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Development of Transgenic North American White Ash (Fraxinus americana) Expressing a Bacillus thuringiensis Protein for Management of the Emerald Ash Borer

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ABSTRACT

White ash (WA), *Fraxinus americana*, is an integral part of the hardwood forest ecosystem. Economically, WA provides wood for important products such as baseball bats, tool handles, and hardwood flooring. Ecologically WA provides cover and mast to support wildlife. The emerald ash borer (EAB) is a significant threat to all ash species because of a lack of native resistance in North American ash trees, its rapid spread, and the ineffectiveness and expense of control measures. EAB is a non-native beetle that consumes tree vascular tissue while in the larval stage. The development of an *Agrobacterium*-mediated transformation protocol may impart systemic resistance to EAB via the Cry8Da toxic protein, which is lethal to EAB larvae and naturally found in *Bacillus thuringiensis* SDS-502. Development of a transformation system for WA will allow this gene or others imparting resistance to be successfully incorporated into the WA genome. Embryos were extracted, transformed, and cultured on a selection-regeneration medium, killing any non-transgenic tissues while allowing for transgenic shoot formation. Transformed hypocotyls will be further cultured on a selection-elongation medium to allow for shoot development, after which shoots will be rooted and acclimatized to greenhouse conditions. Additional confirmation of transgenic plants will be conducted through quantitative measurements of the FLAG protein, polymerase chain reaction, and finally a feeding assay performed with EAB larvae. WA hypocotyls regenerated shoots after transformation at a 27.8% efficiency rate. The transformed hypocotyls received 6 weeks of exposure to kanamycin in the selection medium, allowing for positive results within the scope of this project. This protocol can be used to further genetically modify *F. americana*, allowing for preservation of this ecologically and economically important species.

KEYWORDS

Fraxinus americana, transformation, EAB, emerald ash borer, agrobacterium

REFERENCES

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