Regeneration of Bigleaf Magnolia by Somatic Embryogenesis

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Abstract. Bigleaf magnolia (*Magnolia macrophylla* Michx.) cultures were initiated from immature seeds on an induction medium containing 9.0 μM 2,4-D, 1.1 μM BA, and 1 g casein hydrolysate/liter. After 2 months on induction medium, one culture produced adventive embryos. Clumps of embryos transferred to liquid induction medium proliferated as nodules, which grew in diameter, but failed to produce embryos while maintained in induction medium. Nodules transferred to basal medium produced clumps of somatic embryos, which continued to produce repetitive embryos with monthly transfer to fresh basal medium. Individual embryos transferred to basal medium lacking casein hydrolysate germinated and leaves expanded. Plantlets derived from these embryos were transferred to potting mix and acclimatized to greenhouse conditions. Chemical names used: (2,4-dichlorophenoxy)acetic acid (2,4-D); *N*-(phenylmethyl)-1H-purin-6-amine (BA).

Bigleaf magnolia has the largest leaves of any magnolia species (Gardiner, 1989) and the largest simple leaves of any dicotyledonous tree in North America (Brown and Kirkman, 1990)—up to 90 cm long and 30 cm wide. The fragrant, cup-shaped flowers are also the largest of any North American tree (up to 30 cm across), with creamy white tepals, often with some purple tint near their bases. The tree, up to 15 m tall, is found in rich wooded ravines. Its distribution is restricted in the southeastern United States, where it is found as a scattered tree primarily in Georgia, Alabama, Mississippi, Louisiana, and northward to Tennessee and Kentucky (Brown and Kirkman, 1990).

Somatic embryogenesis has been reported for three magnolia species native to the southeastern United States: sweetbay magnolia (*M. virginiana* L.), fraser magnolia (*M. fraseri* Walt.), and yellow cucumbertree (*M. cordata* Michx.) (Merkle and Wiecko, 1990). Because bigleaf magnolia is a relatively rare species with substantial potential as an ornamental tree, we believe somatic embryogenesis may have considerable value as a propagation method for this species. Here, we report preliminary results from a study aimed at producing somatic embryos and plantlets of bigleaf magnolia.

Experiments to initiate embryogenic bigleaf magnolia cultures were conducted in the same manner as those described for embryogenic cultures of other magnolia species (Merkle and Wiecko, 1990) with regard to explant preparation, media, and conditions. However,

unlike the other magnolia species with which we have worked, very few fruits (aggregates of follicles) were available in any given year. Therefore, experiments with bigleaf magnolia were conducted in 1988, 1989, and 1991, with explant collection date as the only controlled variable. In all 3 years, fruit were collected from a small population of trees growing in

Jackson County, Georgia. In 1988, fruit were collected from four bigleaf magnolias on 18 July, $\approx$ 8 weeks postanthesis. In 1989, fruit were collected from the same four trees on 21 June and 10 July, $\approx$ 5 and 8 weeks postanthesis, respectively. In 1991, fruit were collected from three of the same trees on 18 June, $\approx$ 3 weeks postanthesis. Individual seeds were dissected from the aggregates using a grafting knife and disinfested using the following sequence: 70% ethanol, 20 sec; 10% Roccal (National Laboratories, Montvale, N.J.), 1 min (repeating the ethanol and Roccal steps once); 5.25% sodium hypochlorite, 5 min; sterile water rinse, 3 min; 0.01 M HCl rinse, 3 min; and three additional 3-min sterile water rinses. Seeds were bisected longitudinally with a scalpel, and the halves were placed cut surface downward on semisolid yellow-poplar induction medium (Merkle et al., 1990) in 60-mm plastic petri dishes (three seeds per dish). During explanting, no zygotic embryos could be observed inside the seeds using a dissecting microscope, and endosperm, if present, was still in the liquid stage. Induction medium contained 9.0 μM 2,4-D, 1.1 μM BA, 1 g casein hydrolysate (CH)/liter, and 40 g sucrose/liter. Cultures were maintained at 22C in darkness and transferred to fresh medium monthly.

In the 1988 (99 seeds cultured) and 1989 experiments (162 seeds cultured), microbial contamination exceeded 50%. Of the remain-

Fig. 1. Nodules, somatic embryos, and plantlets derived from embryogenic cultures of bigleaf magnolia. (A) Nodules derived from culturing somatic embryos in liquid induction medium (bar = 1 mm). (B) Proembryonic masses and somatic embryos produced from nodules transferred to semisolid basal medium (bar = 500 μm). (C) Repetitive embryo production on semisolid basal medium (bar = 1 mm). (D) Somatic embryo-derived plantlet $\approx$ 11 weeks following transplanting to potting mix and transfer to the acclimatization chamber.

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ing explants, all but a few darkened within a few weeks and displayed no growth. Surviving explants produced a slow-growing callus composed of vacuolate cells. Of the 90 seeds explanted in 1991, 32 were contaminated. Of the remaining 58 seeds, 25 produced callus, and one of these produced a cluster of somatic embryos, which developed at the micropylar end of the seed. Although multiple embryos were produced on semisolid induction medium, they did not develop beyond the globular stage. Further, repetitive embryogenesis was discontinued when the embryos were transferred to fresh semisolid induction medium. Instead, callus was produced from the embryo surfaces. In an attempt to sustain repetitive embryogenesis, a clump of embryos was transferred to 40 ml of liquid induction medium in a 125-ml Erlenmeyer flask and maintained on a gyratory shaker at 90 rpm under cool-white fluorescent light ($11 \text{ W}\cdot\text{m}^{-2}$) at 22C. Instead of generating more embryos, however, this material produced hundreds of smooth, round nodules that grew up to 5 mm in diameter (Fig. 1A). A few of the ≈ 40 nodules plated on semisolid "yellow-poplar basal medium and grown in darkness (Merkle et al., 1990) produced somatic embryos within 1 month (Fig. 1B). Repetitive embryogenesis could be maintained by transferring embryo clumps to fresh semisolid basal medium monthly, which eventually produced 50 to 100 new proembryos per 60-mm petri dish per month (Fig. 1C). Embryo production could be maintained at similar levels in suspension culture by transferring embryo clumps to liquid yellow-poplar basal

medium, and growing them in darkness on a gyratory shaker at 22C, with transfer of embryo clumps to fresh liquid medium every 3 weeks.

Although the majority of embryos produced were malformed, with fused cotyledons, those with two to four distinct cotyledons germinated. Their leaves expanded following transfer to semisolid basal medium lacking CH and subsequent culture under cool-white fluorescent lights ($10 \text{ W}\cdot\text{m}^{-2}$) at 30C. Germinants with expanding cotyledons transferred to yellow-poplar plantlet development medium (Merkle et al., 1990) with 2% sucrose and no growth regulators continued to grow slowly. They produced a strong tap root and one new leaf about every 4 weeks. Plantlets transferred to peat-lite (Fafard, Agawam, Mass.) potting mix and grown in an acclimatization chamber at 100% relative humidity, 25C, and 16-h photoperiod under cool-white fluorescent lights ($20 \text{ W}\cdot\text{m}^{-2}$) continued to produce new leaves (Fig. 1D) and were acclimated to greenhouse conditions by gradually lowering the relative humidity.

Of the magnolia species with which we have worked to date, bigleaf magnolia is the most recalcitrant with regard to embryogenic culture initiation and production of several somatic embryos capable of converting to plantlets. We believe that our first two experiments to initiate embryogenic cultures were unsuccessful because the seed explants we used were either infertile or past the optimal developmental stage for repetitive embryogenesis. Only in the 1991 experiment, in which

we used seeds collected 3 weeks postanthesis, did we see callus production from a substantial number of explants, one of which produced somatic embryos. It is also likely that prolonged exposure to 2,4-D inhibited somatic embryo production, since the culture that ultimately did produce embryos could not be maintained on medium containing 2,4-D. Instead, repetitive embryogenesis could be maintained (for at least 7 months) by regular transfer of embryos to fresh basal medium. The ability to maintain repetitive embryogenesis on basal medium and not on 2,4-D-supplemented medium is different from other magnolias and yellow poplar (*Liriodendron tulipifera* L.), all of which were maintained continuously on 2,4-D-supplemented medium (Merkle and Wiecko, 1990; Merkle et al., 1990). Future culture initiation experiments with bigleaf magnolia should test "pulse" treatments on 2,4-D-supplemented medium, followed by culture on media with much lower auxin levels or none at all.

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