

BEYOND HYBRID BACKCROSS BREEDING: THE INTERSECTION OF THE AMERICAN CHESTNUT FOUNDATION'S BREEDING PROGRAM AND VARIETAL FORESTRY

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Since its inception in the early 1980s, The American Chestnut Foundation (TACF) has made substantial progress on developing blight-resistant chestnut trees through its hybrid backcross breeding program with American chestnut (*Castanea dentata*; AC) and Chinese chestnut (*Castanea mollissima*; CC). Following the generation of the original F1 hybrids, the hybrids were backcrossed with AC for three generations, with selection at each generation for blight resistance and AC form, to obtain the BC3 generation, followed by two generations of intercrosses to obtain the BC3F3 (or B3F3) generation. B3F3 trees, which should average 15/16 AC genes and 1/16 CC genes, are known as the "Restoration 1.0" chestnuts. B3F3 nuts are currently being produced in open-pollinated (OP) seed orchards, and thus make use of "half-sib" technology. While the broad range of phenotypes produced by half-sib technology may be useful for restoration purposes, we believe there will likely be an increasing demand for B3F3 or similar hybrid chestnut trees by landowners who wish to grow them on a commercial scale for production of durable (i.e. decay-resistant) timber for outdoor uses (decks, play structures), short-rotation biomass energy plantations and/or nuts. Since landowners will want to plant the best genotypes, rather than a collection of variable genotypes, TACF may want to consider other means of producing planting stock besides open-pollinated seed orchards (i.e. full-sib or varietal technologies) for this market. Southern pine breeding programs have already moved from OP seed orchards significantly in the direction of full-sib technology, producing millions of seedlings per year via mass controlled pollination (MCP) and varietals are also beginning to be deployed in southern pine plantations (McKeand et al. 2007). We know of no studies in which MCP has been tested with chestnuts, so the applicability of this tool to producing elite families of chestnuts is unknown. Thus, varietal (i.e. clonal) approaches may be a more realistic option for production of elite chestnuts.

The advantages of deploying clonal trees in plantations (compared to half-sib or even full-sib seedlings) include the capture of all components of genetic variance of the ortets, the ability to apply very high selection differentials and enhanced product uniformity. Disadvantages of applying this approach include relatively high labor and operating expenses, the requirement for specialized equipment and facilities, the fact that propagules may need to be containerized and the high variation in success rate, depending on species, ortet age and other factors. Varietal approaches that might be applied for propagation of elite chestnuts include rooted cuttings and *in vitro* propagation via axillary shoot multiplication (micropropagation) or somatic embryogenesis (SE). Compared to the other two methods, SE has a number of advantages, including potentially very high multiplying power and the amenability of embryogenic cultures to cryostorage. Propagation of American chestnuts by all three of these methods has been reported over the years, with varying levels of success, but to our knowledge, B3F3 chestnuts have not been clonally propagated previously. We conducted a preliminary experiment testing different

hormone treatments for rooting stump sprouts collected from TACF B3F3 stumps in 2012, with a very low (0.6%) success rate (unpublished data).

For the past three years, as part of our lab's participation in the Forest Health Initiative (FHI), we have tested our chestnut SE protocol for its applicability to propagate a range of chestnut hybrids (Holtz et al. 2013). One particular goal of the FHI was to establish embryogenic cultures of TACF B3F3 material to facilitate clonal testing. Over the past two decades, we have developed a scalable, suspension culture-based system for production of chestnut somatic embryos and somatic seedlings (Andrade et al. 2005, Merkle et al 2011). However, prior to the beginning of the FHI project, only pure AC material had been propagated via SE, so the potential to propagate advanced generation hybrid material using this approach was unknown. Open-pollinated B3F3 seeds, representing both the Clapper and Graves lines of blight-resistance, were collected from B3F2 seed orchard parents by TACF cooperators and used to initiate cultures in 2010 and 2011. Average embryogenesis induction percentages were 0.85% for nine OP B3F3 families in 2010 and 1.63% for 11 OP B3F3 families in 2011. These "capture" percentages were not significantly different from those for AC cultures initiated in those years. The first of these B3F3 somatic seedlings were planted in a clonal field test by Virginia Tech cooperators in Virginia in June 2013. Of course, in order to take full advantage of SE for production of elite varieties, it needs to be combined with full-sib breeding. Selected B2F3 parents were crossed to for this purpose in 2012. While the average embryogenesis induction percentage for the full-sib material (0.5%) was lower than for OP seeds, at least one embryogenic culture was produced for eight of the nine crosses. Now that we have confirmed that SE can be applied to propagate B3F3 material, all the pieces are in place to apply the varietal forestry approach to blight-resistant chestnuts. Somatic seedlings derived from B3F3 cultures initiated from crosses between the best B3F2 parents can be rigorously field tested while the cultures from which they were produced are held in cryostorage. Once the best varieties are identified, those cultures can be recovered from cryostorage and scaled-up for mass somatic seedling production of elite planting stock. The decision as to whether to apply this approach to produce elite chestnut varieties for purpose-grown trees is, of course, up to TACF.

With additional research, we hope to be able to directly clone elite, blight- and Phytophthora-resistant chestnuts by initiating embryogenic cultures from mature tree tissues. This has been accomplished using leaves from epicormic shoots of mature trees of cork oak (*Quercus suber*; Hernandez et al. 2003) and English oak (*Quercus robur*; Toribio et al. 2004). Since oaks and chestnuts are both in the Fagaceae, a similar approach may be applicable to clone elite chestnut trees.

Acknowledgements: This research was supported by funding from the Forest Health Initiative. We would like to thank Fred Hebard and Jeff Donahue of The American Chestnut Foundation for supplying chestnut material for culturing, Dana Nelson for advice and Paul Montello, Heather Gladfelter, Frank Henning and Eric Johnson for technical assistance.

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