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A Laboratory Exercise to Demonstrate Adventitious Shoot Formation Using Stem Internodes of Parrot-feather

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Plant tissue culture instructors face a dilemma when selecting laboratory exercises for large classes. Class size and equipment limitations may require that the exercises be performed without a laminar flow hood. The problem is further complicated when one attempts to choose an exercise to demonstrate adventitious shoot formation. There are few plant species from which suitable tissue explants can be obtained to demonstrate rapid adventitious shoot formation. Problems associated with surface sterilization, extended culture duration, or sporadic shoot regeneration limit the usefulness of most explants for classroom demonstrations.

A simple laboratory exercise, free of these limitations, has been developed using stem internodes of parrot-feather [*Myriophyllum aquaticum* (Vellozo) Verdcourt], a popular water garden plant that grows as a submerged and aerial plant. The exercise has several advantages. Stock plants, generated in vitro from surface-sterilized aerial nodal segments, are the source of internode explants used by the students. Adventitious shoot regeneration occurs within 7 days and is further promoted on cytokinin-supplemented medium. Cultures thus can be inoculated during one laboratory period and evaluated for shoot development and cytokinin effects the following week. Finally, the exercise can be performed in classroom without the use of a laminar flow hood.

Stock plant culture. Cuttings of parrot-feather can be purchased from many aquatic plant nurseries or collected locally in the southeastern United States and California. Aerial shoots are rinsed in running tap water for 1 hr. Shoots then are subdivided into nodal segments and surface-sterilized in 1.0% (w/v) sodium hypochlorite for 12 min, followed by three 5-min rinses in sterile distilled water. Individual nodal segments are transferred into closure-capped 150 × 25 mm glass culture tubes containing 15 ml of sterile medium consisting of half-strength Murashige & Skoog (MS) mineral salts (1) and 87.6 mM sucrose. The medium is solidified with 15 g·liter^{-1} TC agar (Hazleton Research Products, Inc., Lenexa, Kan.) The medium pH is adjusted to 5.7 with 0.1 N KOH prior to addition of the agar. The medium then is autoclaved at 1.2 kg·cm^{-2} for 20 min at 121°C. The basal end of each nodal section is partially embedded into the medium to prevent tissue desiccation. Cultures are maintained at room temperature (21° to 25°) under a 16-hr photoperiod provided by 40-W cool-white fluorescent lamps at 90 μmol·s^{-1}·m^{-2} (≈750 fc). A shoot consisting of five to six usable internodes will develop from each nodal section within 2 weeks (Fig. 1A). Nodal segments from these shoots are subcultured to fresh medium to generate additional stock plants.

Media preparation. Media used for the exercise are more complex than the stock plant medium. The control medium consists of sterile full-strength MS mineral salts (1) supplemented with 87.6 mmol sucrose, 1.2 μM thiamine·HCl, 0.56 mmol *myo*-inositol, solidified with 8 g·liter^{-1} agar, and adjusted to pH 5.7. The cytokinin medium has the same ingredients as the control medium plus 10 μM (2 mg·liter^{-1}) *N*-(3-methyl-2-butenyl)-1*H*-purin-6-amine (2iP). Each medium is dispensed as 10-ml aliquots into 20-ml glass scintillation vials and labeled. The vials are covered loosely with heat-resistant screw caps before autoclaving. Scintillation vials are recommended as culture vessels because their size and shallow depth (Fig. 1B) allow the student to quickly orient and partially embed the internode tissue onto the medium with standard-size forceps.

The exercise. Each student is supplied with the following: two culture tubes containing sterile, 2-week-old shoot cultures of *M. aquaticum*; two sterile covered petri dishes; one pair of sterile forceps and a scalpel, both autoclaved wrapped in aluminum foil; and 10 labeled culture vials, five containing control medium and five containing 2iP-supplemented medium. Shoots are removed from the tubes with the forceps and quickly placed into separate covered petri dishes. Each shoot is subdivided into five to six 10-mm internodal segments with the scalpel. The internodal segments are transferred quickly into individual culture vials and placed horizontally on the medium. Cultures are maintained as stated previously. Shoot buds develop directly from the internode epidermis and are macroscopically visible by day four. On day seven, the number of shoots generated on control and 2iP-supplemented media are counted and compared (Fig. 1C). Adventitious shoot formation can be verified by stained histological section (Fig. 1D) or scanning electron microscopy (Fig. 1E). Single copies of the expanded laboratory protocol are available upon request.

Literature Cited

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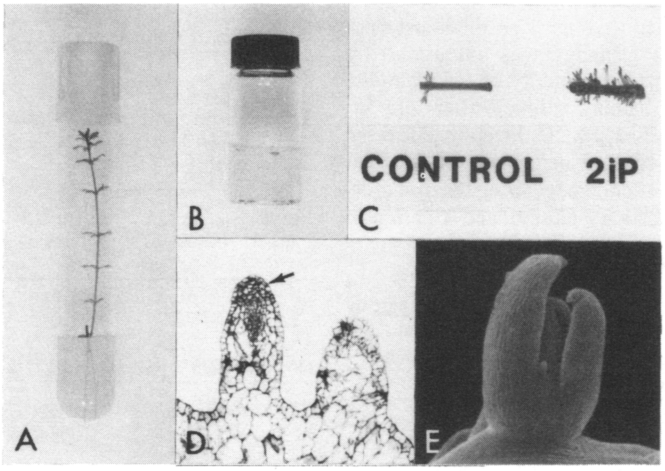

Fig. 1. (**A**) 2-week-old in vitro shoot culture of *Myriophyllum aquaticum* used as the source of internode explants, ×0.4. (**B**) Scintillation vial culture vessel with internode explant, ×0.5. (**C**) Typical adventitious shoot regeneration from 10-mm internode explants cultured on control and 2iP-supplemented media for 7 days, ×1.2. (**D**) Toluidine blue-stained median longitudinal section through an elongated adventitious shoot (arrow) at day 7, ×51. (**E**) Scanning electron micrograph of day 7 adventitious shoot bud, ×40.

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