Micropropagation of Pacific Dogwood

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Abstract. Idaho's population of Pacific dogwood (*Cornus nuttallii* Audubon) has declined. Propagation of disease-resistant clones would be useful to horticulturists and conservation biologists. In vitro-derived microshoots, incubated for 1 month on woody plant medium supplemented with 6.04 mM calcium gluconate and 4.44 μ M benzyladenine, produced an average of 3.1 axillary microshoots per explant. Up to 62% of the elongated microshoots had rooted ex vitro 5 weeks following a 4.5% IBA talc dip. Plantlets resumed shoot growth within 2 months of acclimatization, and 70% survived after 1 year. This protocol is more rapid and efficient than propagation by layering or rooting the difficult-to-root stem cuttings of this species. Chemical names used: 2,3,4,5,6-pentahydroxy-caproic acid (calcium gluconate), benzyladenine (BA), 3-indolebutyric acid (IBA).

Pacific dogwood, also known as western flowering dogwood, is a small, showy tree native to the forests of the U.S. Pacific coast and one location in northern Idaho. Pacific dogwood cultivars (Santamour and McArdle, 1985) are primarily propagated by budding and grafting (Macdonald, 1984). However, dogwood anthracnose (*Discula destructiva* Redlin) (Redlin, 1991), a disease of North American flowering dogwoods, and a root rot caused by *Phytophthora* sp. may advance the decline of Idaho's Pacific dogwood population (Hibben, 1992; Partridge, 1990). If selections of symptom-free Pacific dogwood show genetic resistance to dogwood anthracnose, then a procedure to rapidly propagate the resistant clones would be useful to horticulturists and conservation biologists. Because there are no published reports on the micropropagation of Pacific dogwood to our knowledge, we describe methods for proliferating shoots in vitro and rooting microcuttings ex vitro.

Materials and Methods

Softwood branch tips (10 to 15 cm long) were collected from the following sources: 20 forest trees in the Puget Basin of western Washington; 20 trees in northern Idaho; and 75 greenhouse-grown, 2-year-old seedlings from the Idaho population. Defoliated shoot tips were washed under running tap water for 1 h, agitated for 30 min in a 3-liter Erlenmeyer flask by bubbling air through an aqueous solu-

tion of two drops detergent/liter (Liquinox, New York), and surface-sterilized in a continuously stirred 1.5% (w/v) NaOCl solution for 15 min. After four rinses in sterile distilled water, each shoot was sectioned into a tip and several stem segments that contained a single node. The cultures were initiated on hormone-free Murashige and Skoog (MS) medium (Murashige and Skoog, 1962), supplemented with 3% sucrose and 0.7% agar (Sigma Chemical Co., St. Louis) at pH 5.7. Contamination percentages were compared for forest and greenhouse sources. Cultures contaminated with bacteria underwent up to four, short-interval (1 to 2 weeks) subcultures to reduce bacterial growth.

All experiments used microshoots derived from one to four subcultures of the previous shoot tip and stem segment explants. Microshoots were incubated individually for 1 month in 25 $\times$ 150-mm glass test tubes, loosely covered with cotton-filled caps (Magenta Co., Chicago). Cultures were placed on shelves under an 18-h photoperiod provided by cool-white fluorescent lights (40 W) that produced 20 μ mol-m⁻²-s⁻¹ photosynthetically active radiation at the leaf surface of the cultures. Diurnal temperatures in the laboratory ranged from 27/22C (day/night).

To assess the effect of BA on multiplication, 120 in vitro-derived microshoots from the forest sources were assigned to the MS (0.8% agar) medium supplemented with 0.0, 0.44, 2.22, or 4.44 μ M BA in a completely randomized design. To compare the influence of basal media on shoot elongation and proliferation, 116 in vitro-derived, 0.5-cm-long microshoots (forest sources) were assigned to two replicates of MS and woody plant medium (WPM) (Lloyd and McCown, 1980) in a randomized complete-block design (RCB). WPM contained 2% sucrose and 0.6% agar and was adjusted to pH 5.2. Both media (MS and WPM) contained 4.44 μ M BA and 6.04 mM calcium gluconate. To determine proliferation on WPM supplemented with combinations of BA and α -naphthaleneacetic acid (NAA), 150 microshoots (greenhouse source) were assigned

to two replications of three treatments on WPM: a control (0 BA and NAA), 4.44 μ M BA, and 4.44 μ M BA in combination with 0.54 μ M NAA in an RCB design. Data were collected 1 month after initiating experiments. To establish multiplication over time, 10 in vitro-derived microshoots (greenhouse source) were multiplied and subcultured monthly on WPM with 4.44 μ M BA for five consecutive months. Shoot counts and height increments were processed by analysis of variance, and the treatment means were separated by Tukey's HSD multiple comparison procedure at α = 0.05. Maximum-likelihood analysis of variance and treatment contrasts using SAS category modeling procedure (SAS Institute, 1989) were performed on health scores (0 = dead, 1 = necrotic, 2 = chlorotic, 3 = vigorous). Hand sections of new shoots were examined microscopically to evaluate axillary or adventitious origin.

To evaluate ex vitro rooting, 160 in vitro-derived, 1.5- to 3.0-cm-long microshoots (greenhouse source) were assigned to two replicates of talc dips with 0%, 0.1%, 0.8%, 1.6%, or 4.5% IBA. The bases of the treated microshoots were inserted in 6-cm³ plug containers filled with a 2 perlite : 1 peat : 1 vermiculite mix (by volume). The microshoots were placed in fog (88% to 92% relative humidity), at 20 to 25C, under 70% shade, and with light intensities $\leq$ 12,000 lx and natural photoperiod. Twenty plantlets were fertilized weekly for 12 weeks with 20N–20P–20K at 150 ppm N (16-h photoperiod) in the greenhouse.

Results and Discussion

Fewer explants from the greenhouse seedlings were contaminated with fungus than from the forest trees (18% vs. 75%). Trigiano et al. (1992) reported high contamination rates with explants from forest trees of *C. florida* L. All explants from the forest sources and $\approx$ 25% from the greenhouse source developed slow-growing bacteria that generally disappeared after two to four subcultures. The average number of new shoots per explant (forest source) on the MS medium supplemented with BA at 4.44 μ M was higher than on hormone-free MS ($P \leq 0.0010$). The shoot counts were (mean $\pm$ SD) 0.6 $\pm$ 0.5, 1.8 $\pm$ 1.2, 2.3 $\pm$ 1.0, and 3.3 $\pm$ 1.6 for 0, 0.44, 2.22, and 4.44 μ M BA, respectively. Comparing microshoot response on WPM vs. MS, the new shoots produced per explant were more numerous (2.6 vs. 0.7) ($P = 0.0003$), taller (1.2 vs. 0.9 cm) ($P = 0.0001$), and less chlorotic (health score 2.7 vs. 2.1) ($P = 0.0002$) on WPM. Bacterial growth during this experiment may have depressed proliferation to 0.7 shoots per explant vs. 3.3 in the previous experiment (both results on MS with 4.44 μ M BA). Shoot proliferation was slightly higher in the microshoots (greenhouse source) incubated on WPM supplemented with 4.44 μ M BA alone vs. 4.44 μ M BA combined with 0.54 μ M NAA ($P = 0.0051$) (Table 1). Moreover, the explants on growth regulators were less chlorotic than on hormone-free WPM ($P = 0.0006$). Multiplication over the five monthly

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subcultures resulted in an average of 2.9 shoot tips produced per culture per month. Pacific dogwood on WPM supplemented with 4.44 μM BA proliferated at rates similar to those reported by Trigiano et al. (1992) for *C. florida* on Knop's medium. New microshoots produced an opposite or whorled habit typical of Pacific dogwood's axillary growth, and vascular traces examined were continuous with the main stem.

In contrast to recalcitrant rooting of *C. florida* (Trigiano et al., 1992), Pacific dogwood microshoots rooted ex vitro after 5 weeks, with final rooting rates of 62% and 43% in the two replicates treated with 4.5% IBA. Microshoots receiving 0% or 0.1% IBA failed to root, and only 18% of the microshoots treated

with 0.8% or 1.6% IBA rooted. Plantlets resumed shoot growth within 2 months of transfer to the acclimatization greenhouse and survived at 70% after 1 year.

Pacific dogwood can be micropropagated by 1) using greenhouse stock plants to minimize contamination and enhance in vitro growth, 2) multiplying microshoots on WPM containing 4.44 μM BA, 3) rooting microshoots ex vitro using a 4.5% IBA talc dip, and 4) acclimatizing in a greenhouse. This propagation protocol is more rapid and efficient than layering or rooting the difficult-to-root stem cuttings of this species. Furthermore, rooting microshoots ex vitro lowers overall propagation costs and enhances the feasibility of commercial production.

Table 1. Effects of BA and NAA combinations on multiplication of Pacific dogwood microshoots derived from greenhouse seedlings.^z

Growth regulator treatment (μM)		Shoots		
BA	NAA	Mean no. ^y	Mean elongation (mm) ^y	Mean health score ^x
0	0	0.2 a	2 a	1.8 a
4.44	0	3.1 b	6 b	2.4 b
4.44	0.54	2.2 c	8 c	2.3 b

^zFifty explants were used per treatment.

^yMeans separation in columns by Tukey's HSD at $\alpha = 0.05$.

^xShoot health rated on a scale from 0 to 3 (0 = dead, 1 = necrosis, 2 = chlorosis, and 3 = vigorous growth). Treatment mean separation determined by contrasts of maximum likelihood estimates.

Literature Cited

- Hibben, C.R. 1992. Anthracnose in a coastal disjunct population of Pacific dogwood in Idaho: An evaluation based on comparisons with the disease in Eastern flowering dogwood. U.S. Dept. Agr. For. Serv. Rpt., Missoula, Mont.
- Lloyd, G. and B. McCown. 1980. Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. Proc. Intl. Plant Prop. Soc. 30:421-427.
- Macdonald, B. 1984. Two noteworthy *Cornus* introductions. J. Royal Hort. S. 109:151-153.
- Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.
- Partridge, A.D. 1990. Mortality of Pacific dogwood, a coastal disjunct population in northern Idaho. U.S. Dept. Agr. For. Serv. Rpt., Missoula, Mont.
- Redlin, S.C. 1991. *Discula destructiva* sp. nov., cause of dogwood anthracnose. Mycologia 83:633-642.
- Santamour, F.S. and A.J. McArdle. 1985. Cultivar checklists of the large-bracted dogwoods: *Cornus florida*, *C. kousa*, and *C. nuttallii*. J. Arboriculture 11:29-36.
- SAS Institute. 1989. SAS/STAT user's guide. Version 6, 4th ed. vol. 1. SAS Inst., Cary, N.C.
- Trigiano, R.N., R.M. Beaty, and K.W. Lowe. 1992. Micropropagation of dogwoods (*Cornus* spp.), p. 81-90. In: Y.P.S. Bajaj (ed.). Biotechnology in agriculture and forestry. vol. 20. Springer-Verlag, Berlin.