

ously listed in this frequency grouping. The remaining genera listed and the other unlisted genera each represented approx 33%.

The 10 most problem prone genera (Table 7) have an interesting relationship to the plant product mix being offered in the central Florida area (3). The most frequent problem genera, *Peperomia*, resulted in 13.5% of the cases but only accounts for 4% of the foliage plants sold, while the no. 2 problem genera, *Philodendron*, was diagnosed with 12.4% of the total with a 28% share of the market. *Scindapsus* accounted for 4% of the market but 9.6% of the problems. The total of the top 3 again represented approx 33% of the problems and 33% of the market. However, there was a disproportionate number of problems for both *Peperomia* and *Scindapsus* in relationship to their market position. The remaining genera generally had approx the same percentage of the market as their percentage of plant problems.

Table 7. Ten genera of foliage plants most frequently diagnosed with problems (1/76-6/77) and the proportion each genus displaces in the total foliage plant product mix in Central Florida (1976).

Genus	No. of plant problems	% of total plant problems	% product mix in Central Fla.
<i>Peperomia</i>	162	13.5	4
<i>Philodendron</i>	148	12.4	28
<i>Scindapsus</i>	115	9.6	4
<i>Dieffenbachia</i>	80	6.7	6
<i>Maranta</i>	71	5.9	5
<i>Dracaena</i>	51	4.3	6
<i>Brassaia</i>	47	3.9	2
<i>Aglaonema</i>	42	3.5	2
<i>Aphelandra</i>	41	3.4	3
<i>Syngonium</i>	41	3.4	4
Others	398	33.3	—

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TISSUE CULTURE PROPAGATION OF SOME FOLIAGE PLANTS¹

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Additional index words. *Dieffenbachia*, *Yucca*, cloning.

Abstract. Rapid in vitro propagation systems have been determined for *Dieffenbachia* sp. and *Yucca* sp. Sterile, axillary buds were used as the primary explants, and these were inoculated onto modified Murashige and Skoog basal medium containing 6-benzyl amino purine (BA) (*Dieffenbachia*) or α naphthalene acetic acid (NAA) and 6-benzyl amino purine (*Yucca*). Details of the procedures and proliferation rates are presented.

The foliage plant industry of Dade County has become very much aware of some of the commercial benefits of plant tissue culture propagation. *In vitro* systems for the mass propagation of many foliage plants have been published, and are currently being exploited (1). However, many plants have not yet been considered, and some of the currently utilized tissue culture propagation systems are slow or otherwise unsatisfactory. Although many of the Liliaceae and Araceae have been successfully propagated using plant tissue culture, there have been no published reports in which systems for *Yucca* and *Dieffenbachia* sp. have been described.

Because many growers are interested in improving their production systems, and in rapidly increasing disease indexed stock, formulations were devised and tested for the clonal propagation of these plants.

Materials and Methods

Yucca. Small, unopened lateral buds from stem pieces of *Yucca* sp. were removed and any discolored leaves were excised. After a brief rinse in absolute alcohol, the buds were sterilized by immersion in 20% (v/v) Clorox for 10-12 minutes, stirred occasionally and were rinsed with three changes of sterile, distilled water. The explants were transferred into test tubes containing modified Murashige and Skoog basal medium (2) with 30 g/liter sucrose, to which had been added various concentrations of kinetin (5-50 μ M), 6-benzyl amino purine (0.4-10 μ M) and α naphthalene acetic acid (0.5-50 μ M).

Dieffenbachia. Primary explants were obtained from vigorously growing *Dieffenbachia* plants. The leaves were removed from the main stem, and the small lateral buds at the base of each leaf were excised. The tissue pieces were transferred into 20% (v/v) Clorox for 12-15 minutes, and were subsequently rinsed in three changes of sterile distilled water. Following sterilization, the lateral buds were further dissected with the aid of a binocular microscope until buds 2.5-3.0 mm in length were obtained. The buds were re-immersed in 5% (v/v) sterilant for 2-3 minutes, subsequently rinsed with sterile, distilled water, and were placed into culture media. Growth media based on the modified Murashige and Skoog formulation with 30 g/liter sucrose and added cytokinin (0.2-9 μ M BA) were used.

The media were solidified with 8 g/liter Difco Bacto-agar and the pH was adjusted to 5.7 with KOH before sterilization. The cultures were maintained in an air conditioned room at 28° C with 16 hr light (3500 lux) and 8 hr darkness.

Results

Yucca. There was little difficulty in obtaining sterile

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Yucca explants from lateral buds. During the first month and a half in culture, there was no evidence of proliferation, although some extension growth of the foliage occurred. Many cultures, regardless of medium, had begun to proliferate by the end of the second month. As a result of experimentation the optimum medium for establishment and proliferation consisted of the Murashige and Skoog basal medium with 3 μM BA and 1 μM NAA. Proliferation was the result of the induction of axillary buds at the base of each leaf (Fig. 1). Average biweekly increases over a 3 month period varied between one- and three-fold with some lines (Table 1). Occasionally, hard callus masses were induced from which adventitious shoot formation resulted. Young plantlets were excised from culture at regular intervals, and were subcultured on fresh media. Plantlets were transferred either to basal medium without hormones or to basal medium supplemented with 0.5 μM NAA to induce roots and to inhibit further proliferation. Roots were initiated within three weeks. Plants have been successfully established under intermittent mist.

Dieffenbachia. Successive dissections and sterilizations in dilute Clorox were of the utmost importance in obtaining sterile explants of *Dieffenbachia*. The rate of microbial contamination was very high, and this procedure reduced the number of cultures that ultimately would have been destroyed. Media containing auxins caused excessive callus

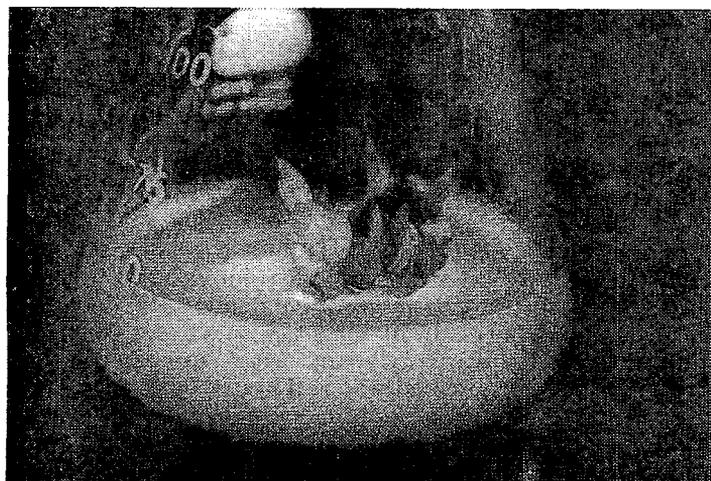


Fig. 1. A proliferating *Yucca* culture 2 weeks after sub-culturing on modified Murashige and Skoog basal medium with 3 μM BA and 1 μM NAA.

Table 1. Theoretical proliferation data* for *Yucca* sp. on Murashige and Skoog basal medium supplemented with 3 μM BA and 1 μM NAA.

Date	No. cultures
Day 1	1
Day 60 ^y	2
75	4
90	8
105	16
120	32
135	64
150	128
345	1,048,576
360	2,087,152

*These figures are based on observations of proliferating cultures during 3 months.

^yThe establishment period for the primary explant was approximately 60 days.

induction. Furthermore, the explants responded more rapidly to BA than to kinetin. The most satisfactory growth response occurred on media containing 10 μM BA.

The initial pattern of development favored the growth of small plantlets with the typical leaf shape. After approximately 4 months in culture, the stimulation into growth of the axillary buds had occurred (Fig. 2), and the cultures were subdivided. Subsequent increases occurred that resulted in doubling of the number of plants after each four week interval (Table 2). Adventitious roots were initiated both in proliferation media and after transfer to basal medium without growth substances.

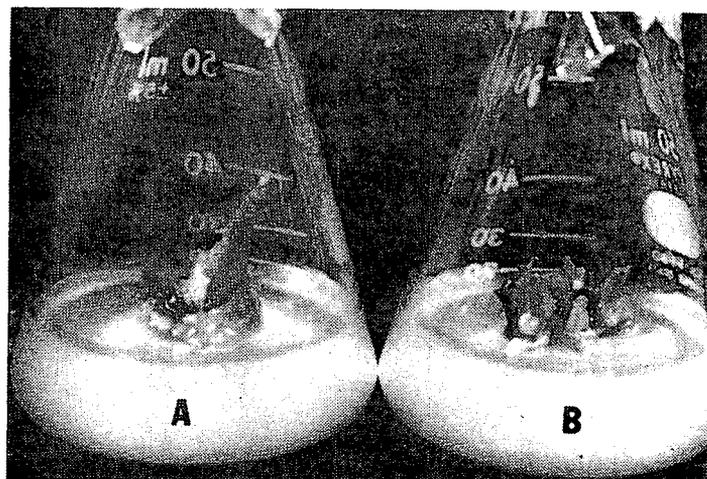


Fig. 2. Proliferating *Dieffenbachia amoena* cultures 1 month (A) and two months (B) after sub-culturing on modified Murashige and Skoog basal medium with 10 μM BA.

Table 2. Theoretical proliferation data* for *Dieffenbachia* sp. on Murashige and Skoog basal medium supplemented with 10 μM BA.

Date	No. Cultures
Day 1	1
120 ^y	2
150	4
180	8
210	16
240	32
270	64
300	128
330	256
360	512

*These figures are based on observations of proliferating cultures during three months.

^yThe establishment period for the primary explant was approximately 4 months.

Discussion

Rapid, *in vitro* propagation systems have been described for *Yucca* and *Dieffenbachia* sp. The theoretical increase from a single *Yucca* plant would yield in excess of two million plants after one year (Table 1), while *Dieffenbachia* could be increased in excess of five hundred (Table 2). These procedures should provide a convenient tool for the rapid clonal propagation of these two genera.

Moreover, this propagation method would provide an aseptic method for increasing the number of disease-indexed *Dieffenbachia* sp. using the *in vitro* screening procedure developed by Knauss (3).

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IN VITRO PROPAGATION OF CRYPTANTHUS SPP.

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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*	4,340.0 [†]	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 ₂ O	80.0	80.0	80.0
NaH ₂ PO ₄ · H ₂ O	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 (α-naphthaleneacetic acid)	—	—	1.0
Citric acid	150.0	150.0	150.0
Difco Bacto-Agar	—	—	8,000.0

*GIBCO, Grand Island Biological Co., Grand Island, NY.

[†]mg/l