GENETIC TRANSFORMATION OF *FRAXINUS* SPP. FOR RESISTANCE TO THE EMERALD ASH BORER

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The emerald ash borer (EAB; *Agrilus planipennis* Fairmaire) (Coleoptera; Buprestidae) is a wood-boring beetle that poses substantial risk to the ash resource in North America. Ash species native to the United States and known to be susceptible to EAB are *Fraxinus pennsylvanica* (green ash), *F. americana* (white ash), and *F. nigra* (black ash) trees, which are major components of the landscape; and blue ash (*F. quadrangulata*) and pumpkin ash (*F. profunda*), which are less common species. Ash timber is valued for applications requiring strong, hard wood. Green ash is used both for solid wood applications, such as crating, boxes, and tool handles, and for fiber in the manufacture of fine papers. White ash is the primary commercial hardwood used in the production of baseball bats; it also is made into furniture, flooring, doors, and cabinets. Black ash is typically used for interior furniture and cabinets, and Native Americans require this species for the art of basketry. Beyond manufacturing, ash trees play an important ecological role by providing aesthetic value, and shelter and food for wildlife. EAB threatens these resources and may permanently alter forest ecosystems throughout North America. The development of transgenic *Fraxinus* spp. exhibiting resistance to EAB attack will have great economic and ecological benefits for landowners, the nursery and forest products industries, and the U.S. foreign trade of ash lumber and logs.

This research will result in the development of an *Agrobacterium*-mediated transformation system for *Fraxinus* spp. with recovery of transgenic plants resistant to EAB. In addition, this research will initiate studies of effective deployment strategies for transgenic ash trees. Data generated will be used for risk assessment, which is necessary for commercial release. Another major focus of future studies will be to examine the use of sterility genes to further modify transgenic ash to alter flowering and prevent gene escape.

An adventitious shoot regeneration and rooting protocol was first developed for green ash and is being optimized for black and white ash. The best regeneration medium for freshly isolated green ash hypocotyls was Murashige and Skoog (MS) medium supplemented with 13.3 µM 6-benzylaminopurine (BA) plus 4.5 µM thidiazuron (TDZ). The effect of in vitro-germinated seedling age on adventitious shoot regeneration from hypocotyl explants was also studied. Results showed that hypocotyl explants from freshly isolated embryos exhibited a higher organogenic potential than 4- to 15-day-old explants. Adventitious shoots from hypocotyls were established as proliferating shoot cultures following transfer to MS basal medium with Gamborg B5 vitamins supplemented with 10 µM BA plus 10 µM TDZ. A high rooting percentage was achieved when adventitious shoots were rooted on woody plant medium containing 4.9 µM indole-3-butyric acid (IBA) plus 5.7 µM indole-3-acetic acid (IAA) with a combination of a 10-day dark culture period followed by a 16-h photoperiod. Rooted plants were successfully acclimatized to the greenhouse, and 100 percent survived after overwintering in cold storage. This regeneration system provided a foundation for developing a genetic modification protocol for green ash. We are also investigating an adventitious shoot regeneration system using leaf or internodal explants.

A genetic transformation protocol was first developed for green ash hypocotyl explants and is currently being optimized for black and white ash. Green ash hypocotyls

were transformed using *Agrobacterium tumefaciens* strain EHA105 harboring binary vector pq35GR containing the neomycin phosphotransferase (*nptII*) and β-glucuronidase (GUS) fusion gene. After a 1-day pre-culture on regeneration medium, hypocotyl explants were transformed in the presence of 100 µM acetosyringone using 90-sec sonication plus 10-min vacuum-infiltration. Kanamycin at 20 mg l^{-1} was used for selecting transformed cells. Adventitious shoots regenerated on MS medium supplemented with 13.3 µM BA, 4.5 µM TDZ, 50 mg l^{-1} adenine sulfate, and 10 percent coconut water. GUS- and polymerase chain reaction (PCR)-positive shoots from the cut ends of hypocotyls were produced via an intermediate callus stage. Presence of the GUS and *nptII* genes in GUS-positive shoots was confirmed by PCR, and copy number of the *nptII* gene in PCR-positive shoots was determined by Southern blotting. Three transgenic plantlets were acclimatized to the greenhouse and then successfully survived overwintering in cold storage. Studies are underway using a construct containing the Cry8Da protein of *Bacillus thuringiensis* for genetic transformation of ash for resistance to EAB.