Accelerated Propagation of *Chionanthus virginicus* via Embryo Culture

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Abstract. Traditional seed propagation (warm/cold stratification) was compared to embryo culture of *Chionanthus virginicus* L. to determine if germination could be promoted and time necessary to produce a sizable plant could be reduced. Embryos of *C. virginicus* were extracted from immature fruit collected 9, 16, and 23 Aug. 1995 and grown in vitro on Anderson's rhododendron medium. They germinated in 4 weeks and were transferred ex vitro to flats. Mature fruit from the same source were grown simultaneously using warm/cold stratification. The two groups were evaluated periodically over a 2-year period for percent germination, plant size, and seedling success. The embryo-cultured plants had a lower survival rate (16% vs. 44%) and were more labor intensive. After 2 years, embryo-cultured plants were 13.4-fold the mass and 4.7-fold taller than traditionally grown plants. Ten-month-old cultured plants were comparable in size to 2-year-old plants grown traditionally.

White fringetree (*Chionanthus virginicus* L.) is a small tree or shrub native to the United States from southern Pennsylvania and New Jersey south to Florida and west to Texas. Its ability to adapt to a variety of landscape situations and its floral display in late spring make it a valuable ornamental. *Chionanthus* is traditionally propagated from seed using warm/cold stratification. Two years are required to break double dormancy, caused by a hard endocarp, endosperm inhibitors, and shoot dormancy (Dirr, 1990). Propagation by cuttings (Dirr and Heuser, 1987; Nicholson, 1990) or grafting has not been particularly successful (Dirr, 1994; Fagan and Dirr, 1980; Young and Young, 1992). Additional work related to the dormancy of *C. virginicus* has included removal of the epidermis, pericarp, and endocarp to accelerate germination (Carpenter et al., 1991) and unsuccessful attempts at culturing embryos on a medium containing gibberellic acid (Redcay and Frett, 1990). Embryo culture has been used to surmount seed dormancy in many other species (Arrillaga et al., 1992; Bridgen, 1994). These reports suggest a possible method to overcome dormancy and reduce the time required to obtain marketable plants. The objective of this investigation was to compare traditionally propagated and embryo-cultured *C. virginicus* for percent germination, plant size, and general seedling success over 2 years.

Materials and Methods

Immature *C. virginicus* fruit were collected on 9, 16, and 23 Aug. 1995 from 4-year-old

specimens growing at The Holden Arboretum, Kirtland, Ohio. The fruit were surface sterilized with 95% ethanol for 30 s, followed by 1.05% sodium hypochlorite and 0.1% polyoxyethylenesorbitan monooleate (Tween 80) with agitation for 20 min, rinsed three times for 4 min each in sterile distilled water, and left in the final rinse until excision. Embryos were excised under sterile conditions. The stem end of the fruit was cut, removing about one-third of the endocarp, then cut longitudinally and the embryo dislodged. The embryo was positioned on 30 mL of Anderson's rhododendron medium (Anderson, 1978), pH 5.8, with the radicle below the surface and the cotyledons above. Ninety embryos were excised for each collection date with three placed in each of 90 jars (270 embryos). In addition, 15 fruit were selected randomly from each collection date, embryos excised, and lengths measured.

Cultures were held at 25 °C and a 16-h photoperiod with 40-W cool-white fluorescent bulbs [average photosynthetic photon flux density 32.0 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$]. After 4 weeks of incubation, the germinated embryos were transferred to Kadon flats (10 cm deep) containing sterile 1 perlite : 1 peat mix, watered-in, covered with clear plastic, and placed in a humid greenhouse at 18 to 24 °C for 4 months. The plastic was removed after 1 week. Flats were moved to cold storage (4 °C) in Jan. 1996 for 2 months, and then placed in a greenhouse at 18 to 24 °C for 8 months. The seedlings were transplanted to 2 peat : 2 perlite : 6 bark mix in 6 × 6 × 12.5 cm tree bands (Anderson, Portland, Ore.) in June 1996. The pots were moved into cold storage (4 °C) in Nov. 1996, then into a polyhouse in May 1997. Plants were transplanted to 3.8-L polybags (Menne Nursery Corp., North Tonawanda, N.Y.) in June 1997, and kept under shade cloth for the summer.

To compare embryo culture vs. traditional propagation, 158 mature fruit were collected

from the same source in late Sept. 1995. Seventy-nine seeds were warm-stratified in sterile 1 perlite : 1 peat in a mist room at 21 to 27 °C for 5 months, moved to cold storage (4 °C) in Feb. 1996 for 1 month, and then returned to a warm greenhouse. Additional cold treatments are reported to be ineffective in shortening the time between radicle elongation and seedling emergence (Carpenter et al., 1992); therefore, the seed pans remained in the greenhouse until the appearance of cotyledons. In late Apr. 1997, the seedlings were transplanted from the seed pans to tree bands and soil mix as described above. Percent germination, seedling height, numbers of leaves, and stem diameter of all seedlings were measured at regular intervals. In Oct. 1997, 10% of each of the two groups of seedlings were randomly selected, cleaned, dried in a 95 °C oven for 24 h, and weighed (roots, stems, and leaves). Data for dry weight were evaluated using a *t* test.

Results and Discussion

The average embryo lengths (Fig. 1A) at excision were 2.9 (9 Aug.), 5.0 (16 Aug.), and 6.9 mm (21 Aug.). Larger embryos were easier to work with, but more mature fruit had a harder endocarp, making excision difficult. In culture, radicles of excised embryos started to grow within 3 d; most of the embryos had true leaves and radicles >4 cm in length after 1 month, and were transplanted. Some embryos (30%) became contaminated during culture, while others (55%) failed to survive ex vitro. Initial survival was (from early to late collection times) 41, 68, and 79, but was reduced to 5, 16, and 19 at 7 months. On 16 May 1996, 1 month after removal of the traditional group from cold stratification, survival of embryo-cultured seedlings was unchanged and plantlets were growing; radicle growth was evident in the traditional seed group. Shoots started to appear in the traditional group in Mar. 1997.

Fig. 1. (A) Excised *Chionanthus virginicus* embryos. Bar = 2 mm. (B) Traditionally germinated (left) and embryo-cultured (right) *Chionanthus virginicus* seedlings 2 years post-initiation.

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By Oct. 1997, survival of excised embryos was 6% (9 Aug.), 18% (16 Aug.), 21% (23 Aug.), and 44% for traditionally grown seedlings.

On 15 May 1997, mean height of embryo-cultured seedlings was 4.7-fold (12.7/2.7 cm) as great as that of traditional seedlings, and mean number of leaves was 3.1-fold (8.8/2.8) as great. The maximum height was 31.5 cm for embryo-cultured vs. 6 cm for traditional; maximum number of leaves was 22 for embryo-cultured vs. 10 for traditional. The mean stem caliper of embryo-cultured plants was 3-fold that of traditional (7.9/2.6 mm).

Dry weight of stems, leaves, roots, and total (Fig. 2) were all significantly different at $P \leq 0.01$, with the embryo-cultured group having 13.4-fold the mass of the traditionally germinated seedlings. Twenty-four months from seed collection, differences in size between traditional and embryo-cultured seedlings were dramatic (Fig. 1B).

In conclusion, *Chionanthus virginicus* can be successfully grown from extracted embryos. When the embryo was removed from the endosperm and testa, germination proceeded within 7 to 10 d. Ten months after extraction, embryos had developed into seedlings comparable in size with 2-year-old seedlings obtained by traditional germination. Additional time and labor were required for embryo-cultured seedlings, with survival about half that for traditionally germinated seedlings. However, the increase in both size and rate of development suggests that embryo-culture may be a viable alternative to traditional propagation of *Chionanthus*.

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Fig. 2. Average dry weight of leaves, stem, roots, and total plant of *Chionanthus virginicus* seedlings derived from traditionally germinated and from embryo-cultured plants 24 months post-initiation. Vertical bars represent standard deviation.

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