into premoistened potting medium in a 10-cm-diameter plastic "community" pot. Immediately after outplanting, the pot is covered loosely with a plastic bag (humidity tent), the upper corners of which have been cut off diagonally to create two 1-inch long ventilation holes. Community pots are placed under 80% shade in a greenhouse at 24C. Acclimatization is accomplished by gradually reducing the atmospheric relative humidity and increasing the light level. At the end of the first week in the greenhouse, the top edge of the plastic bag humidity tent can be cut off along its entire length. After 3 and 5 weeks, shading can be reduced to 70%, and then 50%, respectively, for *Phalaenopsis*, but *Dendrobium antenatium* can be lowered to, and permanently grown at, 30% shade.

We have found that many students who are training for a profession in horticulture or other aspects of plant science are especially curious about and interested in orchids. We have found this laboratory exercise involving orchids to be pedagogically useful because of the important principles and practices that it conveys, and also because it affords students the satisfaction of propagating and eventually taking home their very own orchids.

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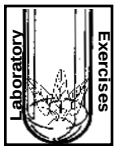
A Laboratory Exercise to Demonstrate Direct and Indirect Shoot Organogenesis using Internodes of *Myriophyllum aquaticum*



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Additional index words. adventitious shoot formation, plant tissue culture, teaching, aquatic plant

Summary. Only a few plants are suitable for reliably demonstrating rapid direct and indirect shoot organogenesis in vitro. A laboratory exercise has been developed using internodes of *Myriophyllum aquaticum*, an amphibious water garden plant. Stock shoot cultures are established and maintained in vitro from nodal explants cultured on agar-solidified medium consisting of half-strength Murashige & Skoog salts (MS) and 30 g·liter^{-1} sucrose. Students use these cultures as the source of internode explants. Explants are cultured on agar-solidified full-strength MS with 30 g·liter^{-1} sucrose, 100 mg·liter^{-1} *myo*-inositol, and 0.4 mg·liter^{-1} thiamine·HCL and factorial combinations of 0 to 10 μM 2iP and 0 to 1.0 μM NAA. Adventitious shoot development occurs directly from the explant epidermis within 4 days and is promoted in media supplemented with 2iP alone. Cytokinin-supplemented media amended with NAA induce organogenetic callus formation, but reduce 2iP promotion of direct shoot organogenesis. After 4 weeks, shoot organogenesis on the various media is quantified and can be analyzed statistically. Chemical names used: *N*-(3-methyl-2-butenyl)-1*H*-purin-6-amine (2iP); α–naphthaleneacetic acid (NAA).

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The most commonly used method for the micropropagation of most horticultural crops is shoot-tip culture. The primary feature of this method is that shoots are produced from shoot-tips or axillary buds that possess preexisting shoot meristems. Plants are produced following subsequent multiplication via enhanced axillary branching and rooting of these shoots (Chu and Kurtz, 1990; George and Sherrington, 1984). Shoots also can be formed adventitiously from tissues through the process of shoot organogenesis. The two types of shoot organogenesis are indirect and direct shoot formation. Indirect shoot organogenesis involves the development of shoots indirectly from an intervening callus derived from the explant. Indirect shoot organogenesis may be less desirable for commercial clonal propagation because plants produced by this method may exhibit greater genetic variation (Cassells, 1985; Cassells and Morrish, 1987; Geier, 1991).

During direct shoot organogenesis, shoot meristems develop directly from the explant. Adventitious meristems arise from the epidermis or subjacent layer and usually are of single-cell origin. Direct shoot organogenesis is more desirable because the probability of genetic variability among the plants produced is lower than with indirect organogenesis (Geier, 1991). However, one limitation to this method is that chimeras cannot be propagated true-to-type through production of adventitious shoots (Lineberger and Druckenbrod, 1985).

Plants such as *Saintpaulia*, *Begonia*, and *Salpiglossis* can be propagated readily in vitro by shoot organogenesis from pieces of internode, leaf blade, or petiole explants (Cooke, 1977; Lee et al., 1977; Start and Cumming, 1976; Takayama, 1983). For these genera, shoots can arise directly from the explant epidermis, but indirect shoot organogenesis also may occur if callus is promoted. The levels and combinations of cytokinin and auxins used can affect significantly the type of shoot organogenesis observed (Start and Cumming, 1976).

For commercial micropropagation, students must recognize the relationship between media selection, subsequent mode of plant regeneration, and resultant genetic stability of the plants produced. However, instructors encounter a dilemma when selecting laboratory exercises to demonstrate direct and indirect shoot organogenesis. Problems associated with poor surface sterilization success, extended culture duration, or sporadic shoot regeneration limit the usefulness of many species for teaching purposes. A reliable

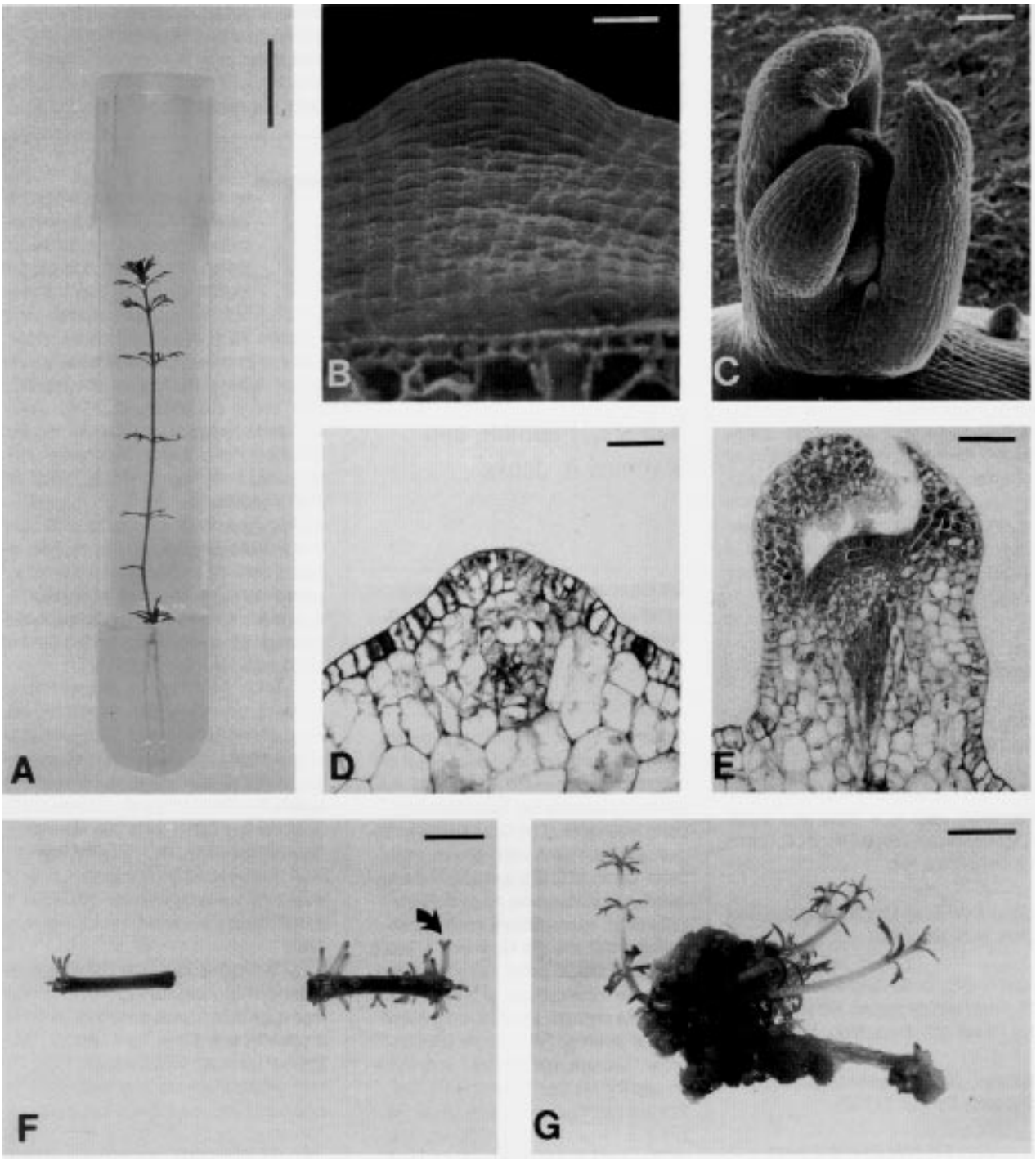

Fig. 1. (A) Two-week-old *Myriophyllum aquaticum* shoot culture used as a source of internode explants. Scale bar = 20 mm. (B,D) Direct formation of dome-shaped adventitious shoot meristem from within the epidermal layer of a 10-mm internode explant after 2 days of culture. Scale bars = 100 µm. (C,E) Adventitious shoot consisting of a distinct apical meristem flanked by numerous leaf primordia after 7 days of culture. Scale bars = 100 µm. (F) Direct shoot organogenesis from a 10-mm internode explant cultured on basal (left) and 2iP-supplemented (right) media for 7 days. During early development, some adventitious shoots (arrow) may morphologically resemble dicotyledonous somatic embryos. Scale bar = 10 mm. (G) Induction of organogenetic callus on internode segment cultured for 28 days in presence of 2iP and NAA. Scale bar = 2.5 mm.

undergraduate laboratory exercise, free of these limitations, has been developed using stem internodes of Parrot-feather [*Myriophyllum aquaticum* (Vellozo) Verdcourt], a popular water garden plant. This laboratory exercise represents a significant enhancement to a simpler exercise we described previously (Kane et al., 1988). The exercise has several key advantages. Stock plants, generated in vitro from surface-sterilized aerial nodal segments, are the source of internode explants used by the students. This procedure eliminates the need for students to perform an often unsuccessful surface-sterilization step. Shoots form via direct organogenesis within 7 days on stem internodes cultured on basal medium, and shoot formation is promoted further on cytokinin-supplemented basal medium. In addition, the influence of 2iP and NAA concentration and combinations on direct and indirect shoot organogenesis can be demonstrated readily within 28 days.

Laboratory exercise preparation

Establishment and maintenance of stock plant cultures. Cuttings of Parrot-feather

(*Myriophyllum aquaticum*) can be purchased from biological supply companies or aquatic plant nurseries. Parrot-feather, being amphibious, grows both as a submerged and aerial plant. Stock cultures are established best using aerial shoots because they are more resistant to tissue damage during the surface-sterilization procedure. Aerial shoots are rinsed in flowing tap water for 1 h. Shoots are then subdivided into nodal sections and surface-sterilized in 1.05% (w/v) sodium hypochlorite for 12 min, followed by three 5-min rinses in sterile distilled water. Nodal sections are transferred aseptically into closure-capped 150 × 25-mm glass culture tubes containing 15 ml of sterile medium consisting of half-strength Murashige and Skoog mineral salts (Murashige and Skoog, 1962) and 30 g·liter^{-1} sucrose with no vitamin supplement. The medium is solidified with 15 g·liter^{-1} TC™ agar (JRH Biosciences, Inc., Lenexa, Kan.). The medium pH is adjusted to 5.7 with 0.1 N KOH prior to addition of the agar and autoclaving at 1.2 kg·cm^{-2} for 20 min at 121C. The basal end of each nodal section should be embedded partially into the medium to prevent desiccation. The cultures are maintained at 21 to 25C under a 16-h photoperiod provided by cool-white fluorescent lamps at about 45 μmol·m^{-2}·s^{-1}. A shoot consisting of five to six usable internodes will develop from the axillary buds of the nodal sections within 2 weeks (Fig. 1A). Nodal sections from these shoots can then be subcultured onto fresh medium at 3- to 4-week intervals to generate additional stock plant cultures.

Preparation of shoot organogenesis media. A basal medium consisting of full-strength Murashige and Skoog mineral salts, 30 g·liter^{-1} sucrose, 100 mg·liter^{-1} *myo*-inositol, and 0.4 mg·liter^{-1} thiamine·HCL is prepared. The basal medium is subdivided and supplemented with factorial combinations of 0, 1, or 10 μM 2iP and 0 or 1.0 μM NAA (Fig. 2). Media are solidified with 8 g·liter^{-1} TC agar. Medium pH is adjusted to 5.7 with 0.1 N KOH before adding the agar and autoclaving at 1.2 kg·cm^{-2} for 20 min at 121C. Autoclaved molten (45C) media are dispensed in 25-ml volumes into separate 100 × 15-mm sterile disposable petri dishes. Petri dishes are labeled with the appropriate shoot organogenesis medium number (Fig. 2).

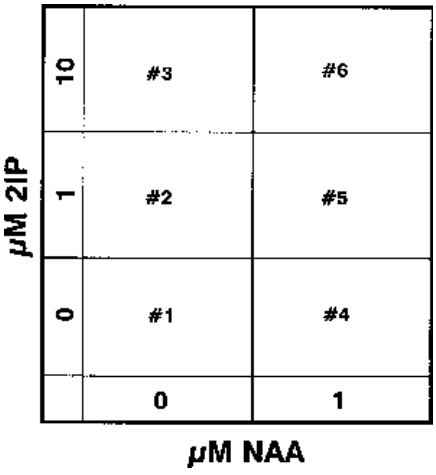

Fig. 2. Treatment matrix for shoot organogenesis laboratory exercise. Specific 2iP/NAA combinations are designated by treatment numbers in blocks.

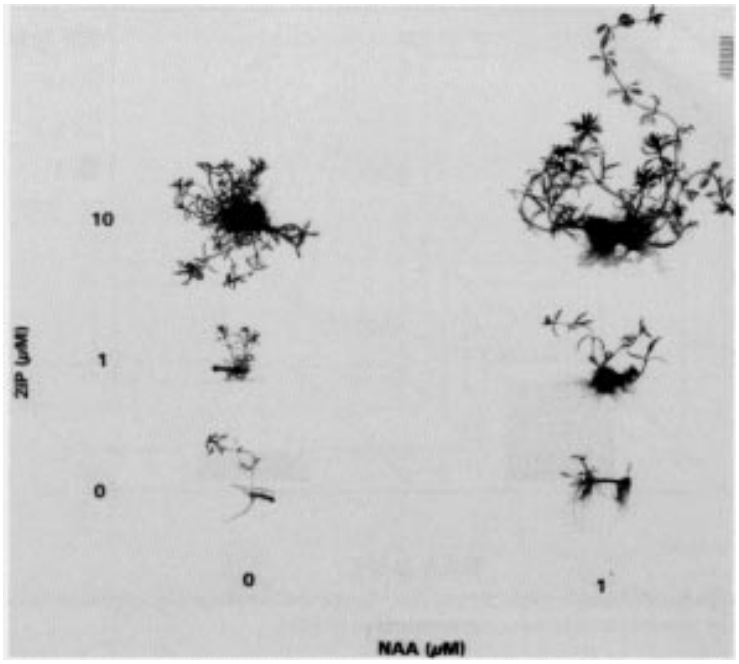

Fig. 3. Effect of 2iP and NAA combinations on organogenetic response of internode explants cultured for 28 days. Note presence of adventitious root formation on NAA-supplemented media. Scale bar = 10 mm.

Materials and procedures

Materials

1) Five culture tubes containing sterile shoot cultures of *Myriophyllum aquaticum*.
2) Forceps and surgeon's No. 7 scalpel handle with No. 10 blade.
3) Sterile petri dishes.
4) Two petri dishes each of Shoot Organogenesis Medium #1, #2, #3, #4, #5, and #6.
5) Sterile, distilled deionized water.

Procedures

1) Obtain five *Myriophyllum aquaticum* shoot cultures.
2) In the transfer hood, remove the closure from one of the cultures and flame the top of the tube.
3) Remove the shoot from the tube by gently grasping it about halfway down the stem with sterile forceps and then pulling it out of the tube.
4) Transfer the shoot into a sterile petri dish.
5) Add enough sterile water to the dish to partially cover the shoot.
6) Cut the shoot above and below each node using a sterile scalpel. This should yield five to six 1-cm-long internode explants.
7) Repeat Steps #3 through #6 using with the remaining shoot cultures.
8) Using sterile forceps, transfer three of the internode explants into each of the petri dishes containing the media. Be sure to place the explants horizontally in firm contact with the medium.
9) Seal each petri dish with a single layer of sealing film (Parafilm or Handiwrap).
10) Initial and date each dish. Using the treatment matrix (Fig. 2) as a guide, write the corresponding 2iP and NAA concentrations on the top of each plate.
12) Maintain the cultures in an incubator or on a culture table at 21 to 25C under a 16-h photoperiod provided by cool-white fluorescent lamps at ≈45 μmol·m^{-2}·s^{-1}.
13) Make weekly observations of your cultures. Be sure to compare the explant responses on each of the six media. Also look for the presence of callus formation. After 4 weeks, determine the average number and length of shoots produced per explant on each medium. Remember that you have a total of six explants per medium (three subsamples in each duplicate dish). Construct a table or graph using your final data.

Expected results and instructional opportunities

Adventitious shoots first develop within 7 days from the epidermis at the basal cut ends of the internode segments cultured on basal medium (Fig 1B–F). Regardless of stem position on the donor plant, internode explants are usually 100% responsive. Direct shoot organogenesis is promoted significantly in the presence of 2iP alone (Fig. 1F, Fig. 4). During the early stages of shoot organogenesis, some adventitious shoots may morphologically resemble dicotyledonous somatic embryos (Fig. 1F). This result provides the opportunity to discuss the morphological and anatomical differences between adventitious shoots and

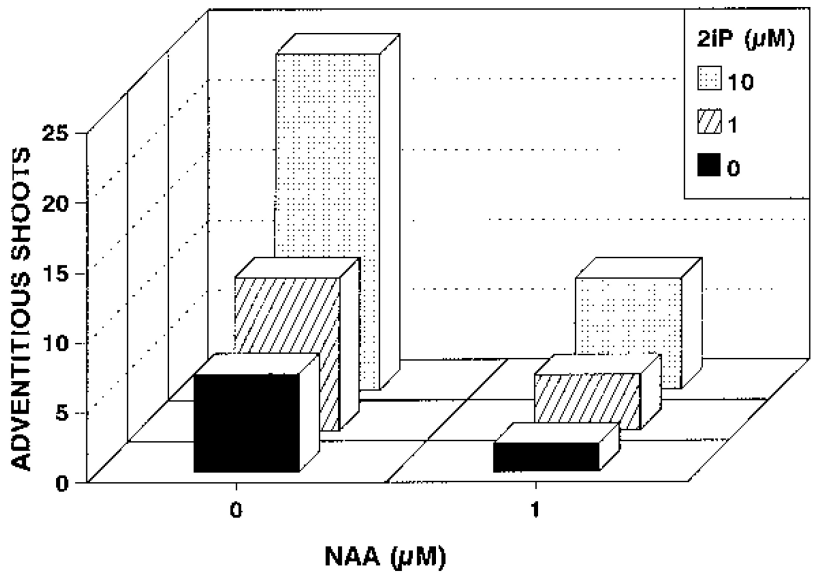

Fig. 4. Typical effect of 2iP and NAA combinations on shoot organogenesis from internode explants cultured for 28 days. Each histobar represents the mean response of six explants per treatment.

somatic embryos. The origin of shoot organogenesis can be verified easily by stained histological section (Fig. 1E) or examined using scanning electron microscopy (Fig. 1 B and C).

Medium supplementation with 1 μM NAA alone promotes adventitious root formation (Fig. 3). Cytokinin-supplemented media amended with NAA induce organogenetic callus formation while reducing 2iP promotion of direct shoot organogenesis (Fig. 2G, Fig. 4). Complete development of organogenetic calli and subsequent indirect shoot organogenesis requires ≈3 weeks of culture. Both direct and indirect shoot organogenesis will occur on the same explant. Typical explant responses for a 28-day exercise are shown in Fig. 3. Main treatment effects and interactions can be analyzed statistically using analysis of variance (ANOVA) procedures. Data can be presented in either a tabular or graphic format (Fig. 4).

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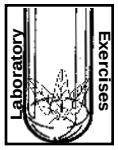
A Laboratory Exercise to Demonstrate Direct and Indirect Shoot Organogenesis from Leaves of *Torenia fournieri*



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Additional index words. adventitious shoots, in vitro, micropropagation, plant tissue culture, teaching, wishbone flower

Summary. A laboratory exercise on direct and indirect organogenesis from leaf explants is presented for students of plant tissue culture or plant propagation. *Torenia fournieri*, the wishbone flower, is used for this laboratory exercise because the in vitro production of adventitious shoots from *Torenia* is easy to control, seeds are easy to obtain, and plants are easy to grow. Direct shoot organogenesis results from leaf explants without an intervening callus phase, and indirect shoot organogenesis is possible after 4 to 6 weeks of callus production from leaf explants. The basal medium for all forms of organogenesis contains Murashige and Skoog (MS) salts and vitamins, 30 g sucrose/liter, and 7 g agar/liter at pH 5.7. To obtain direct shoot organogenesis, leaf explants should be placed on the MS basal medium with 1.1 μM (0.25 mg·liter^{-1}) 6-benzylaminopurine (BAP) and 0.25 μM (0.05 mg·liter^{-1})

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