*HortScience* 14(1):21–22. 1979.

# Homogenization as an Aid in Tissue Culture Propagation of *Platycerium* and *Davallia*[1]

**Ron C. Cooke**
*Flow Laboratories, Inc. 936 W. Hyde Park Blvd., Inglewood CA 90302*

*Additional index words.* rabbit's foot fern, staghorn fern

*Abstract.* ***Platycerium* (staghorn fern) and *Davallia* (rabbit's foot fern) plantlets initiated *in vitro* were aseptically homogenized and dispensed volumetrically into plastic tissue culture containers. The resultant cultures grew rapidly and were successfully transferred to soil. This method offers an alternative for separation and distribution of previously multiplied plantlets in the culture step prior to transplantation in soil.**

Commercial plant tissue culture laboratories have become numerous in the United States. Most labs are designed to partially fill the plant propagation needs of the parent nursery; some do custom tissue culture for other firms and have little or no greenhouse space. A few make plants in test tubes as novelty items. Murashige (1) has described 3 steps that are usually utilized by these commercial laboratories: 1) *explantation* (the preparation of a surface sterilized explant on a medium that will support growth and vitality of the tissue), 2) *proliferation* (the induction of multiple shoots or plantlets by adjustment of growth regulators and/or lighting and other physical requirements) and 3) *plant establishment* (the rooting, growth and final hardening of individual plantlets in preparation for transfer to soil). This last step is accomplished by changes in media formulation and lighting requirements. Many commercial laboratories go directly from step 2 (multiplication) to soil.

Certain of these steps are labor intensive. For example, a laboratory might place 10 initial explants onto a medium that is suitable for steps 1 and 2. In 30 days each of these 10 explants would have developed 10 shoots. If the aim is continuous propagation, a technician would then be required to manually remove 10 clumps of multiple shoots. These clumps would be manually dissected into 100 separate shoots and innoculated onto separate media. The multiple shoots are often small and delicate, and the labor may become a very expensive factor in the overall scheme.

Knauss (2) has developed a "partial tissue culture" method for propagation of ferns. He aseptically germinated fern spores on agar solidified medium; clumps of prothallia were divided on a regular basis to provide a source of gametophytic tissue. This tissue was ground in a blender with one-half strength Murashige and Skoog (MS) salts and poured onto the soil surface. This soil suspension would develop into lush prothalli and eventually into sporophyte plants.

The following is an adaptation of this method using tissue culture propagated sporophyte plants and does not require the time factor involved for fertilization and subsequent development of sporophyte plants.

*Platycerium* and *Davallia* plantlets were initiated and multiplied on MS salts and minimal organics medium (Flow Laboratories, Inc., 1710 Chapman Ave. Rockville, MD 20852) (consisting of MS salts plus mg/liter: sucrose 30,000; agar 8,000; inositol 100; thiamine-HCl 0.4). These cultures had been subcultured by the traditional manual method of removal of multiple plantlets, dissection, and transfer to individual tubes for subsequent multiplication.

The mass of plantlets from one 25 × 150 mm test tube of each species (the *Platycerium* tube contained about 40 plantlets and the *Davallia* tube contained about 10) was aseptically removed and placed in a sterile blender with 50 ml of sterile medium cooled to 40°C. The contents were ground for 5 sec and 10 ml aliquots were aseptically transferred (by a pipet with the tip constriction removed) into a 10 × 10 × 9.5 cm plastic disposable plant culture container. Additional cooled medium was added to bring the volume up to 100 ml. A considerable amount of the homogenate settled to the bottom during the gelling phase of the agar. The containers were kept in a controlled environment of 27° and 1000 lux illumination for 16 hr per 24 hr period. Growth was evident at all depths of the agar in 2-3 weeks, and excellent multiple plant production was noted in 2 months for both *Platycerium* (Fig. 1) and *Davallia* (Fig 2). Each container of *Platycerium* contained about 200 plantlets that were 10-20 mm in length, easily separated and had well developed root systems. The *Davallia* container had about 80 plantlets that were 10-15 mm in length. The *Davallia* grew in the container as groups of multiple plantlets with poorly developed root systems.

The same results were obtained by blending the multiple plantlets with distilled water and pouring an aliquot over solidfied medium. This latter method would have an advantage with temperature sensitive plant material.

The plants were removed from the plastic container, manually transferred to soil, and covered with a plastic bag. The plants grew well and the bag was

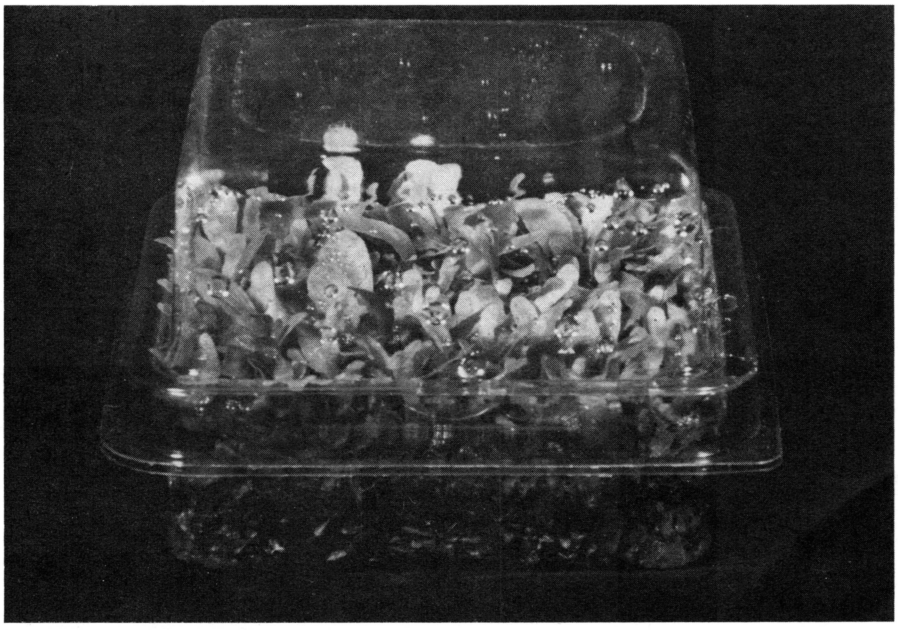

Fig. 1. *Platycerium* multiple growth after homogenization

[1]Received for publication September 28, 1978
**The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper must therefore be hereby marked *advertisement* solely to indicate this fact.**

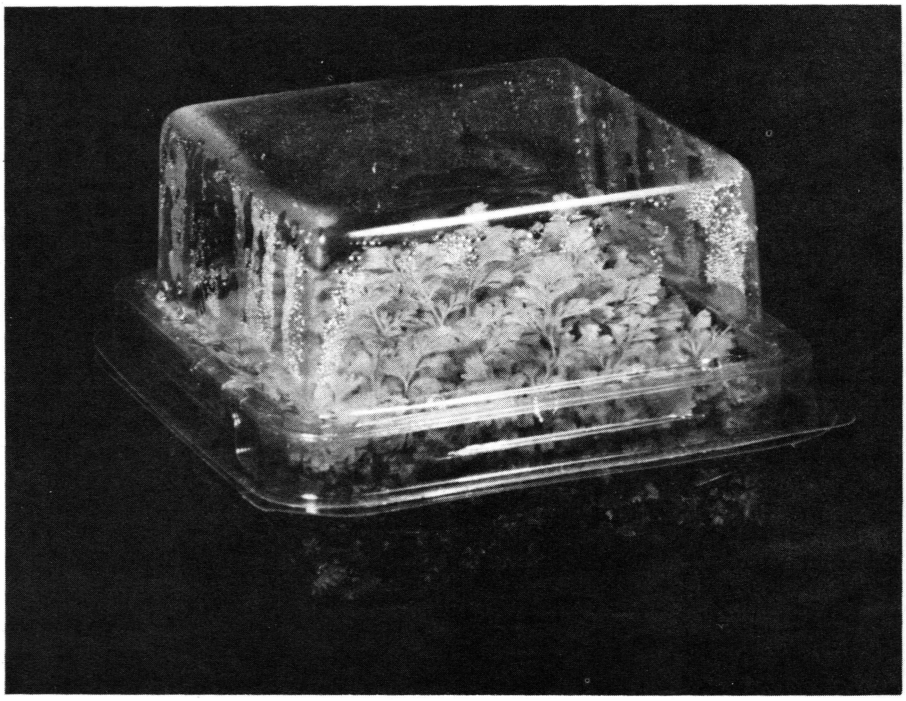

Fig. 2. *Davallia* multiple growth after homogenization

removed in 2 weeks. The survival rate for both plants was about 80%.

The number of plantlets produced in the containers by this process is comparable to standard methods utilizing manual dissection and transfer of plantlets, however, it eliminates labor intensive work and thus may save on operating costs of the laboratory. In addition, the reduction of many manual transfers may decrease contamination spread by contact.

This method 1) employs a single medium for all steps, 2) eliminates many laborious transfers and 3) carries the potential to be mechanized on a large scale. It remains to determine which species currently being propagated by tissue culture methods can be adapted to subculture by mechanical blending.

### Literature Cited


1. Murashige, T. 1974. Plant propagation through tissue culture. *Annu. Rev. Plant Physiol.* 25:135-166
2. Knauss, J.F. 1976. A partial tissue culture method for pathogen-free propagation of selected ferns from spores. *Proc. Fla. State Hort. Soc.* 89:363-365



*HortScience* 14(1):22–23. 1979.


# *In Vitro* Propagation of *Cucumis sativus* L.[1]


**Levis W. Handley and Oyette L. Chambliss[2]**

*Agricultural Experiment Station, Auburn, AL 36830*



*Additional index words.* tissue culture, cucumber



*Abstract.* **Axillary buds of gynoecious cucumber (*Cucumis sativus* L.) were cultured on a semi-solid medium containing Murashige and Skoog salts plus thiamin, pyridoxin, nicotinic acid, myo-inositol, and sucrose with 0.1 mg/liter naphthaleneacetic acid (NAA) and 0.1 mg/liter kinetin. Both shoots and roots were proliferated with little callus formation. Plants produced *in vitro* were successfully transferred to soil.**


Gynoecius hybrids are being used in increasing amounts in commercial cucumber production and a large percentage of pickling cucumber cultivars are gynoecious hybrids (10). In order to develop gynoecious inbreds for use in a gynoecious hybrid breeding program, single selections must be vegetatively propagated to provide enough plants for inbreeding. The current practice involves rooting 3 or 4 cuttings in aerated water containing indolebutyric acid (1,2,3). Gibberellic acid is then used to induce production of staminate flowers on at least 1 plant to furnish pollen for propagation of the gynoecious selection. In some cases, however, plants will fail to root or will be lost, particularly when transferring to soil. This becomes especially significant when only 3 or 4 cuttings can be obtained from 1 plant and at least 2 (1 staminate and 1 pistillate) must survive to maintain the selection. A tissue culture method of propagation that could produce numerous plants from a single gynoecious selection would be desirable. Such a procedure has not been previously reported for cucumbers although shoot regeneration from callus of pumpkin has been reported (4,5). Maciejewska-Potapczykowa et al. (7) succeeded in initiating callus from cucumber stem fragments and floral buds but their main interest was the study of hormonal effects on sex expression rather than propagation.

In the early part of this investigation attempts were made to initiate callus from explants. Callus formation occurred readily on stem pieces of the cultivar 'Carolina' cultured on a medium containing Murashige and Skoog salts (9) and the following organic additives in mg/liter; thiamin, 0.4; pyridoxin, 1.0; nicotinic acid, 5.0; myo-inositol, 100; sucrose, 30,000; and agar, 7000; with NAA, 0.1; and kinetin, 1.0. The use of callus culture was abandoned, however, because no organ regeneration occurred after varying the concentrations of NAA and kinetin in the nutrient medium.

A propagation procedure using axillary buds was developed which avoided callus formation. Axillary buds of 'Carolina' were excised from 1 month old plants grown in a growth chamber at 27°C (day) and 19°C (night) temperature, under a 12 hr photoperiod at 32.3 klx. Each leaf axil was surface-sterilized in a 2.5% sodium hypochlorite solution for 2 min and then rinsed in sterile deionized water. Axillary buds measuring 1-3 mm long were removed with the aid of a dissecting microscope. Explants were placed into culture on a medium based on Murashige and Skoog salts (9) plus the same organic additives as used for callus with various levels of NAA (0-0.5 mg/liter) and kinetin (0-20.0 mg/liter). Twelve axillary buds per treatment were used. The pH was adjusted to 5.7 with 0.1N NaOH prior to sterilization at 121°C for 15 min. Cultures were incubated at 25°C in a growth chamber with 16 hr photoperiod at 4.3 klx provided by cool white fluorescent tubes and incandescent bulbs.


[1]Received for publication October 11, 1978. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper must therefore be hereby marked *advertisement* solely to indicate this fact.



[2]Graduate Research Assistant and Professor, respectively, Department of Horticulture.