embryogenesis.

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