APPLICATION OF HYBRID BREEDING AND SOMATIC EMBRYOGENESIS TO DEVELOP SWEETGUM VARIETIES FOR THE BIOENERGY AND PULP AND PAPER INDUSTRIES

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The application of hybrid breeding to forest trees has resulted in some very useful and productive genotypes for some forest crops. Notable successes include hybrid poplars (e.g. *Populus trichocarpa* x *Populus deltoides*), hybrid Eucalyptus (e.g. *E. urograndis*), and hybrid pines (e.g. *Pinus rigida* x *Pinus taeda*). However, hybrid breeding has not been a widely used tool in U.S. plantation forestry in the southeastern U.S. Similarly, somatic embryogenesis, which at one time appeared to have great potential for forestry applications, in particular for clonal propagation of elite southern pine genotypes, has yet to attain a significant percentage of southern pine annual regeneration. Combining these two technologies, however, creates a very powerful approach that appears to have created its first product with real economic potential in the southeastern U.S.—in a hardwood tree, hybrid sweetgum. The timing of the appearance of this new hardwood feedstock coincides with an unusual convergence of pine and hardwood pulpwood prices in the region, where prices for hardwood have traditionally trailed those for pine. Trends indicate that this change is due to either rising demand for hardwood, decreasing supply of hardwood or both. In any event, the case for purpose-grown hardwoods in the southeast is becoming stronger, in particular in areas where a reliable supply of hardwood fiber is needed year-round, since purpose-grown trees have the ability to stabilize wood costs and provide predictable price caps for mills. Sweetgum (*Liquidambar styraciflua*) plantations, first established in the southern U.S. in the 1960s, experienced some limitations that have largely been overcome with the development of improved planting stock (Wright and Cunningham 2008). Even more dramatic improvements in performance may be possible by hybridizing *L. styraciflua* with, *L. formosana*, which is native to China and Formosa. Even though the two species have been separated by continental drift for 10 million years they are still inter-fertile. Santamour (1972) reported the first interspecific *Liquidambar* hybrids.

To generate hybrid sweetgum varieties, in 1999, pollen was collected from three mature *L. formosana* trees growing in a U.S. Forest Service demonstration planting near Saucier, MS and used by International Paper Co. breeders to conduct controlled pollinations with three *L. styraciflua* selections from the N.C. State Hardwood Program at IP's Southlands Experiment Forest near Bainbridge, GA, for a total of nine crosses. Immature fruit were collected from the *L. styraciflua* mother trees in June and July and shipped to the Merkle lab at UGA, where the immature seeds were used for culture initiation. Briefly, immature fruits were surface-disinfested using a sequence that included Clorox, then the fruits were dissected and immature seeds removed, nicked with a scalpel blade and cultured on a semi-solid induction-maintenance medium (IMM) that included 2 mg/L 2,4-D (Vendrame et al. 2001). A second round of hybrid

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breeding and culturing was conducted in 2005. Seed explants were transferred to fresh medium after one month.

Approximately 2% of the cultured seeds produced proembryogenic masses (PEMs) within 2 months following culture initiation. PEMs could be maintained by monthly transfer to fresh medium and grew more rapidly once inoculated into liquid IMM to produce embryogenic suspension cultures. Suspension cultures were size-fractionated on stainless steel sieves, and a selected size fraction between 38 µm and 140 µm collected on filter paper using a Büchner funnel produced up to 6000 synchronously developing somatic embryos per 0.5 g of PEMs within 2 months following plating on semi-solid basal medium, which was the same as IMM but lacking 2,4-D (Fig. 1A; Dai et al. 2004). Eighty to 90 percent conversion of the somatic embryos to somatic seedlings could be obtained following an 8-week pre-germination cold treatment at 10º C (Merkle et al. 2010). Recently, further improvements in PEM production were obtained using air-lift-bioreactors and early growth of somatic seedlings was accelerated using RITA® temporary-immersion bioreactors (Lu and Merkle, in preparation). Several hundred hybrid sweetgum somatic seedlings, representing multiple varieties, were produced, transferred to potting mix and planted in field tests and demonstration plantings by IP and ArborGen collaborators over the past 10 years. These trees can be identified as hybrids by their leaf shape, which is intermediate between the five-lobed *L. styraciflua* leaf and the three-lobed *L. formosana* leaf. In addition, embryogenic cultures and trees derived from them were verified to be hybrids using RAPDs (Vendrame et al. 2001).

The hybrid somatic seedlings produced displayed a range of growth rates and habits in field tests on multiple sites. On the oldest planting, established in 2002 on IP property near Aiken, SC, a small number of the varieties showed faster growth rates than elite native sweetgum genotypes, as well as significantly higher wood specific gravity. For example, one variety in the test had a DBH of almost 11 in and a height of 69 ft at age 12, with a wood specific gravity of almost 0.55, for an estimated whole tree MAI of over 12 green tons/acre/yr, compared to an average DBH of 7.3 in, height of 59 ft and specific gravity of 0.45 for native American sweetgum seedlings. This variety and two others displaying similar performance were selected by ArborGen for commercial production. Other hybrid varieties showed potential for use as landscape trees and ornamentals, with dwarf phenotypes and striking fall color. The second round of breeding and culturing in 2005 resulted in the production of another eight hybrid varieties, which were planted in field tests at four locations in 2008. One of the tested varieties showed superior growth rates at all four sites and so was selected as a fourth commercial variety. Copies of the hybrid cultures had been cryostored following the protocol of Vendrame et al. (2001) with the intention of thawing and scaling up production from those cultures that produced the best trees, based on the results of the field tests. However, the desired varieties could not be successfully re-grown following recovery from cryostorage. Instead, a previously published method for initiating embryogenic cultures from sweetgum inflorescence tissues (Merkle and Battle 2000) was used to start new embryogenic cultures from the top hybrid varieties growing in the Aiken, SC field test, which had begun to produce flowers. Plantlets regenerated from these cultures were used to establish hedges for scaled-up production of rooted cuttings (Fig. 1B). In the past year, approximately 200,000 trees, representing four elite

hybrid varieties, were produced by ArborGen and planted by landowners in four states, with the goal of providing a ready source of hardwood fiber for pulp mills in their areas. If the hybrid varieties continue to perform well, they may provide a new fiber crop for pulp and paper as well as biomass energy applications.

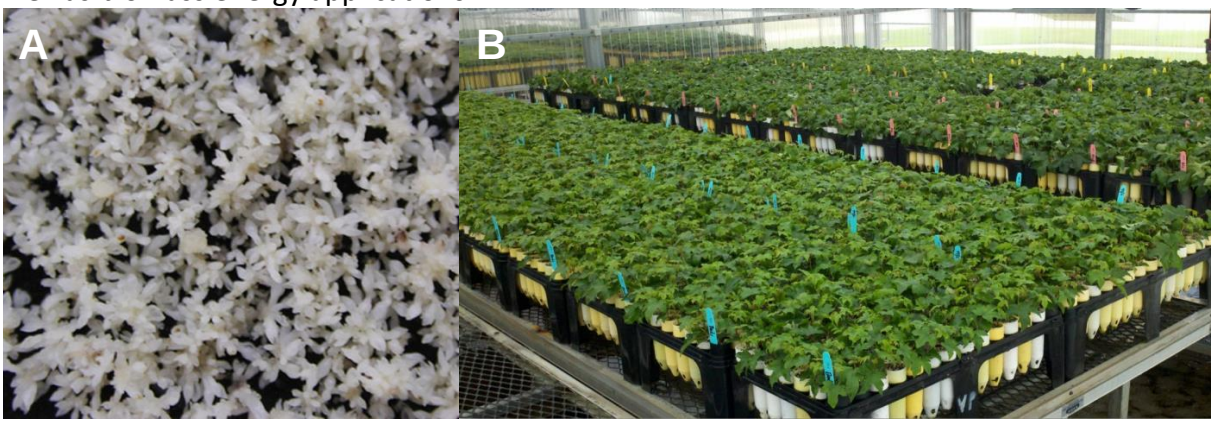

Fig. 1. Hybrid sweetgum varietal production. **A.** Embryogenic cultures produced thousands of hybrid sweetgum somatic embryos. **B.** Rooted cutting production in ArborGen greenhouse.

Acknowledgements: This research was supported by funding from the Consortium for Plant Biotechnology Research, International Paper Co. and ArborGen Inc. We would like to thank Ron Schmidtling for helping us get access to Chinese sweetgum material, Jeff Donahue and Robert Thomas for supplying information on the hybrid variety performance, and Wagner Vendrame, Paul Montello, Jianliang Dai, Siran Lu and Taryn Kormanik for technical assistance.

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