Genetic Transformation of the Ozark Chinquapin Tree (*Castanea ozarkensis*)

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The Downfall of the Ozark Chinquapin

This keystone tree species of the Ozarks region was devasted following the arrival of the invasive fungal pathogen, *Cryphonectria parasitica,* to the range in the 1950s. Billions of trees in this genus were killed. Genetic diversity of the tree is being lost as stump resprouting ability is diminishing with time. Efforts to conserve and restore the tree are critical.

Castanea Comeback Using Biotechnology

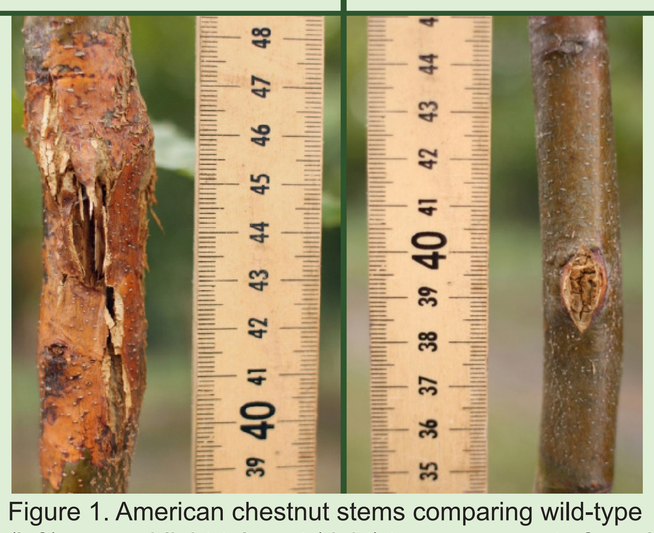


Figure 1. American chestnut stems comparing wild-type (left) versus blight-tolerant (right) tree response to fungal innoculation. Pictures courtesy of Andy Newhouse.

Protection from the fungus is now possible. A blight-tolerant American chestnut (*Castanea dentata*) has been developed by incorporating a plant defense gene, Oxalate Oxidase (OxO), into the tree's genome (Polin et al, 2006, Zhang et al, 2013 & Newhouse et al, 2014). OxO detoxifies the oxalic acid produced by the fungus and prohibits the formation of deadly cambium cankers (Fig 1).

Research Objective

To determine if the methodology used to develop a transgenic American chestnut (*C. dentata*), can be applied to the Ozark chinquapin (*C. ozarkensis*).

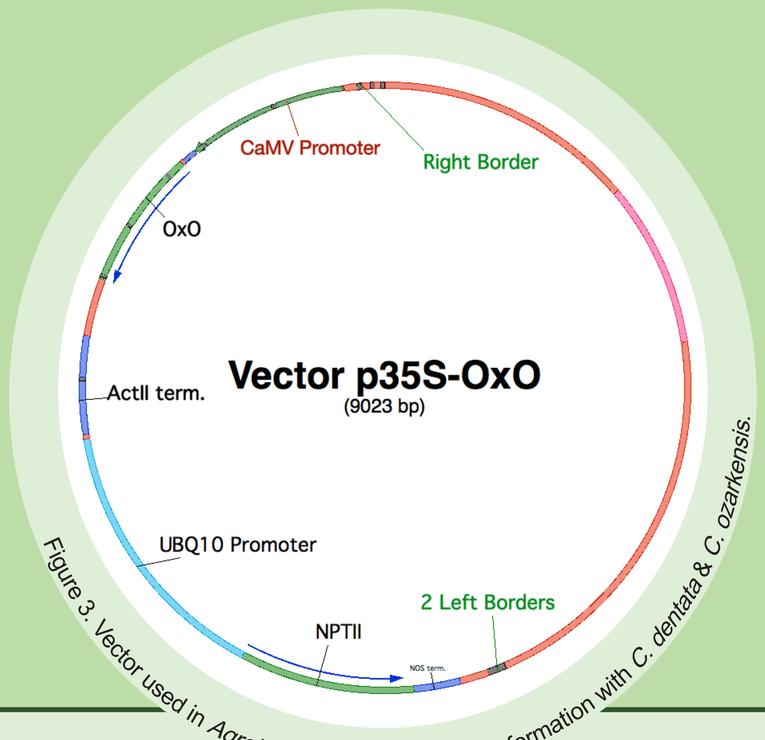
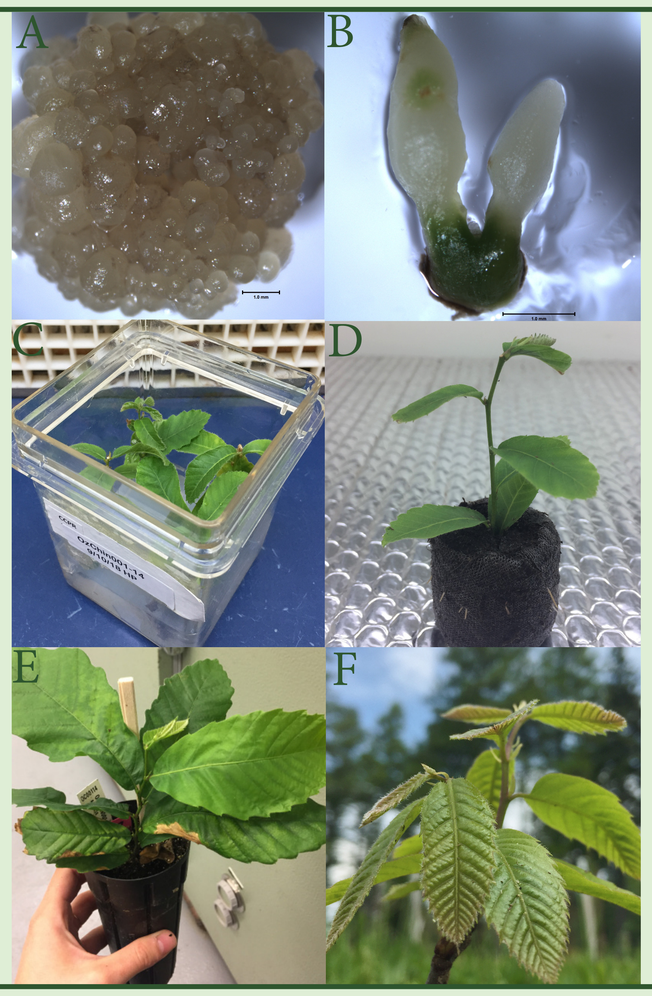


Figure 3. Vector used in *Agrobacterium*-mediated transformation with *C. dentata* & *C. ozarkensis*.

Step 1: Regeneration of Ozark Chinquapin Embryos



Somatic embryos (genotype "1-14") isolated from an immature chinquapin nut are maintained *in vitro* (A). Using a series of growth media, embryogenesis was initated and embryos differentiated through stages such as the cotyledonary stage (B). Germinated embryos were multiplied using axillary buds and apical tips (C). Shoots produced roots using an exogenous auxin dip (D). Once rooted, plantlets were potted into soil and grown in growth chambers followed by the greenhouse (E). Regenerated trees were planted in the field in approximately one year after germination (F). When somatic embryos were shown to regenerate into whole plants, transformation began.

Step 2: Inserting Blight-Tolerance Gene (OxO)

Figure 4A. Embryos were rotated in tubes containing *Agro.* & Acetosyringone solution.

Figure 4B. Embryos in bioreactors were periodically flooded with media & antibiotics.

Agrobacterium (AGL1) solution containing the p35S-OxO binary vector (Fig 3) was mixed with somatic embryos (Fig 4A), followed by a two day desiccation period on sterile Petri dishes with filter paper. Embryos were transferred to bioreactors containing a medium with antibiotics to kill *Agro.* (Fig 4B). After two weeks, paromomycin was added to the medium to select for transformed embryos. After an eight week selection period, surving embryos were transferred to a semisolid medium and subcultured every two weeks until DNA extractions and PCR were performed.

Transgenic Ozark Chinquapin

PCR confirmed one embryo culture was positive for the presence of the OxO gene (Fig 5). Results indicated using the established transformation method is possible for *C. ozarkensis*.

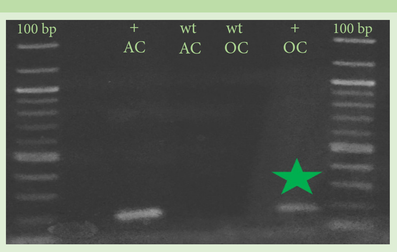


Figure 5. Starred band shows OxO presence in transformed chinquapin DNA (+OC). Transgenic American chestnut (+AC) was used as the postive control.

Next Steps

- Regenerate transgenic embryos to check for copy number and gene expression.
- Perform fungal innoculations on leaves and stems to measure blight tolerance.
- Conduct optimization experiments with the transformation protocol to increase event yield.
- Initiate new embryo cultures *in vitro* to transform new genotypes.

Acknowledgements

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For More Information

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References

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