A TRANSGENIC AMERICAN CHESTNUT SYSTEM FOR EVALUATION OF PATHOGEN RESISTANCE GENES

Campbell J. Nairn[1], Lisheng Kong, Haley Houke, Scott A. Merkle

[1]Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA

Forest tree species are increasingly exposed to novel pathogens introduced as invasive species outside their native ranges. The rapid spread of plant pathogens facilitated through anthropogenic activities circumvents natural evolutionary processes that produce resistance or tolerance in host species. A goal of the Forest Health Initiative is to explore the potential role of biotechnologies, including transgenics, for addressing existing and emerging threats to forest health. With the large number of plant genes that have been implicated in pathogen resistance, a robust system for gene identification, vector construction, plant transformation and regeneration of tree species is needed. We have developed an efficient embryogenic tissue culture, transformation, selection, and regeneration system and combined that with a modular expression vector to rapidly clone and express candidate pathogen resistance genes (PRGs) in American chestnut (*Castanea dentata*). The system facilitates cloning of PRGs from genomic analyses within and between species as well as the incorporation of PRGs from diverse heterologous sources. Approximately 30 PRGs have been cloned and transformed into each of several American chestnut (AC) genotypes. With a goal of producing 10-20 independent transgenic lines (events) for each PRG and AC genotype combination, dozens of transgenic lines for each PRG are screened for transgene integration and expression. Embryogenic cultures representing over 2,000 independent transgenic lines have been screened approximately 6-8 weeks post-transformation for transgene integration. Individual lines are then screened prior to plant regeneration by qRT-PCR to characterize transgene expression levels. Expression profiling reveals a wide range of PRG expression levels in independent transgenic lines, which can vary by more than 100 fold in AC lines harboring the same PRG vector. Using these data, independent lines that represent the full range of moderate to high expression levels are selected for plant regeneration and eventual testing for in vivo pathogen resistance.