## Literature Cited

1. Murashige, T. 1974. Plant propagation through tissue cultures. *Ann. Rev. Plant Physiol.* 25:135-166.
2. ———— and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15:473-497.
3. Knauss, J. 1976. A tissue culture method for producing *Dieffenbachia picta* cv. Perfection free of fungi and bacteria. *Proc. Fla. State Hort. Soc.* 89:293-296.


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# IN VITRO **PROPAGATION OF** CRYPTANTHUS **SPP.**

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*Additional index words.* Tissue culture.

*Abstract.* **One of the primary objectives of commercial plant tissue culture is the rapid increase of various plant populations. Several species of** Cryptanthus **can be successfully propagated** in vitro. **Explants were initiated in a liquid modified minimal organics medium. Proliferation was carried out on the same modified Murashige Skoog salts multiplication medium containing 2.0 mg/l Kinetin and 2.0 mg/l IAA. Preparation for transplant to the greenhouse was done on a solid minimal organics medium which contained 1.0 mg/l NAA. Details of the complete procedure are discussed.**

As production costs and plant volume demands increase, alternate methods of producing plants become necessary. Mass propagation by means of tissue culture is such a method. Burr (1) demonstrated that *Nephrolepis exaltata bostoniensis* could be produced economically in large numbers through tissue culture. Strode and Oglesby (5) effectively produced a variety of *Hemerocallis* that had tremendous sales appeal but was not commercially available in large numbers. Jones and Murashige (2) reported the use of tissue culture techniques to produce *Aechmea fasciata* Baker and indicated that those specific techniques were applicable to various other bromeliads. Many other types of plants may be produced by using similar techniques (4).

The Bromeliaceae family is composed of many tropical epiphytic plants. Two more commonly known members of this family are *Ananas* and *Tillandsia usneoides* while other plants from several genera are commercially propagated. These plants are normally propagated by seed or asexually from pups or divisions. Seed propagation may yield plants that vary in size, coloration and flowering depending upon the species. Division or production of pups is slow and normally does not yield many plants in any given amount of time.

The objectives of this study were: (a) to adapt *Cryptanthus bivittatus minor* to tissue culture techniques, and (b) to produce large numbers of plants commercially on a scheduled basis.

## Materials and Methods

Prior to use, *Cryptanthus* plants were kept 2-4 weeks in a dry, air conditioned room with a 16-8 hr light-dark cycle. All outer leaves were stripped from the plant at the leaf-stem axil to expose the lateral buds. The stem with the buds intact was washed for 10 min in running water to remove loose soil. The stem section was placed for 10 min with constant agitation in a solution of 1:10 Clorox (5.25% sodium hypochlorite), 150 mg/l citric acid, Tween 20 (polyoxyethylene sorbitan monolaurate) and deionized water.

In a laminar flow transfer hood, the buds and a small subadjacent piece of the tissue were removed from the stem using a scalpel with a #11 surgical blade with the aid of a dissecting microscope. Excised plant material was placed in an antioxidant solution of 150 mg/l citric acid in sterile deionized water. Buds were removed from the antioxidant solution, wrapped in sterile cheesecloth and submerged in a solution of 1:20 Clorox plus 150 mg/l of citric acid for 5 min. The buds were then rinsed several times with a soln of sterile deionized water plus 150 mg/l citric acid.

Individual buds were dropped into 16 x 150 mm glass culture tubes (Bellco Co., Vineland, NJ) which contained 5 ml of Stage I medium (Table 1). The tubes were placed on a rotating wheel at 1 rpm and grown under 75 ft-c, 16-8 hr light-dark cycle at 27°C. Within 4-6 weeks, the buds were removed from Stage I medium and placed in a 125 ml baby food jar with 5 ml of Stage II medium (Table 1). The jars were covered with 48S Mylar (DuPont Co., Wilmington, DE) and secured with rubber bands. The cultures were grown under 300 ft-c, 16-8 hr light-dark cycle at 27°C without agitation. With each subsequent subculture every 4 weeks all dominant shoots were cut and placed on Stage III medium (Table 1) in 946 ml mason jars. Forty plantlets were placed in each jar containing 75 ml of medium. Callus with small shoots was placed in new Stage II medium. Stage III cultures were grown for 5-6 weeks under 900 ft-c, 16-8 hr light-dark cycle at 27°C. After removal from the mason jars, the plants were grown in a saran greenhouse in trays on raised benches containing a medium of 1:1 peat and perlite. Light intensity at plant level was 3000 ft-c.

A 3x4 factorial experiment was set up to evaluate the effect of different auxins at 4 concn on the rooting of plantlets on Stage III medium. At concn of 0.0, 0.1, 1.0 and 10.0 mg/l auxin sources of indole-3-butyric acid (IBA),

Table 1. Media for propagation of *Cryptanthus bivittatus minor in vitro.*

| Compound | Establishment Stage I | Multiplication Stage II | Rooting Stage III |
|---|---|---|---|
| Murashige-Skoog salts[z] | 4,340.0[y] | 4,340.0 | 4,340.0 |
| Thiamine • HCl | 0.4 | 0.4 | 0.4 |
| i-Inositol | 100.0 | 100.0 | 100.0 |
| Adenine Sulfate, $H_2O$ | 80.0 | 80.0 | 80.0 |
| $NaH_2PO_4 \cdot H_2O$ | 170.0 | 170.0 | 170.0 |
| Sucrose | 30,000.0 | 30,000.0 | 30,000.0 |
| IAA (Indole-3-acetic acid) | 2.0 | 2.0 | |
| Kinetin ($N^6$-furfurylamino) purine | 2.0 | 2.0 | 2.0 |
| NAA ($\alpha$-naphthaleneacetic acid) | — | — | 1.0 |
| Citric acid | 150.0 | 150.0 | 150.0 |
| Difco Bacto-Agar | — | — | 8,000.0 |

[z]GIBCO, Grand Island Biological Co., Grand Island, NY.
[y]mg/l

Table 2. Effect of auxin source and concn on root initiation of *Cryptanthus bivittatus minor*.

| Compound | Concn mg/l | | | |
|---|---|---|---|---|
| | 0.0 | 0.1 | 1.0 | 10.0 |
| IAA | 1.7[z] | 2.1 | 2.0 | 2.7 |
| IBA | 2.6 | 1.6 | 2.2 | 3.0 |
| NAA | 2.7 | 2.5 | 6.7 | 3.0 |

[z]Average number roots per plantlet.

indole-3-acetic acid (IAA) and $\alpha$-napthaleneacetic acid (NAA) were incorporated into a minimal organics agar medium (4). Data were collected at 4 and 6 week intervals (Table 2).

### Results and Discussion

Preliminary experimental work comparing establishment of explants in a rotating versus stationary liquid medium showed that rotation was necessary for survival and growth. At no stage of growth could the explants be submerged. The addition of citric acid to the medium and disinfecting soln resulted in increased growth in a shorter period of time. Average loss of the initial explant was 18%. Results indicated that by placing the plant to be used as a source of explants in a dry, air conditioned room the percentage loss in Stage I was decreased. The loss in Stage I varied from crop to crop and could be attributed to the vigor of the mother plant or possibly to daylength.

The successful production of this plant in culture necessitated the establishment of a callus piece in a multiplication medium (Table 1) to produce shoots. At 4 week intervals, shoots 7-8 mm and larger were harvested. As callus pieces grew, more medium was put into each culture vessel up to a maximum of 20 ml liquid. With each subculture the number of shoots produced increased markedly. Some of the cultures showed an unidentified bacterial contaminate. Knauss (3) reported several organisms associated with plants grown *in vitro* resulted in reduced vigor, color and in some cases death of tissue. A definite difference was noted in production between contaminated and apparently clean cultures. Apparently clean cultures yielded 36.0 plantlets while obviously contaminated produced only 14.6 plantlets. In a period of 9 months, 10,000 plantlets were produced from 1 mother plant with 25 buds. Normal production by asexual means under greenhouse conditions is 6-9 plantlets per year from an established stock plant. Callus pieces were kept in culture through 10 subcultures with a very low frequency of mutation. Plantlets were almost entirely green at this stage and the rooting stage. NAA had the greatest effect on root initiation with an average of 6.7 roots per plantlet (Table 2). Jones and Murashige (2) found that IAA promoted the best root formation of the various bromeliads they examined.

Plantlets taken from State III to be planted into beds or flats were ready for transplant in 4 months. The loss in this phase of growth was less than 1%. At the higher light intensities in the growing areas the *Cryptanthus* quickly resumed its normal pink-brown coloration.

This study demonstrated that the growth of *Cryptanthus bivittatus minor* by plant tissue culture methods is economically feasible with the production of over 60,000 plants within 10 months. The need to establish clean cultures was evident from the difference in production between clean and contaminated cultures. Root formation was directly related to auxin source and concn.

### Literature Cited


1. Burr, R. W. 1975. Mass propagation of Boston fern through tissue culture. *Proc. Int. Plant Prop. Soc.* 25:122-124.
2. Jones, J. B. and T. Murashige. 1974. Tissue culture propagation of *Aechmea fasciata* Baker and other bromeliads. *Proc. Int. Plant Prop. Soc.* 24:117-126.
3. Knauss, J. F. 1977. Bacterial contaminants affecting commercial plant tissue cultures. *In Vitro* 13(3):147.
4. Murashige, T. 1974. Plant propagation through tissue culture. *Ann. Rev. Plant Physiol.* 25:135-166.
5. Strode, R. E. and R. P. Oglesby. 1976. Commercial tissue culturing at Oglesby Nursery, Inc. *Proc. Fla. State Hort. Soc.* 89:367-368.


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# EFFECT OF SUCROSE LEVEL, MEDIUM COMPOSITION AND pH ON THE IN VITRO GERMINATION OF POLLEN FROM SPATHIPHYLLUM FLORIBUNDUM (LINDEN & ANDRE) N. E. BR. MAUNA LOA AND VRIESEA MALZINEI E. MORR.[1,2]

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*Additional index words.* Calcium, boron, nutrition.

*Abstract.* **Attempts were made to germinate pollen from** Spathiphyllum floribundum **(Linden & Andre) N.E. Br. cv. Mauna Loa and** Vriesea Malzinei **E. Morr. in various artificial media. Optimum germination of** Spathiphyllum **pollen occurred in a nutrient medium with sucrose levels of 5 or 10% while** Vriesea **pollen germinated well at levels from 5 to 30%. The addition of boron or calcium to the germination medium was essential for germination of** Vriesea **pollen. Germination of** Spathiphyllum **pollen was also increased by the addition of boron and calcium but not to the extent of** Vriesea **pollen.** Spathiphyllum **pollen germinated satisfactorily only when the medium pH was between 4.0 and 7.0, whereas good germination of** Vriesea **occurred in the pH range of 4.0 to 8.0.**

Pollen germination *in vitro* has been studied in many horticultural crops (4, 5, 7, 8). The ability to germinate pollen *in vitro* is important to plant breeding programs where it is often necessary to determine pollen viability after long periods in storage. *In vitro* germination and tube

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