Section 3 Abstracts: Chestnut Tree Breeding, Propagation and Physiology

Somatic Embryogenesis and Gene Transfer in American Chestnut. Scott Merkle, D.T. Carraway, B.A. Watson-Pauley and H.D. Wilde. School of Forest Resources, University of Georgia, Athens, GA 30602, USA

Somatic embryogenesis is a plant propagation technique that may be combined with current gene transfer techniques for application in the production of blight-resistant American chestnut *(Castanea dentata).* Two experiments in consecutive years tested effects of explant source and culture conditions on initiation of American chestnut embryogenic cultures. In a preliminary study, developing ovules from nuts collected from 3 trees approximately 6 wk postanthesis and cultured on semisolid induction medium containing 0.25 mg BM and either 6 mg NAM or 4 mg 2,4-D/1 produced embryogenic cultures. Ovules pulsed for 1 or 2 wk on auxin-containing media and subsequently transferred to media without auxin produced multiple embryos directly from the radicle end of the zygotic embryo, while those maintained on auxin-supplemented media initially produced proembryogenic masses, which formed globular and heart-stage embryos as they aged. Transfer of clusters of somatic embryos from auxin-supplemented media to hormone-free medium promoted maturation of embryos to the cotyledon stage. In a more extensive study the following year, cultures were initiated from nuts collected at 5 different stages from 13 different source trees at 4 locations in the current range of the species. Ovules and immature zygotic embryos from nuts of 11 or 13 trees produced embryogenic cultures, while no embryogenic cultures were obtained from mature zygotic embryo explants. A histochemical assay demonstrated transient expression of B-glucuronidase (GUS) in embryogenic cultures following microprojectile bombardment. This reporter gene can be employed in gene transfer experiments directed at obtaining stable integration of foreign DNA into the American chestnut genome.