

The American Chestnut Founder Line *OxO* Transformation Project

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Production of American chestnut (*Castanea dentata*) trees expressing the wheat oxalate oxidase gene (*OxO*) to provide resistance to the chestnut blight fungus (*Cryphonectria parasitica*) has been adopted by The American Chestnut Foundation (TACF) as a promising means to restore the tree to forests of the eastern U.S. The primary path chosen by TACF for spreading the transgene to multiple genetic backgrounds for restoration is via pollinating American chestnut trees with pollen produced by Darling 58 transgenic *OxO* trees. An alternative approach is to directly insert the *OxO* gene into multiple American chestnut genotypes representing the natural genetic diversity of the species. The resulting trees would already be adapted for growth in their native regions. We began pursuing this approach by initiating new somatic embryogenic (SE) culture lines (“Founder Lines”) from nuts collected by TACF cooperators from large surviving American chestnut trees (LSAs) growing in different parts of the range from Maine to Georgia. In 2020, over 100 new embryogenic cultures representing eight source trees from five regions (New England, Pennsylvania, Maryland, Virginia, Georgia) were captured. Copies of all the new Founder Lines were placed in cryostorage. The cultures were then screened for their abilities to produce abundant somatic embryos and high-quality somatic seedlings, to facilitate choosing those to target for transformation with *OxO*. The selected Founder Lines showed a range of sensitivities to the selection agent geneticin in liquid medium, necessitating that selection be customized for each line. Transformation experiments with these lines using the pFHI-OXO vector have produced over 80 PCR-positive events in nine culture line backgrounds thus far. Some of these PCR-positive events are currently being grown up in suspension culture for somatic embryo and somatic seedling production. Future plans call for the transgenic lines to be screened for *OxO* transgene copy number, mRNA expression level and production of a functional *OxO* enzyme. We have also begun working on transforming selected Founder lines with the pWIN3.2-OXO vector to produce events that will have *OxO* expression under the control of a wound-inducible promoter. We hope these Founder Line transformations will eventually lead to restoration of regionally-adapted, blight-resistant American chestnut trees.