Suspension Culture and Plant Regeneration of *Typha latifolia*

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Additional index words. cattail, tissue culture, growth curve, wetlands, plant regeneration

Abstract. *Typha latifolia* L. (broadleaf cattail) callus was initiated from leaf sections, as well as from pistillate and staminate spikes. Two basal media in combination with three growth regulator regimes were tested for their capacity to induce callus from the explants. Pistillate spikes maintained in the dark on B5 medium supplemented with 5 mg·L⁻¹ dicamba and 1 mg·L⁻¹ BA produced the fastest growing cell line compared to other explants and media combinations. A growth curve in suspension culture was generated for this cell line on B5 medium. The mass of the callus increased by 150% by the end of the growth curve. Upon transfer of the callus to MS medium without growth regulators but with 3% sucrose and 3% phytagel, plants could be regenerated from 22% of the cultures. Chemical names used: 3,6-dichloro-2-methoxybenzoic acid (dicamba); N⁶-benzyladenine (BA).

Typha latifolia is a native perennial herb that can grow up to 3 m tall and forms inflorescences from June through August (U.S. Dept. of Agriculture, 1999). It grows from thick, underground rhizomes that survive extreme winters and produce shoots in the spring. This plant can produce >2000 g·m⁻² per year of biomass (Mitsch and Gosselink, 1986). Many consider *T. latifolia* to be a nuisance because it grows and reproduces rapidly; however, researchers have reported that this species can effectively remove pollutants from stormwater wetlands (Breed, 1993; Kadlec and Knight, 1996). *Typha latifolia* can dominate large areas, especially where water levels fluctuate (U.S. Dept. of Agriculture, 1999). *Typha latifolia* grows in freshwater marshes, wet swales, streams, ponds, and along lake shores (Reddington, 1994). This species is found in all 50 states and is among the most common aquatic plants. In the early spring, *T. latifolia* rapidly forms dense colonies that slow down stormwater-associated flows and allow particles to settle into the sediment (Stockdale, 1991).

Typha latifolia is also known to take up nutrients and heavy metals, such as P, N, Cu,

Ni, Zn, and Mg, from soil, water, and stormwater wetlands (Breed, 1993; Prentki et al., 1978; Taylor and Crowder, 1983). These pollutants are then stored in all parts of the plant, including the flower (Taylor and Crowder, 1983). These attributes of *T. latifolia* make it an excellent candidate for employment in constructed stormwater wetlands. This study had two main objectives: 1) to develop a medium that could induce callus formation routinely from explants placed in culture, and 2) to develop a medium that would regenerate cattail plants.

Material and Methods

Typha latifolia leaves and inflorescence segments were collected on 11 June 1999 from Johnson Park on the Cook Campus of Rutgers Univ. in New Brunswick, N.J. These plant parts were selected because they could be successfully disinfected in preliminary experiments. When collected, the developing inflorescence segments were totally enveloped in the foliage. Before disinfection, the outer leaves were removed and 20- to 31-cm-long shoot sections containing the inflorescence were cut and rinsed with tapwater. The shoot sections were rinsed in ethanol for 1 min, then soaked in 0.5% chlorine bleach containing 1% Tween-20[®] (Carolina Biological Supply Co., Burlington, N.C.) for 10 min, followed by three sequential rinses of 1 min in sterile distilled water. The inflorescence sections were then cut in 0.5-cm-thick cross-sections.

The disinfection procedure for mature leaves was as described for the spikes. The explants were placed into petri dishes (six explants per petri dish) containing 20 mL of either MS or Gamborg B5 basal medium supple-

mented with plant growth regulators (PGRs) dicamba or 2,4-D (2,4-dichlorophenoxy acetic acid) at 1, 2.5, or 5 mg·L⁻¹ (Gamborg et al., 1976; Murashige and Skoog 1962). All media contained 0.3% phytagel (Sigma, St. Louis), 3% sucrose, and were adjusted to pH 5.6 with 1 N HCL or 1 N KOH prior to autoclaving at 121 °C for 20 min at 1 kg·cm⁻². The petri dishes were placed in the dark at 25 ± 1 °C. Once established, callus (4 weeks) was subcultured onto similar fresh basal medium with the addition of 0, 0.5, 1, 2.5, or 5 mg·L⁻¹ BA. At the time of subculture, the diameter of the callus was estimated by placing a ruler against the top of the petri dish. After measurement, the callus was subcultured every 4 weeks onto similar medium from which it was derived.

Suspension cultures were generated from actively growing callus isolated from 3-week-old stock cultures maintained in the dark on B5 basal medium. Callus had been subcultured three times onto fresh medium of the same composition before it was placed in suspension culture. Callus (5 g) was placed into a 125-mL Erlenmeyer flask containing 60 mL basal medium without phytagel. Thirty-six flasks were set up for this experiment and placed on a gyratory shaker at 110 rpm in the dark at 25 ± 1 °C. Six flasks were harvested on 4, 8, 12, and 16 d after initiation, and three flasks were harvested on days 20, 24, 28, and 32. Once removed from the shaker, the flask contents were vacuum-filtered to separate the callus cells from the medium. The cells were harvested, and their fresh weights were recorded. The mean weight and the standard deviation of the cells were determined using the SigmaStat[®] software package.

Plantlets were formed by transferring callus from semisolid medium to petri dishes containing MS medium without growth regulators supplemented with 3% sucrose, 3% phytagel, and adjusted to pH 5.6. The petri dishes were placed under continuous light (≈50 μm·m⁻²·s⁻¹) for 6 weeks. Regenerated plantlets were transferred to small, sterile baby food jars (10.2 cm high × 5-cm diameter) containing 10 mL of the same basal medium for an additional 6 weeks. The plantlets were then transferred to 2.5-cm-square plastic liners for 8 weeks, then to 7.6-cm pots. A mix of 1 vermiculite : 1 peat was used as potting soil. After they had been transplanted to the 7.6-cm pots, the plantlets were placed in a mist chamber for 1 week, then moved to the greenhouse. Eight weeks later, the plants began to form additional shoots and were transferred to 15.2-cm pots.

Results

All three types of explant produced callus after 3 to 4 weeks in culture; however, the immature pistillate spikes were the superior explant source. Callus initiation from pistillate spikes occurred between 7 and 10 d and doubled in size every 4 weeks. The callus mass formed only on the outer edge (Fig. 1). This is similar to what was observed in *Sporobolus virginicus* (Straub et al., 1992). The cells were initially white, off-white, or beige on both MS

Received for publication 20 July 2001. Accepted for publication 5 Nov. 2001. We gratefully acknowledge the technical assistance of Dr. Chee-Kok Chin from the Dept. of Plant Science at Rutgers Univ. This study was conducted in the Dept. of Plant Science, Rutgers Univ., Cook College, New Brunswick, NJ 08903, through a contract from the U.S. Environmental Protection Agency's National Risks Management Research Laboratory, Water Supply and Water Resources Division, Urban Watershed Management Branch. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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Fig. 1. Callus initiation from female spikes of *Typha latifolia* on B5 basal medium supplemented with 5 mg·L⁻¹ dicamba and 1 mg·L⁻¹ BA at 2 weeks (bar = 0.51 cm).

Fig. 2. Root formation from *Typha latifolia* callus grown on B5 basal medium supplemented with 5 mg·L⁻¹ dicamba and 1 mg·L⁻¹ BA after 2 weeks under a 12-h photoperiod (bar = 1.3 cm).

Fig. 4. Regenerated *Typha latifolia* plants after 6 months in a greenhouse (bar = 0.3 m).

Table 1. Callus growth of *T. latifolia* callus on B5 basal medium supplemented with various plant growth regulators. Callus growth represents the mean of 20 plates per treatment and was measured 3 weeks after subculture.

BA concn (mg·L ⁻¹)	Auxin concn	Callus growth (mm)
0	2.5 mg·L ⁻¹ 2,4-D	7
	5 mg·L ⁻¹ dicamba	14
0.5	2.5 mg·L ⁻¹ 2,4-D	11
	5 mg·L ⁻¹ dicamba	16
1	2.5 mg·L ⁻¹ 2,4-D	12
	5 mg·L ⁻¹ dicamba	28
2.5	2.5 mg·L ⁻¹ 2,4-D	15
	5 mg·L ⁻¹ dicamba	18
5	2.5 mg·L ⁻¹ 2,4-D	(No callus growth)
	5 mg·L ⁻¹ dicamba	12

Fig. 3. Fresh weight as a function of incubation period of a suspension culture of *Typha latifolia* callus grown in B5 medium supplemented with 5 mg·L⁻¹ dicamba and 1 mg·L⁻¹ BA.

and B5 media but darkened after the third subculture. The callus cells were a hard, compact-to-loose mass. Callus initiated from pistillate spikes on both B5 and MS basal medium at all PGR concentrations tested.

Callus initiation from immature staminate spikes was low (33%), and grew slowly. Only MS basal medium supplemented with 5 mg·L⁻¹ 2,4-D and MS basal medium supplemented with 1 mg·L⁻¹ dicamba induced callus cells from the staminate spikes. The cells formed after 12 to 14 d in culture and were beige, loose, and hard. These cells were subcultured once, but eventually died.

Callus initiation from leaf explants was slightly greater than from immature staminate spikes. The leaf produced callus at a rate of 66% and 33% on MS and B5 basal media, respectively. Most of these cells were hard and white and grew slowly. A period of 14 to 21 d was required for callus initiation from the leaf tissue. Several months were required for leaf-derived callus to reach a size sufficient for subculturing.

Only callus derived from pistillate spikes was subcultured 4 weeks after initiation. Callus in petri plates that had been under a 12-h photoperiod formed shoots and was not used in this study. Less than 10% of the callus was placed under a 12-h photoperiod, and all formed shoots. Callus initiated on B5 basal medium was larger (doubled in size every 4 weeks) and had a more uniform growth and color than that derived from MS basal medium. Callus derived from the immature staminate spikes was subcultured onto B5 basal medium containing various PGR concentrations.

After the third subculture, callus on B5 medium containing 1 or 5 mg·L⁻¹ dicamba began to form roots (Fig. 2). To prevent root formation and stimulate callus growth, we subcultured callus onto similar medium with the addition of BA. BA prevented root formation and accelerated the rate of callus growth. B5 basal medium supplemented with 5 mg·L⁻¹ dicamba and 1 mg·L⁻¹ BA produced the largest callus mass at the fastest rate (Table 1). After the addition of BA to the media, the cell mass doubled in 3 weeks, as opposed to 4 weeks. This formulation was selected as the optimum growth medium.

Suspension cultures were established on B5 medium supplemented with 5 mg·L⁻¹ dicamba and 1 mg·L⁻¹ BA (Fig. 3). The lag phase lasted 4 d, during which the callus mass increased by 2%. Subsequently, the callus entered an exponential growth phase, which lasted 20 d. During this time, the callus mass increased 150%. The doubling time for the cells in suspension culture was 16 d. Compared with other wetland plants grown in suspension culture, the growth rate of this callus was slow. For example, *Distichlis spicata* (seashore saltgrass) exhibited a doubling time of ≈40 h, and *Catharanthus roseus* (madagascar

periwinkle) doubled in 2 d (MacCarthy et al., 1980; Warren and Gould, 1982). The rapidly dividing *D. spicata* had a lag period of 2 d and an exponential growth phase of 7 d (Warren and Gould, 1982); *C. roseus* has a lag period of 4 d and an exponential growth phase of 14 d (MacCarthy et al., 1980). The growth rate of callus cells in suspension culture may increase after initiation. However, the growth rate of cattail cells did not show an increase in growth rate even after continuous culture for more than 2 years. The *D. spicata* and *C. roseus* cultures were in callus culture for 7 and 3 months, respectively, before being placed in suspension culture. This demonstrates that the slow growth of the cattail cells is not a reflection of the age.

The mode of regeneration was organogenesis, as observed for other wetland monocots (Sarma and Rogers, 1998). Of cattail callus cultures placed on MS basal medium, 22% regenerated plants, which is less than the 37% regeneration rate reported by Rogers et al., (1998). However, the survival rate of the regenerated plants formed in this study was >90%. After 8 weeks in the greenhouse, the plants produced large compact root balls. They were transferred to 15-cm pots after 8 weeks because new shoots formed in the pots. At this point, the shoots were 20 to 31 cm long. The regenerated plants did not show any abnormal phenotypic traits after more than 1 year in the greenhouse (Fig. 4).

Discussion

Our study indicates that explant source is the most important factor in callus formation in *T. latifolia*; immature pistillate inflorescences produced callus cells that could be used to establish a cell line when grown on several different media, whereas staminate inflorescences and leaf sections did not produce viable cell lines on any of the media tested. Zimmerman and Read (1986) concluded the type and concentration of PGR were critical for callus formation from male or female inflorescences of *T. latifolia*. They reported callus initiation from 65% and 49% of the *T. latifolia* immature staminate and pistillate inflorescences, respectively (Zimmerman and Read, 1986). The optimum basal medium developed in our study induced callus formation from 100% of the female spikes used as explants (Table 1).

Typha latifolia is one of the most frequently employed marsh plant species in stormwater and wastewater wetland sites in the United States, because of its effectiveness in absorbing environmental pollutants from the environment (Kadlec and Knight, 1996). This study reports on a regeneration system that can allow researchers to introduce genes into *T. latifolia* that may enhance its pollutant absorption capacity. Enhancing the pollutant

absorption capacity of a plant such as *T. latifolia*, which grows rapidly and has a relatively large size, could produce a plant that may be of tremendous benefit in stormwater and wastewater wetland projects.

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