Long-Term Preservation of American Chestnut Germplasm By Cryostorage of Embryogenic Cultures

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Since the chestnut blight, *Cryphonectria parasitica,* invaded the Eastern United States almost a century ago, the American chestnut (*Castanea dentata*) tree has been transformed from a dominant canopy and timber-producing species into a relatively scarce, understory tree. Recent evidence suggests that the tree may be disappearing entirely from the southern part of its original range as stumps cease to resprout. The ability to cryostore embryogenic chestnut cultures may aid in the preservation of germplasm from a shrinking gene pool of existing American chestnuts and help to ensure the distribution of blight resistant material. Two embryogenic American chestnut culture lines, initiated from seed explants, were subjected to a cryostorage protocol originally developed for radiata pine. Prior to freezing, cultures were given an osmotic pretreatment in medium supplemented with 0.4 M sorbitol, then cryoprotected with either 5% or 10% dimethylsulfoxide (DMSO) to prevent cell dehydration and freezing injury. Cultures were gradually frozen to -80 C using Nalgene freezing containers and an ultra-low freezer, after which they were immersed in liquid nitrogen (-196 C) for 24 hours. Following thawing, evaluation of recovery of cryostored material was based on visual assessment of regrowth and fresh weight gain. Within 2-3 weeks, globular-stage embryos appeared and after 4-6 weeks heart, torpedo, and cotyledon stage embryos formed. Both DMSO treatments tested yielded 100% recovery for both genotypes. However, cultures protected with 5% DMSO recovered more rapidly. Fresh weight gain data also suggested a genotypic effect on recovery, with one line recovering more readily than the other, regardless of DMSO concentration.