In Vitro Propagation of *Drosera* spp.

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Carnivorous plants are being depleted from their natural environment due to large-scale collection of plants and development of sites that formerly supported carnivorous plant populations (Campbell, 1983; Juniper et al., 1989). Although there are now reputable dealers of carnivorous plants who do not collect plants from the wild, some species are already considered threatened or endangered (Ayensu and DeFilipps, 1978). If these plants are to continue to be made available to the public without danger to existing natural stands, it is imperative that alternative methods of production be investigated.

In vitro propagation of carnivorous plants is indicated because many plants may be rapidly produced from a small amount of tissue, thereby minimizing collection from natural populations. Several types of carnivorous plants have been successfully propagated in vitro, including the Venus fly trap *Dionaea muscipula* Ellis (M. Crehan, personal communication) and others (Adams et al., 1979a, 1979b; Carroll, 1982; Mohan Ram et al., 1972). This paper describes a simple method of propagating *Drosera* spp. in vitro.

The sundews, *Drosera rotundifolia* L., *D. capensis* L., and *D. binata* La Billardière, were chosen for this study because of their varying growth characteristics: *D. rotundifolia* has a prostrate growth habit with leaves in contact with the soil, and *D. binata* and *D. capensis* have upright growth. Plants were obtained from Carnivorous Gardens (Hamilton, N.Y.) and grown in 100% sphagnum under fluorescent lights (Philips 40-W Agro-Lite fluorescent bulbs) with a 12-h photoperiod.

Whole plants and whole leaves were sterilized in 10% (v/v) Clorox (0.5% sodium hypochlorite) with one drop of Tween 80 added to each 50 ml of diluted Clorox. Tissue was sterilized for 5 min followed by three 5-min sterile distilled water rinses. Whole leaves were used as explants and were placed in 25 × 150-mm culture tubes on 20 ml of medium consisting of Murashige and Skoog mineral salts and vitamins (Murashige and Skoog, 1962) supplemented with 30 g sucrose/liter and solidified with 8 g Difco agar/liter. Medium was adjusted to pH 5.7 before autoclaving. Because carnivorous plants normally grow in nutrient-poor areas, 1/2, 1/4, and 1/8 normal salt and vitamin con-

centrations (1/2 MS, 1/4 MS, and 1/8 MS, respectively) were examined in addition to full-strength medium. Dilutions of full-strength medium containing vitamins were used for 1/2 MS, 1/4 MS, and 1/8 MS. Explants were grown on media without phytohormones and on media containing BA at 0.02 mg·liter⁻¹ and NAA at 0.01 mg·liter⁻¹, according to Adams et al. (1979b). Cultures were grown at 25°C with fluorescent lighting (as described above) under a 16-h photoperiod.

After ≈4 weeks, shoots were visible on leaf explants, apparently forming directly on leaf surfaces without intermediate callus. Leaf explants grown on 1/2 MS with or without hormones produced multiple plantlets that entirely covered the original leaf explant (Fig. 1A). In many cases, too many plantlets were produced to count accurately. All but one leaf explant grown on 1/2 MS and 1/4 MS produced plantlets, but more plantlets were produced on 1/2 MS. Plantlets were produced on 60% and 38% of the explants on 1/8 MS and full-strength medium, respectively.

Plantlets were readily subcultured on 1/2 MS without hormones. All subcultured *D. rotundifolia* and *D. capensis* plantlets produced extensive root systems after 6 to 8 weeks (Fig. 1B), in contrast to the generally weak-rooted plants found in the wild (Juniper et al., 1989). Only 10% of the *D. binata* plantlets rooted on medium without hormones, whereas 30% rooted on 1/2 MS containing BA and NAA. About one-half of the *D. rotundifolia* and *D. capensis* cultures produced flowers on 1/2 MS with or without

hormones, and 90% of the *D. binata* cultures produced flowers on 1/2 MS containing hormones. *D. binata* cultures on medium without hormones did not produce flowers.

Plantlets were removed from culture following root development and planted in 100-liter pots in 100% sphagnum or peatmoss. Pots were placed in magenta boxes (Magenta Corp., Chicago) and acclimated by gradually removing the covers, thereby reducing the relative humidity over 4 weeks. Growth conditions were the same as for cultures, and mature plants flowered and produced viable seed.

About 75% of the original cultures were contaminated. When explants were treated with 20% Clorox for 10 min or 10% Clorox for 20 min, tissue damage was extensive and no regeneration occurred. Occasionally, cultures were contaminated in the immediate area surrounding the explant even when the tissue was dead. No clean cultures were obtained using whole plants as the tissue source.

About 95% of the original *D. rotundifolia* cultures were contaminated. *Drosera rotundifolia* has a prostrate growth habit as compared with *D. capensis* or *D. binata*, and it is likely that leaves in close proximity to the soil would be infested with more soil-borne organisms than leaves of plants with a more upright growth habit. Contamination problems could also be due to a symbiotic relationship that may exist between some carnivorous plants and certain fungi or bacteria that may have a role in the digestive process. After a culture appeared to be clean, contamination of subcultures was much reduced but not eliminated, indicating that microorganisms may remain in intercellular spaces in cultured plants and may occasionally be stimulated to resume growth.

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Fig. 1. (A) Numerous small plantlets forming on leaf explant of *Drosera binata*. (B) Extensive root system developing on culture of *D. capensis*.

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