CALADIUM — A CANDIDATE FOR MICROPROPAGATION?

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Members of the family Araceae have attracted considerable attention as subjects for micropropagation.

Seeds are used for propagation of some species (e.g. *Monstera deliciosa*, *Anthurium* spp.), but the progeny are variable. Other species are normally propagated by stem cuttings (e.g. *Philodendron scandens*, *Dieffenbachia maculata* (Syn.: *D. picta*), *Epipermnum aureum* (Syn.: *Scindapsis aureus*) and *Syngonium podophyllus*), but multiplication rates are not very high and large numbers of stock plants must be maintained. Propagation by crown, rhizome, or tuber division is also used with other species (e.g. *Anthurium* spp., *Caladium* × *hortulanum* and *Zantedeschia* spp.), but propagation by these means is even slower.

Faster rates of multiplication have been achieved by micropropagation than can be achieved by the traditional methods listed above. At the 1981 meeting, Cohen (1) reported that the proliferation rate for *Zantedeschia* in culture is approximately 5-fold/month (i.e. potentially 100,000 in 10 months), compared with approximately 10-20 fold/year by rhizome division. Multiplication rates for *Dieffenbachia* are considerably lower, 500-fold/year (8) compared with approximately 8-10 fold/year by cuttings. Pierek (13) recommended a callus phase for *Anthurium andreanum* which achieved rapid multiplication rates, but this method has lost favour because of undesirable variability in the progeny. Kunisaki (7) suggests a slower procedure involving proliferation of existing vegetative buds in order to maintain genetic uniformity.

Many aroids are susceptible to a number of systemic bacterial, fungal, and viral pathogens which are readily spread using conventional vegetative propagation techniques, resulting in inferior plants and loss of production.

In vitro methods are an established means for the elimination of pathogens and subsequent rapid multiplication of high-health plants. Knauss (5) developed a tissue cultured method to produce *Dieffenbachia* free of bacteria and fungi. Hartman (4) eliminated dasheen mosaic virus and other phytopathogens by shoot-tip culture of *Caladium* × *hortulanum*.

A survey of procedures recommended for araceous genera has been made (Table 1). The mineral salt formation of Murashinge and Skoog (12) and the vitamin supplements of either

Murashige and Skoog or Linsmaier & Skoog have been used by most workers.

Table 1. Hormone recommendations for the multiplication phase of various Araceous genera *in vitro*.

Species	Reference	Auxin	Cytokinin
Anthurium andreanum	(6,7)	—	0.2 mg/l BA
	(13,14,15,17)	—	1.0 mg/l PBA
A. scherzerianum	(16)	—	0.1 mg/l PBA
Caladium × *hortulanum*	(4)	15 mg/l TAA	1 mg/l K
Dieffenbachia maculata	(8)		2 mg/l BA
	(18)	2 mg/l IAA	16 mg/l IPA
Monstera deliciosa	(3)	2 mg/l IAA	10 mg/l PBA
Scindapsis aureus	(10)	—	10 mg/l IPA
Spathiphyllum clevelandii	(2)	—	2 mg/l PBA
Syngonium podophyllum	(10)	—	20 mg/l IPA
	(9)	3 mg/l IAA	20 mg/l IPA
Zantedeschia	(1)	—	3 mg/l BA

Abbreviations: BA — benzyladenine
IPA — isopentyladenine
K — kinetin
IAA — indoleacetic acid
PBA — Shell Development — SD 8399

The procedure for *Zantedeschia* hybrids recommended by Cohen (1) relied on the control of bud multiplication, elongation, and rooting by the alteration of a single medium component, the cytokinin benzyladenine (BA). Other workers often use BA to induce shoot multiplication, but a wide range of other cytokinins are also used. In some methods the auxin, indoleacetic acid (IAA), is added and it has been suggested by Kunisaki (6) that auxin is required when petiole or leaf blade explants are used, but not when entire leaves or buds are cultured. The addition of coconut water and adenine sulphate is sometimes recommended and some workers prefer to use liquid media rather than solidified media for some stages.

MICROPROPAGATION OF *CALADIUM* × *HORTULANUM*

There has recently been interest in caladiums as houseplants in New Zealand. Although they are easily produced by the importation of dormant rhizones from Florida, there are several situations in which micropropagation could be advantageous.

1) Exportation of *in vitro* material to Australia (or elsewhere), as their quarantine laws do not allow the importation of rhizomes.

2) Rapid multiplication of a new cultivar, which would be considerably faster than the conventional division of rhizomes (4).

3) Possibility of the development of a lower cost method of production.

We were interested in the micropropagation of *C.* × *hortulanum* for several reasons:

1) To determine whether the procedure developed for *Zantedeschia* would be suitable for other araceous plants.

2) To investigate whether plants remained true to type when produced from compressed bud tissue.

We have developed a method for the tissue culture of caladiums similar to that used for the *Zantedeschia*, but more economical in terms of vessels and media. There are two stages involved rather than three, and space saving, inexpensive petri dishes are used for all stages, rather than jars.

There are slight changes in the hormone levels used for initiation and multiplication, and no separate elongation medium.

CULTURE INITIATION

Pieces of rhizome (approximately 2×2×1 cm) with dormant buds were cut off plants growing in the lab, washed under running tap-water and dried out on the lab bench for about an hour. These were then dipped in 95% ethanol and flamed twice.

Using a stereomicroscope, buds approximately 1 to 2 mm long, with some basal rhizome tissue and several leaf primordia attached, were dissected out and placed base down on the multiplication medium. Contamination was less than 20%.

MEDIA AND CULTURE CONDITIONS

For initiation and multiplication, the medium used contained MS salts, LS vitamins, 3% sucrose, 0.6% agar and benzyladenine (BA) at 1 mg/l.

Cultures were grown at 26°C under continuous diffuse light of approximately 1000 lux.

MULTIPLICATION

In the initial establishment phase of 8 to 10 weeks, growth was fairly slow. Two types of tissue developed. The apical bud grew into a small shoot, and a clump of suppressed bud tissue formed at the base. When the shoot was cut off and subcultured, clumps of shoots were formed and, in the first month, the multiplication rate was approximately 2 to 3 fold, but this increased to approximately 6-fold/month thereafter. When clumps of suppressed bud tissue were subcultured, there was

an increase in size of about 4 to 5 fold and some small shoots formed on most pieces.

At a higher concentration of BA (3 mg/l) shoots and meristematic tissue were more suppressed, and less shoots were formed later on the elongation/rooting medium. The addition of the auxin naphthaleneacetic acid (NAA) at 1 or 3 mg/l reduced shoot multiplication considerably and stimulated the excessive formation of roots.

When the medium recommended by Hartman (4) containing 1 mg/l kinetin and 15 mg/l IAA was tested, multiplication rates were lower and the shoots were large with many roots, making the material much harder to handle during subculture. Suppressed bud tissue formed at the base of the shoots, which Hartman referred to as "callus".

SHOOT ELONGATION AND ROOTING

The BA concentration for this stage was lowered to 0.1 mg/l, but all other factors were the same as for the multiplication stage.

Single shoots or small clumps of shoots were transferred to petri dishes containing the elongation/rooting medium, and after 4 to 6 weeks they had elongated, formed roots, and were able to be transferred out of culture.

Small pieces of meristematic tissue were also subcultured onto the elongation medium, but these generally needed a second subculture before transfer. At the rate of ten pieces of meristematic tissue/plate, single plates yielded from 40 to 60 shoots in the first subculture.

TRANSFER TO POTTING MIX

Plantlets were washed under tap-water to remove agar, planted into a 50:50 fine pumice:peat mix, watered with Hoagland's complete nutrient solution. They were then placed under high humidity in the 26°C culture room with the light increased to 3000 lux.

Survival on transfer was 100% and subsequent growth has been excellent.

Several unrooted shoots transferred to potting mix have rooted. This was not achieved with unrooted *Zantedeschia* shoots. There is a possibility that rooting in culture will not be important and clumps of unrooted shoots could be transferred directly to potting mix. This is currently being tested.

DEVELOPMENT OF COLOUR AND VARIEGATION

The pink colour and variegation has developed in some of

the faster growing clones. We are continuing to observe colour development and, in particular, we are interested in whether variegation is increased by repeated subculture or the use of the compressed bud tissue.

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