DEVELOPMENT AND OPTIMIZATION OF A CRYOSTORAGE PROTOCOL FOR EMBRYOGENIC ASH CULTURES

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Efforts to conserve ash germplasm and eventually restore ash species to their native ranges and as landscape trees following their devastation by emerald ash borer (*Agrilus planipennis*; EAB) could be greatly aided if ash genotypes could be safely archived for later propagation and out-planting. One approach for storing forest tree germplasm indefinitely is cryopreservation. Embryogenic cultures of several forest tree species have been shown to be highly amenable to cryostorage and recovery. The goal of this study was to develop and optimize cryostorage and recovery protocols for white ash (*Fraxinus americana*) embryogenic cultures for use in ash germplasm conservation and species restoration. Three experiments were conducted testing different cryoprotection agents and concentrations, which included sorbitol, sucrose, glucose, polyethylene glycol and DMSO, using six different ash embryogenic culture lines. Following pre-treatment, all cultures were stored in a liquid nitrogen cryofreezer for at least 48 h before removing them for thawing and recovery. Regrowth of cryostored cultures was scored visually 3-5 weeks following their removal from cryostorage. Experiment 1 and its replication, Experiment 2, which tested three different DMSO concentrations, indicated that 10% DMSO provided higher recovery rates than 5% or 15% DMSO across the six tested genotypes, although the only statistically significant difference was between 10% and 5% DMSO in Experiment 2. Recovery varied widely among genotypes, with some genotypes re-growing with multiple DMSO concentrations, and some failing to regrow regardless of DMSO concentration. The failure of some genotypes to regrow following cryostorage prompted us to test different cryoprotection treatments in Experiment 3, employing sucrose or glucose and PEG as osmotica in place of sorbitol. Results from this experiment indicated that, compared to sorbitol, neither sucrose/PEG nor the glucose/PEG treatment improved regrowth of the cultures following cryostorage, and that the major determinant of potential for regrowth remained the genotype of the culture line.