In Vitro Propagation of *Byblis filifolia* (Byblidaceae)

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Introduction

Byblis filifolia Planch. belongs to the family Byblidaceae Domin. (a family closely related to Lentibulariaceae L. Rich. which includes the genera *Pinguicula* L., *Utricularia* L., and *Genlisea* St. Hil.), the genus Byblis also includes the species *B. gigantea* Lindl. (a perennial), and *B. liniflora* Salisb., *B. aquatica* Lowrie & Conran, and *B. rorida* Lowrie & Conran (annuals or short-lived perennials) (D'Amato, 1998; Lowrie & Conran, 1998). *Byblis filifolia* has a long, meandering central stem with occasional branching and numerous linear leaves. The form of *B. filifolia* used in this study has mauve flowers with a white outer surface. Members of *Byblis* Salisb. are considered to be a carnivorous plants because the outer surface of the stems and leaves are covered with perpendicularly oriented trichome-like glands (the traps) that each exude a droplet of a clear, sticky substance from the distal end. The secretions of a second type of laterally oriented glands purportedly digest the soft portions of trapped insects (D'Amato, 1998). The digestive secretions of the second gland-type contain neither enzymes nor bacteria, and some conjecture that *Byblis* taxa may actually be subcarnivores and that the digestive process is actually facilitated by fungi or, as is the case with *Roridula* L., by assassin bugs (D'Amato, 1998).

Byblis is commonly propagated with seed; however, consistent germination can be difficult to achieve due to the necessity of smoke and/or plant growth regulator treatments, and the seedlings that do germinate are commonly killed by damping off (D'Amato, 1998; Schnell, 1975). Also, seedling populations can exhibit phenotypic variation limiting the value of seed propagation in producing large numbers of plants that consistently exhibit desired characteristics such as specific flower color or size. In vitro propagation methodologies can be used to further improve seed germination (and seedling viability) and to vegetatively propagate (via micropropagation) large numbers of true-to-type clones of plants exhibiting especially desirable characteristics (Bunn, 1985; Stoutamire, 1972).

Materials and Methods

Byblis filifolia seed purchased from Allen Lowrie (6 Glenn Place, Duncraig, 6023, Western Australia) were soaked in a 2.9 mM (1,000 mg·L^{-1}) solution of GA_3 (gibberellic acid) for 24 hours. The seed were then disinfected for 20 minutes in a calcium hypochlorite solution (prepared by stirring 10 grams of calcium hypochlorite in 140 mL of water for 1 hour and then decanting the solution through a Buchner funnel to remove the sediment), rinsed with sterile deionized water, and aseptically transferred to an in vitro germination medium (Stoutamire, 1972).

The in vitro germination medium consisted of one-fifth strength Murashige and Skoog (MS) basal salts with the full strength MS vitamin complement (100 mg·L^{-1} myo-inositol, 0.1 mg·L^{-1} thiamine, 2.0 mg·L^{-1} glycine, 0.5 mg·L^{-1} nicotinic acid, and 0.5 mg·L^{-1} pyroxidine), 30 g·L^{-1} sucrose, and 0.5 g·L^{-1} MES (2-*N*-morpholinoethanesulfonic acid)

(Murashige and Skoog, 1962). The medium was solidified with 6.8 $mg \cdot L^{-1}$ agar, and the pH was adjusted to 5.7 with 1 M potassium hydroxide. No PGRs (plant growth regulators) were added. Culture vessels were 125-mL glass jars capped with Magenta-B® lids (Magenta Corp., Chicago, Illinois.), and the interface between the lid and the jar was sealed with a single layer of parafilm to prevent excessive moisture loss. The vessels contained 30 mL of the medium. Cultures were maintained at 24°C under bright white fluorescent lights (55 $mmol \cdot s^{-1} \cdot m^{-2}$, approximately 4400 lx for our light source) for a 16-hour day-length. Seedlings were transferred to fresh media after 35 days.

Propagules (isolated shoot tips or shoot masses used to continue propagation) were transferred to media with the same composition as the germination medium except that full strength MS salts were used with the Linsmaier and Skoog (1965) vitamin complement (100 $mg \cdot L^{-1}$ myo-inositol and 0.4 $mg \cdot L^{-1}$ thiamine), 0.1 µM IBA (4-[3-Indolyl]butyric acid) was added to the medium, and either 1.0 µM or 2.0 µM BA (*N*-[phenylmethyl]1*H*-purine-6-amine) was added. Propagules were also transferred to a medium with the aforementioned composition except that 2.0 $g \cdot L^{-1}$ of Phytagel™ (Sigma, St. Louis, Missouri) was used in place of agar.

In an effort to determine if *Byblis* shoots excised from shoot masses would be amenable to ex vitro rooting and acclimation, thirty 2 to 3 cm shoots were removed from in vitro culture, dipped in Clonex© (Growth Technology™, Freemantle, Australia)—a commercial rooting formulation containing 14.7 µM IBA—to facilitate root formation, and placed into Bio sponge plugs (Park's Seed Company, Greenville, S.C.) that were then enclosed in the Park's Bio Dome seed starter box. After 15 days, vents on the cultivation box were opened halfway to begin acclimating the plantlets to lower humidity. *Byblis* plants were observed for three months after rooting and acclimation were initiated.

Results, Observations, and Discussion

Only 8 of the 20 seed placed on the in vitro media germinated; however, this result was better than our efforts at germinating *B. filifolia* seed on a peat-based soil free mix in which 50 seed were sown with only two seed germinating (which promptly died from the damping-off syndrome). All seedlings that germinated in vitro survived and grew on the medium. Plants that were left on the germination medium for the duration of a second 35-day interval enlarged from less than 0.5 cm in height to 4 to 6 cm and produced roots in vitro.

Removing the apical meristem (disrupting apical dominance) from a plant on the germination medium caused the plant to produce 2 or 3 lateral branches that could be removed and used as propagules for further propagation. Placing shoots onto media containing PGRs caused the shoots to develop into 3 to 5 cm high shoot masses (see Figure 1) from which 5 to 8 shoots could be typically isolated after 35 days. The addition of 1.0 µM BA produced more new shoots than 2.0 µM BA, but the 2.0 µM level caused the shoots produced to be larger. The addition of PGRs and the full-strength MS salt complement also caused the shoot leaves to be thicker and greener than shoots that had been maintained on the PGR-free germination medium. Shoot masses on the PGR-free germination invariably exhibited yellowing and senescing leaves after 35 days. Microshoots produced on media solidified with Phytagel™ were hyperhydric (structures were translucent and water-logged), so the use of Phytagel™ was discontinued because hyperhydricity can interfere with acclimating the microshoots to ex vitro conditions

Twenty-eight of the original 30 microcuttings that had been dipped in Clonex© and placed in the Park's Bio Dome seed starter began rooting 15 days later. The two shoots that had not rooted after this interval desiccated and died. The surviving plants, which had attained a length of approximately 10 cm, were photographed one month after rooting was first observed (see Figures 2, 3).

Byblis shoot masses have been maintained in in vitro culture for one year at

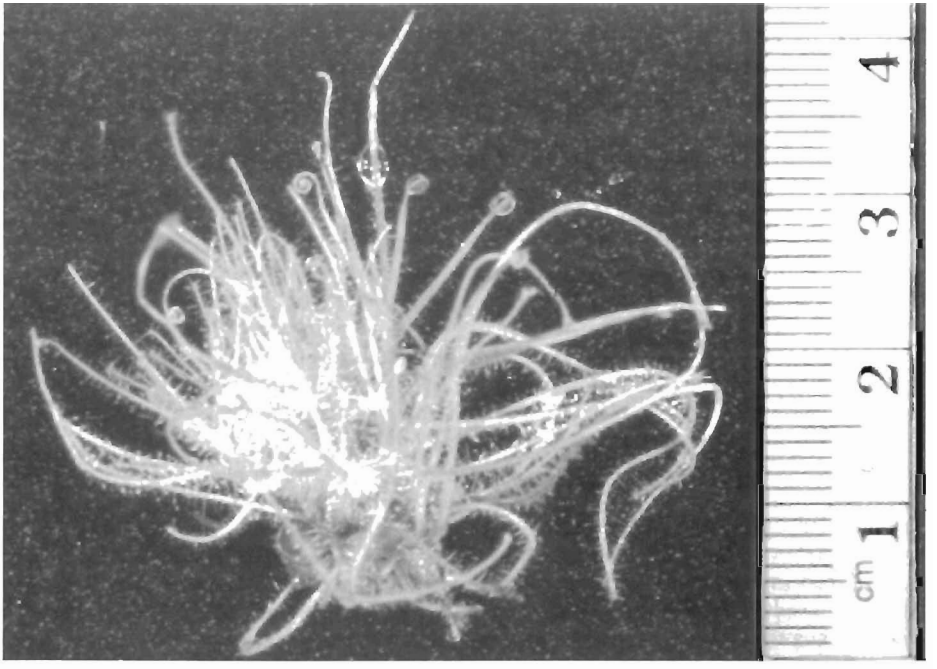

Figure 1: Shoot mass produced by *B. filifolia* on the proliferation medium.

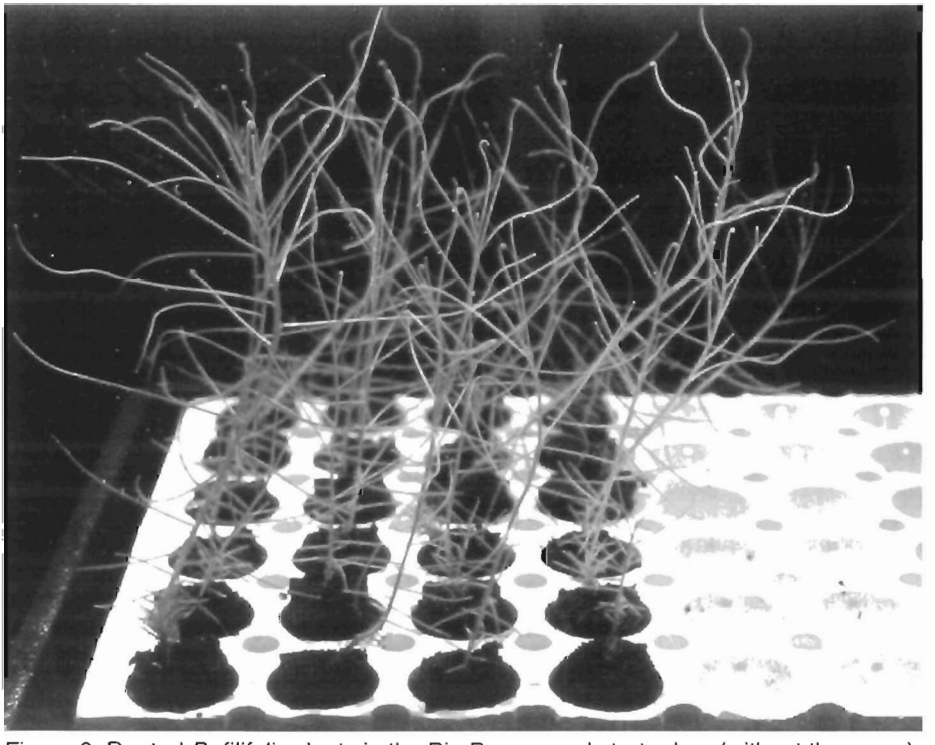
Figure 2: Rooted *B. filifolia* plants in the Bio Dome seed starter box (without the cover).

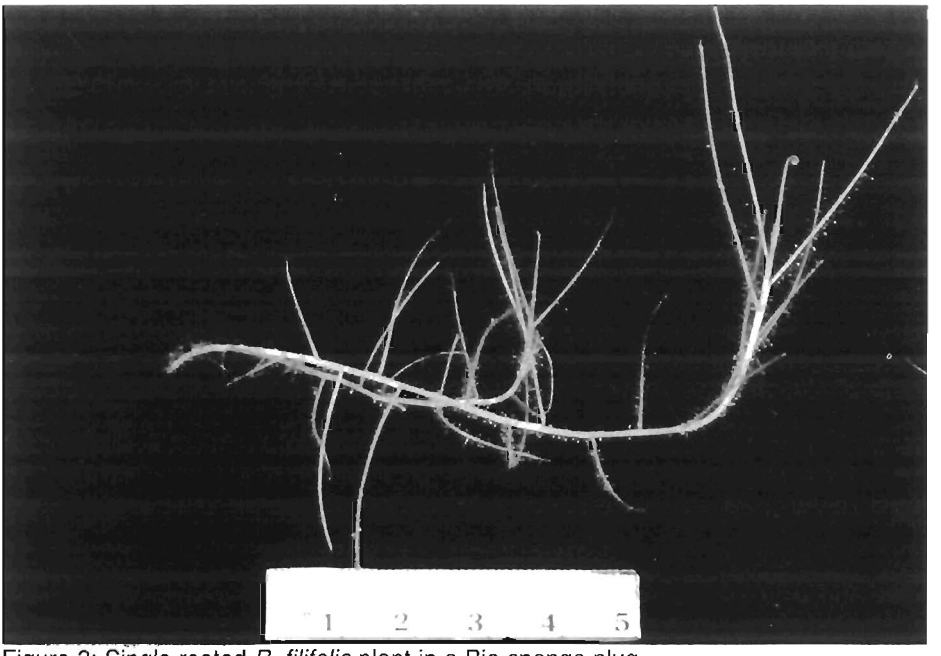

Figure 3: Single rooted *B. filifolia* plant in a Bio sponge plug.

this writing, and we believe that the system of in vitro propagation, ex vitro rooting, and acclimation described herein would permit rapid, continuous production of *B. filifolia* and would prove suitable for rapidly propagating other *Byblis* species. This is the first report on the in vitro proliferation of *B. filifolia*. In vitro rooting of *B. gigantea* had been previously reported by Bunn (1985); however, our observations presented here comprise the first report of successful ex vitro rooting with tissue culture generated *Byblis* microcuttings.

Future work on *Byblis* in vitro culture should entail determining if BA concentrations lower than 1.0 µM are more appropriate for culturing *B. filifolia* and determining if PGR treatments with combinations of PGRs, as suggested by Schnell (1975), might produce better germination of *B. filifolia* seed than GA_3 alone.

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