Progress with American Chestnut Somatic Embryogenesis

G. M. Andrade and S. A. Merkle[1]

Poster Abstract

American chestnut (*Castanea dentata*), once the dominant forest species of the Appalachian Mountains in the eastern United States, was devastated during the early twentieth century by the introduction of the chestnut blight fungus. As part of an effort to restore the species to the forest, we have been working with embryogenic cultures of the species, aiming to establish a reliable somatic embryogenesis system for mass clonal propagation, as well as for genetic transformation with potential anti-fungal genes. While initiation of embryogenic cultures from immature ovules of American chestnut has become routine, bottlenecks still remain for embryo maturation, germination and conversion. Effects of cold stratification, gellan gum concentration and activated charcoal on somatic seedling production were investigated. Studies of other variables, such as the effects of light quality on germination and conversion, are underway. Using five genotypes, clusters of proembryogenic masses maintained on WPM with 2 mg/l 2,4-D were transferred to basal WPM for somatic embryo development. Individual cotyledonary-stage embryos (2-4 mm) were cultured for 10 days on basal medium prior to storage at 4^{o} C for 12 weeks in the dark. These embryos germinated at an average frequency of 23% following transfer to WPM basal medium in GA7 vessels in the light. Embryos that did not receive cold treatment or were stored for only 6 weeks failed to germinate. Embryos on WPM with 5 g/l activated charcoal (AC) germinated at the same frequencies as those cultured without AC, but AC prevented darkening of taproots. Embryos cultured on 3 or 6 g/l Phytagel germinated at higher frequencies following 12 weeks cold storage than did those cultured on 10 g/l Phytagel. To date, over 30 somatic seedlings representing 3 genotypes have been transferred successfully to greenhouse conditions.

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[1] Daniel B. Warnell School of Forest Resources, University of Georgia, Athens, Georgia 30602.