Genomic Tools for American Chestnut Restoration

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The early 20th-century introduction of *Cryphonectria parasitica* in North America caused the functional extinction of American chestnut (*Castanea dentata*), resulting in profound ecological, economic, and societal losses. Two potential solutions—introgressive hybridization with resistant Asian Castanea species and genetic modification—are currently being explored to develop disease-resistant chestnut populations. The hybrid breeding program has produced families with moderate resistance, but the approach is based on a flawed three-gene resistance model. The low heritability of the trait has further complicated the selection process. Genetic modification, specifically the overexpression of an oxalate oxidase (OxO) transgene, produces strong resistance. However, multiple outcrossing generations are required to restore genetic diversity. To overcome these challenges, we have produced several population and functional genomic datasets. We first created a genomic prediction model using progeny-test data, which assisted in selecting the top 1% of backcross families. We also sequenced the whole-genomes of approximately 350 wild C. dentata stump sprouts to characterize neutral and adaptive patterns of variation, thus informing *ex situ* conservation of wild germplasm for breeding with transgenic lines. A subset of backcross families was also sequenced to gauge the breeding program's efficacy in capturing wild adaptive diversity. Finally, although OxO-based resistance is expected to be long-lasting, this is not guaranteed. Hence, we are in the process of identifying additional candidates for transgenic manipulation. This is being done by integrating RNA-seq of a blight canker development time series in resistant and susceptible species, a whole-genome comparative analysis of all Castanea species, and QTL mapping within the backcross breeding program. The outcomes from these diverse projects will underpin our long-term objective of developing disease-resistant, locally-adapted American chestnut populations, thereby aiding in the species' restoration.