TISSUE CULTURE PROPAGATION OF *AECHMEA FASCIATA* BAKER AND OTHER BROMELIADS[1]

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The Bromeliaceae is composed of largely tropical American genera, many of which are herbaceous epiphytes. It includes the pineapple and some commercially important ornamentals. One flowering ornamental, *Aechmea fasciata* Baker, known in the trade as Aechmea, has been popular as a house plant for many years (Fig. 1). In standard practice Aechmea is propagated through seed. This procedure yields plants that are quite variable and a significant proportion may be of unsalable quality. There have been other problems associated with seed propagation, such as a limited supply, seasonal availability, and poor germination. Vegetative propagation of Aechmea can be accomplished by division or suckers, as is done with pineapple, but this method is too slow to be practical. We now describe a tissue culture method of rapid clonal multiplication. The method appears applicable also to some other bromeliads. However, it should be used cautiously, inasmuch as sometimes there is difficulty in reproducing plant type.

Figure 1. Mature plant of *Aechmea fasciata* Baker.

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Some Aechmea plants obtained through tissue culture have shown genetic deviation, and the incidence of aberrant plants has been observed to increase with repeated reculture *in vitro*. Nevertheless, when properly employed, the procedure can yield 500 uniform clonal Aechmea plants from each explant within a 6-month period *in vitro*.

The Starting Tissue. Shoot tips measuring 2-5 mm in height are employed as explants. They can be obtained from buds that are located at the base of the mature plant or from the terminal of an actively growing shoot. The former is preferred, inasmuch as many more explants can be obtained from a given plant. In principle, the procedure of obtaining shoot tips in either instance is the same. When buds are used as source of explants the older leaves and all roots at the base of the plant are first removed to expose the buds (Fig. 2). Usually the buds are buried in the stem tissue and may need to be located systematically. Since the buds on the stem occur in a regular sequence of whorls, once one bud has been found it is relatively easy to locate the others. The outermost 1-2 bud scales are removed and any dead tissue is trimmed away from the bud and the surrounding stem tissue. A 5-10 mm cube of tissue composed of the stem and the bud (Fig. 2) is removed from the plant and transferred immediately to a solution containing 100 mg/1 ascorbic acid and 150 mg/1 citric acid. These acids presumably retard oxidation and deterioration of the tissue. Those experienced with plant tissue cultures would be aware of the browning that results almost immediately in some plants in

Figure 2. Base of Aechmea plant showing exposed buds (left) and bud piece prior to disinfection (right).

response to injury, and these substances appear to delay that browning process.

In using the terminals of growing shoots as shoot-tip explants, all leaves are first removed, leaving only a few embryonic leaves at the extreme stem apex. A 5-10 mm tall structure composed of the embryonic leaves and subjacent stem tissue is severed from the plant and transferred to the ascorbic acid/citric acid solution.

After a sufficient number of buds or shoot terminals have been obtained, they are wrapped in small squares, about 4-inch squares, of cheesecloth and transferred to 25 x 150 mm test tubes for preliminary disinfection. A generous quantity of dilute laundry bleach (Purex or similar bleach diluted 20-fold with distilled water and containing a small amount of detergent, e.g., 1-2 drops of Tween-20 emulsifier per 100 ml of disinfectant solution) is added to the tube, covering all contents within the tube. The tubes are capped with polypropylene closures (Bellco kaputs). After 10 min. the bleach solution is decanted and the contents of the tube are rinsed 3 times with autoclaved water.

Shoot tips of culturable size are isolated with the aid of the dissecting microscope. Usually a 10-20X magnification is adequate. Aseptic procedures should be followed and the surgical and transfer steps should be performed in a laminar-flow clean-air hood to minimize contamination by airborne micro-organisms. The bud or stem-tip section is transferred individually into a sterile Petri dish for further excision. The surgical instruments include a pair of fine-tipped long (about 25 cm) forceps, a pair of smaller (10 cm long) fine-tipped forceps, and a surgeon's scalpel with No. 11 blades. Sometimes the No. 15 scalpel blades may also be helpful. The surgical instruments are sterilized by immersion

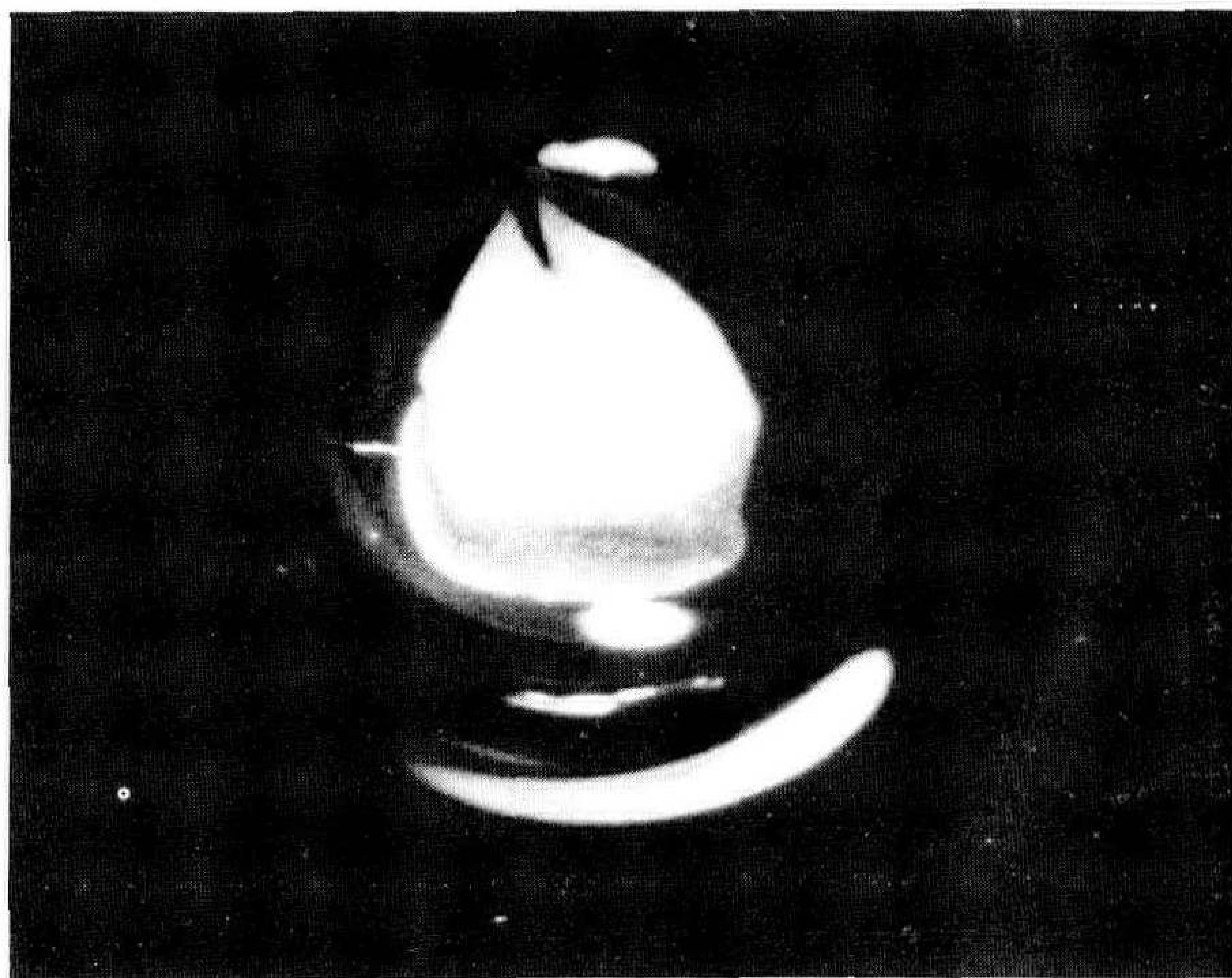

Figure 3. The freshly cultured shoot tip of Aechmea. 16X.

in 70% alcohol. One or 2 of the remaining bud scales or embryonic leaves and a portion of the stem tissue are first removed from the bud or stem tip section. The section is further sterilized by immersing for 3-5 min. in the 20-fold diluted bleach solution. An additional 1-2 scales or leaves and stem tissue are removed, and the final explant measuring 2-5 mm tall is obtained. The explant is dipped in the bleach solution and transferred to the nutrient tube (Fig. 3).

Nutrient Media. Three different nutrient formulations are recommended for the tissue culture propagation of Aechmea, one for each of the three major steps *in vitro:* (1) the establishment of initial culture from freshly excised shoot tips, (2) the subsequent step of rapidly multiplying shoots, and (3) the final step of preparing the multiplied shoots for their transfer to soil. It is possible to accomplish all three steps by using one medium, but maximum efficiency is more likely if the nutrient medium and other conditions *in vitro* are as nearly optimum for each step. The three formulations contain the Murashige and Skoog salt mixture (2); the composition of this mixture is reproduced in Table 1. Table 2 lists other constituents of the medium of the first step; they have been

Table 1. The Murashige-Skoog inorganic salt formulation employed in tissue culture multiplication of *Aechmea fasciata* Baker.

Compound	*mg/l*	*Compound*	*mg/l*
NH_4NO_3	1650.0	H_3BO_3	6.2
KNO_3	1900.0	$MnSO_4 \cdot H_2O$	16.9
$CaCl_2 \cdot H_2O$	440.0	$ZnSO_4 \cdot 7H_2O$	8.6
$MgSO_4 \cdot 7H_2O$	370.0	KI	0.83
KH_2PO4	170.0	$Na_2MoO_4 \cdot 2H_2O$	0.25
$Na_2 \cdot EDTA$	37.3	$CuSO_4 \cdot 5H_2O$	0.025
$FeSO_4 \cdot 7H_2O$	27.8	$CoCl_2 \cdot 6H_2O$	0.025

Table 2. Nutrient addenda employed in initiating cultures from freshly excised Aechmea shoot tips (Step 1).

Addendum	*mg/l*	*Addendum*	*mg/l*
Sucrose	20,000.0	Nicotinic acid	0.5
IBA (Indole-3-butyric acid)	1.75	Pyridoxine•HCl	0.5
NAA (α-naphthaleneacetic acid)	1.75	Thiamine•HCl	0.1
Glycine	2.0	Citric acid	150.0

adapted from the Cattleya orchid culture medium of Reinert and Mohr (3). In Table 3 are found the addenda that are employed for the second, or shoot multiplication, step; and in Table 4, the substances that are added to the nutrient formulation of the third, or pre-transplant, step.

Table 3. Nutrient addenda employed in rapid multiplication of aechmea shoots (Step 2).

Addendum	*mg/l*	*Addendum*	*mg/l*
Sucrose	30,000.0	Adenine sulfate • $2H_2O$	40.0
$NaH_2PO_4 \cdot H_2O$	85.0	Thiamine•HCl	0.4
IAA (Indole-3-acetic acid)	2.0	i-Inositol	100.0
Kinetin	2.0		

Table 4. Nutrient addenda employed in preparing aechmea shoots for transfer to soil (Step 3).

Addendum	mg/l
Sucrose	30,000.0
IAA	2.0
Thiamine•HCl	0.4
i-Inositol	100.0

Liquid formulations are advisable for tissue cultures of Aechmea, although agar-solidified media may be more suitable for some other bromeliads. An initial pH of 5.0 has been satisfactory with liquid media. The pH adjustment is accomplished by adding a few drops of very dilute NaOH or HCl. The media are sterilized by autoclaving 15 min. at 121°C (15 lbs. pressure). The nutrient solution of step 1 is contained in 25 x 150 mm culture tubes, in 5 ml aliquots. The tubes are capped with polypropylene closures (Bellco K-25 kaputs). In the subsequent shoot multiplication step, the nutrient formula is placed in 125 ml Delong or Erlenmeyer flasks, each with 25 ml solution. The Delong flasks are capped with Morton stainless steel closures, whereas the Erlenmeyer flasks are plugged with non-absorbent cotton or sponge. The cotton or sponge plugs may need to be covered with aluminum foil to retard moisture loss. During the final pretransplant step *in vitro*, the nutrient medium is placed in a still larger vessel, a wide mouth 500 ml Erlenmeyer flask or equivalent, and each vessel is provided with 100 ml of medium. The flask is closed with a 2-holed autoclavable rubber stopper, with each of the holes of the

stopper being filled with non-absorbent cotton. Additional protection against microbial entry is provided by covering the stopper and the lip of the flask with aluminum foil.

The Culture Environment. Best results with the freshly excised shoot tip of step 1 are obtained by placing the nutrient tubes on a rotating device and providing gentle agitation. The New Brunswick rollordrum apparatus (Model TC-4) with a constant rotational speed of 1 rpm has been satisfactory in our laboratory. The tubes are held at an angle of 10° from the horizontal. If agitation is not possible the cultures may be maintained in the stationary state. In that case, culture tubes should not be used; small, 50 ml capacity Erlenmeyer flasks are preferred.

None of the cultures in flasks of the second and third steps require agitation. Good cultures of Aechmea have been obtained by simply maintaining them in a stationary state on culture shelves.

All 3 steps of the tissue culture procedure are accomplished by providing a constant temperature of 26-27°C (about 80°F). The cultures are illuminated 16 hr daily with 1000 lux (circa 100 ft-c) light from Gro Lux lamps. It may be desirable to expose the cultures of the pretransplant step to a higher light intensity, perhaps near 10,000 lux (1000 ft-c).

The Multiplication Process. After 4 weeks, the shoot-tip explants should have enlarged considerably and should be ready for transfer to the shoot multiplication medium. Those that show only

Figure 4. Aechmea tissue culture showing shoot multiplication.

little growth, but are still alive, may require further reculture in a freshly prepared step 1 nutrient solution. Transfer of the enlarged shoot tip to the shoot multiplication medium results in the production of numerous new shoots (Fig. 4). After 6 weeks in this medium, each culture is subdivided into quarters or other convenient portions, and each portion is transferred to freshly prepared multiplication medium. After a further reculture of 6 weeks, the tissue cultures should be ready for step 3 and prepared for their transfer to soil.

The Pretransplant Step. The pretransplant step is accomplished by simply subdividing the cultures of the shoot multiplication step and reculturing them in the step 3 medium. A period of 6-8 weeks in this medium has been about optimum for Aechmea. The nutrient medium contains no cytokinin; nevertheless, an increase in the number of shoots occurs. This probably reflects primarily an outgrowth from primordia which have been initiated during step 2. The recultures are placed under a higher light intensity (near 10,000 lux) at this time. The shoots elongate substantially and show well-developed leaves, and some will have roots.

Establishment of Plants in Soil. The clusters of shoots from step 3 are separated into individual shoots, and the shoots are handled hereafter as cuttings. They are set into an equiproportional mixture of peat, perlite and vermiculite. They should be placed under intermittent mist in the greenhouse and shaded during the first 2-3 weeks. By then a good root system should have developed and the plants should be ready for standard culture conditions.

Characteristics of the Plants Obtained through Tissue Culture. The above tissue culture procedure has been tested under commercial conditions and several thousands of *Aechmea fasciata* plants have been established. An average of 500 Aechmea plants of transplantable size have been obtained from each shoot-tip explant in 6 months. The shoots that arise *in vitro* have been rooted successfully and established into plants with a 90-100% survival rate. In general, the plants derived through tissue culture have been uniform in growth and flower characteristics. But there has been a significant incidence of genetically aberrant plants. These aberrant plants are distinct in the morphological appearance, and show plant qualities which in no way resemble the Aechmea (Fig. 5). The frequency of appearance of these aberrant plants seems to be related to the number of times a culture is divided and recultured, and to the length of time a tissue culture is maintained in the laboratory. The greater the number of recultures and the

Figure 5. Plants obtained from Aechmea tissue culture. Type plant on left and variant on right.

longer the period of maintenance *in vitro*, the higher is the incidence of aberrant plants. For example, the practice of utilizing 3 recultures over a period of 6 months resulted in only 2% aberrant plants; in contrast, repeated recultures every 6 weeks for up to a year produced over 20% variant plants.

Cytological examination of root tips from the tissue-culture-derived plants revealed no difference in the chromosome number of the plants, indicating that the aberration did not involve polyploidization. Observations of histogenesis during development of shoots from shoot-tip cultures of Aechmea disclosed that much of the new shoots arose by way of adventitious shoot initiation in the basal region of the explant (Fig. 6). This adventitious origin of the shoots would partially account for the genetically aberrant plants. Indeed, the occurrence of significant frequencies of genetically variant plants through adventitious organogenesis in tissue cultures has not been uncommon (1). The occurrence of sports in tissue culture cannot be avoided entirely. Nevertheless, the incidence can be reduced by keeping the number of recultures to a minimum. It is always tempting to obtain as many plants from a given explant and to maintain stock cultures of established lines for indefinite periods. This practice can lead to tragic consequences when applied to *Aechmea fasciata* and other genetically unstable plants. It is recommended that recultures be done no more than 3 times, with the third one being the pretransplant step. Between the initial stage with the freshly excised shoot tip and the final transfer of shoots into soil, a total of 6 months elapses *in vitro*. In practice, new cultures should be started from fresh shoot-tip explants at prescribed times to coincide with market demands. It is not advisable to maintain stocks of Aechmea tissue cultures indefinitely.

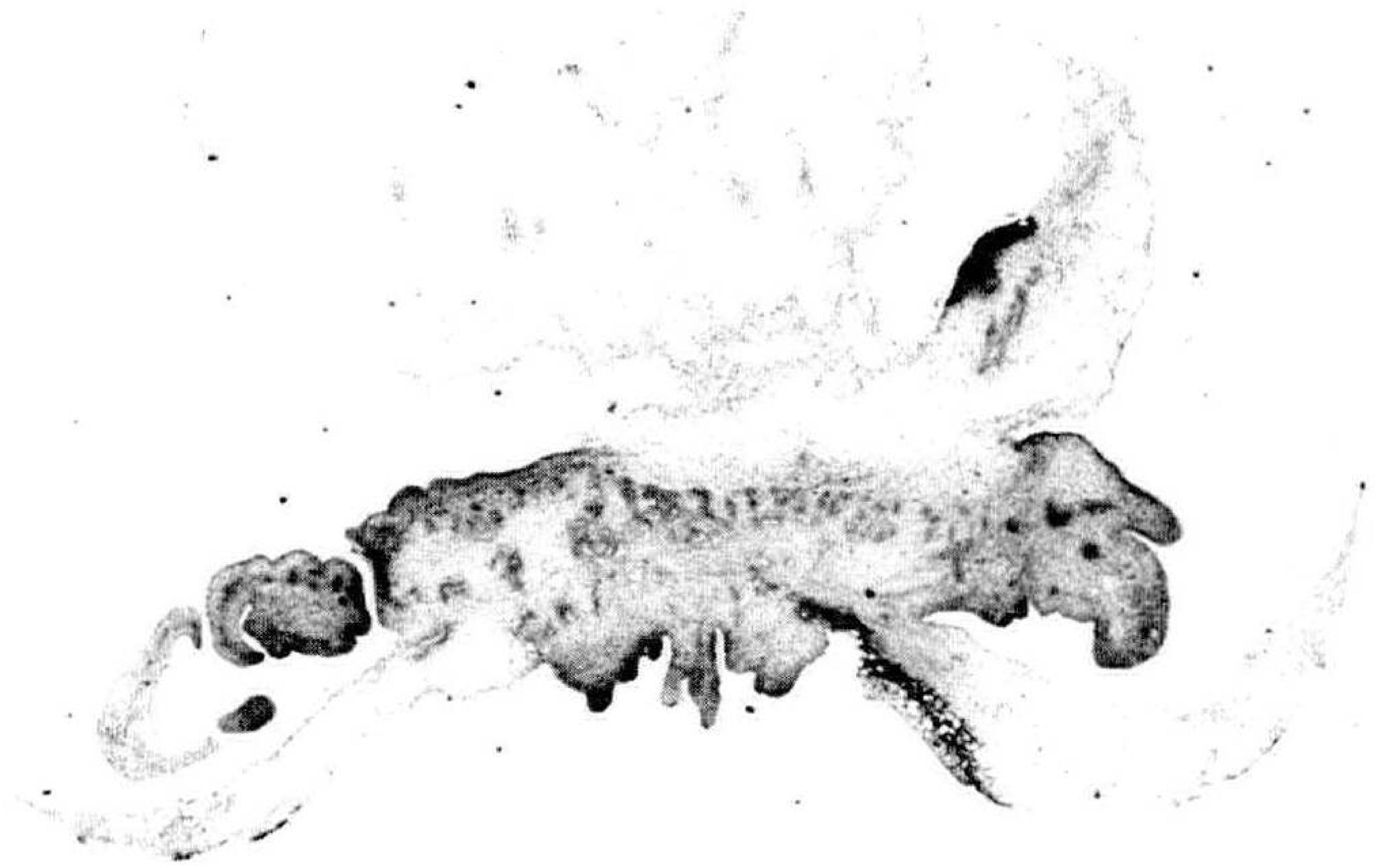

Figure 6. Histological section showing initiation of new shoots in 6-week cultured Aechmea shoot tip. Note shoot primordia arising adventitiously at base of explant. 40X.

Applicability to Other Bromelaids. The procedure has been tried with 19 other genera or species of the Bromeliaceae. Success has been observed with *Ananas comosus* Merrill 'Smooth Cayenne', *Cryptanthus bivitattus* Regel 'Cafe au lait', *Cryptanthus* '*Star*', *Cryptbergia meadii* (*Cryptanthus beuckeri* E. Morr. X

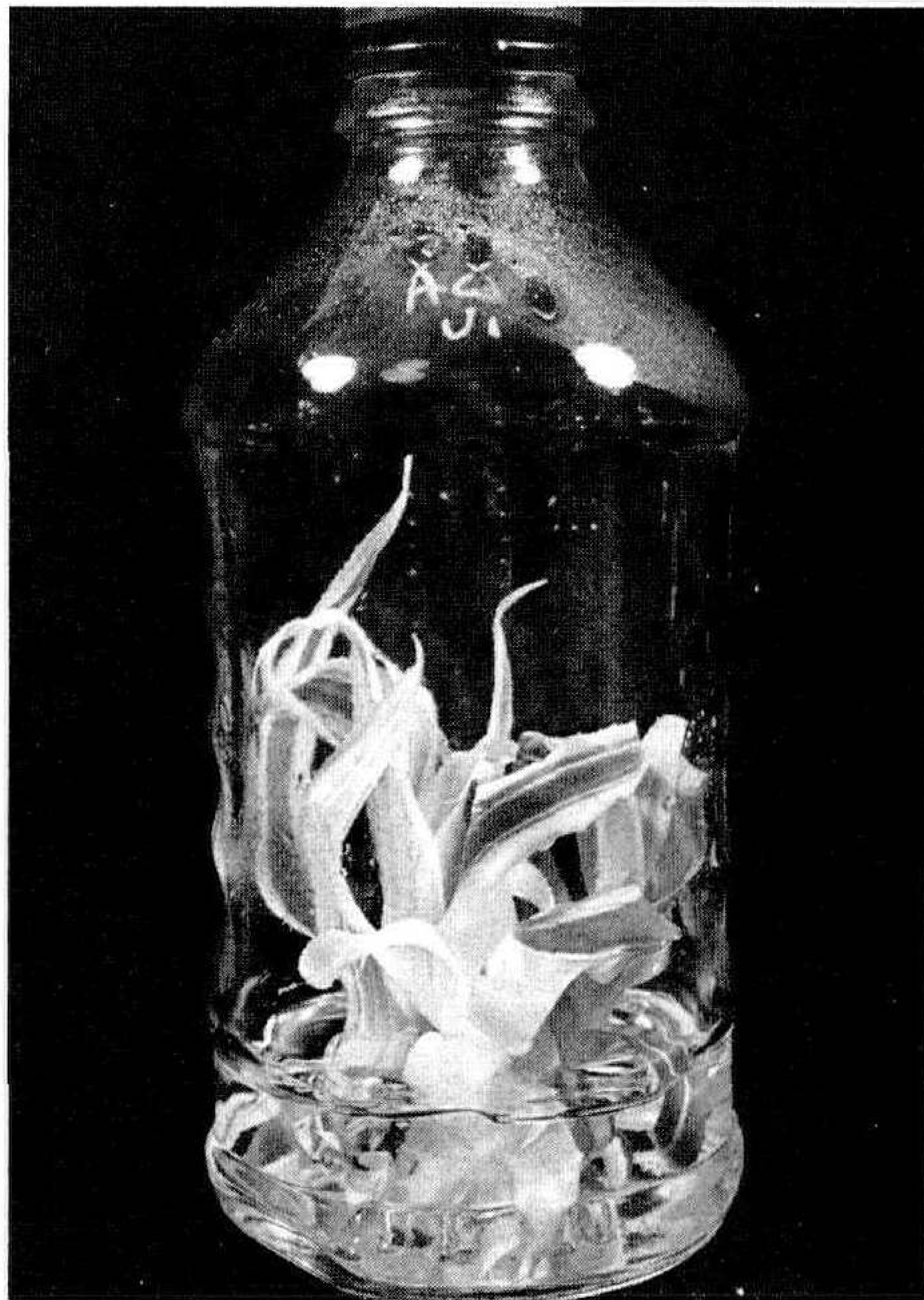

Figure 7. Variegated and non-variegated shoots arising in shoot-tip culture of *Ananas comosus* Merrill 'Variegatus'.

Billbergia nutans H. Wendl.), *Dyckia sulphurea* C. Koch, and *Guzmania* 'Hummel's Supreme'. Whereas the rate of multiplication is satisfactory, the method is not applicable to some of the variegated bromelaids, e.g., *Ananas comosus* Merrill 'Variegatus', *Cryptanthus* 'It' and *Aechmea fasciata* Baker 'Albo-marginata'. The adventitious origin of new shoots causes separation of the chimeral components of these cultivars (Fig. 7).

SUMMARY

A commercially applicable tissue culture procedure for rapid clonal multiplication of *Aechmea fasciata* Baker and certain other bromelaids is described. The procedure starts with shoot tips obtained from buds that are located at the base of the plant or from stem tips of growing shoots. After an initial 4-week culture period, the enlarged shoot tip is transferred to a nutrient medium which enables rapid formation of new shoots. The cluster of new shoots is divided and each portion is transferred to fresh medium and recultured once more to further increase the number of shoots. Subsequently the cultures are divided again and recultured in a medium that enables the new shoots to elongate, harden and initiate roots. Finally, the shoots are separated from the clusters and transplanted into soil in the greenhouse.

The cultures are maintained at a constant 26-27^{0}C. Exposure of the cultures 16 hr daily to 1000 Gro Lux light has been satisfactory for the initial development of shoot tips *in vitro* and for their subsequent multiplication. A higher light intensity, perhaps 10,000 lux, may be desirable for the third step of preparing the multiplied shoots for transfer to soil. The establishment of plants in the greenhouse is enhanced by providing intermittent mists and light shade during the first 2-3 weeks. This procedure, if employed improperly, can result in a high frequency of genetically aberrant plants. It is moreover not recommended for the propagation of some variegated bromeliads.

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