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CLONAL MULTIPLICATION OF CYMBIDIUMS THROUGH TISSUE CULTURE OF THE SHOOT MERISTEM

by

Donald E. Wimber

The propagation of clonal varieties of some orchids is at times exasperatingly slow and occasionally an almost futile effort. Clonal multiplication is generally confined to dividing mature plants and to starting plants from pseudobulbs. There is, of course, the specialized technique for obtaining Phalaenopsis plantlets from the aseptic culture of inflorescence nodes, but this is basically the same thing as propagating plants from pseudobulbs. In certain cases it is highly desirable to rapidly multiply certain clones of orchids. Awarded varieties could thereby be dispersed with great rapidity where now it may take decades for some clones to become fairly common. Commercial flower production would be very much enhanced if certain desirable clones could be multiplied ad infinitum within a short time. Orchid flower production could then be placed more on a par with many of the other cut flowers and the clonal peculiarities of some of the current hybrids could be pampered instead of ignored. This paper describes a tissue culture method for the rapid propagation of Cymbidium clones.

Small shoots approximately 3 cm in length were removed from 3 Cymbidium hybrids. The outermost leaves were stripped from the shoots and the shoots were surface sterilized for 10 min in a freshly prepared 2% solution of calcium hypochlorite. Sterile procedures were observed in all subsequent operations. The rest of the leaves were removed from the shoots and

DISCLAIMER

the apical meristems were exposed; these were immediately cut off and each placed in a 125 ml Erlenmeyer flask which contained 25 ml of a liquid nutrient solution (see Table 1).

TABLE 1

Culture Medium* (modified from Tsuchiya, 1954[1])	
Potassium nitrate	0.525 g
Dicalcium phosphate	0.20
Monopotassium acid phosphate	0.25
Ammonium sulfate	0.50
Magnesium sulfate	0.25
Ferric tartrate	0.03
Tryptone	2.00
Sucrose	20.00
Water	1000.00 ml

* 1.2% agar added for the solid medium

The flasks were sealed with rubber stoppers and some were placed on a rotary shaker. The flasks received constant light (100 foot candles or less) and constant temperature (22° C). All meristems showed greening and noticeable growth within a week and within a month, balls of tissue, very much resembling protocorms, were evident. The meristem tissues in the flasks that were not agitated followed a developmental sequence that superficially resembled that of seed germination; after the ball of tissue reached a size of approximately

[1] Tsuchiya, I. 1954. Na Pua Okika o Hawaii Nei 4: 11-16.

4 mm, a shoot appeared and differentiation followed the usual course that it does in seed germination. Occasionally additional proliferation of the protocorm-like object occurred. The tissue in the flasks that were agitated, however, usually developed no shoots. This tissue grew to approximately 4 mm in diameter and then started proliferating protocorm-like tissue masses that retained their connection with the parental mass (Fig. 1). After 2 months growth several of these masses were removed from the flasks and each was chopped up into 20 or more pieces. These pieces were placed in other flasks with fresh nutrient solution and returned to a rotary shaker. In a month's time most of the fragmented pieces of the original tissue masses had reached a diameter of 1 cm. Some of these were cut up and given fresh nutrient solution and they followed the same growth pattern. At this point some of the tissue masses were removed from the liquid medium, cut up and placed on solid nutrient agar. These mostly formed protocorm-like objects and differentiated, producing normal shoots and roots (see Fig. 2). Up to 5 successive transfers of the tissue have been made. However, it may not be possible to carry the cultures on indefinitely since by the fifth transfer many chlorotic tissue masses were noticed. Yet, using this technique, prolonged culture is unnecessary, since by the third transfer of tissue derived from one shoot apical meristem, it is possible to produce several hundred plants.

It might be argued that chromosomal alterations could be induced in the parental genotype by the culture methods and the original clonal material would not necessarily be recovered. With this in mind, 10 plants derived from 1 meristem after the third transfer were examined for their

chromosome numbers and in all cases they appeared identical to the parent. Unfortunately none of the plants produced through this tissue culture technique have flowered yet.

Morel (1960)[2] reported similar results in producing virus-free Cymbidiums. He noted that the original protocorm-like body produced from the apical meristem often divided into several similar structures when the tissue was grown on a solid medium. Thereby, the stock of that plant could be increased at the same time that the virus was being eliminated. The use of liquid culture methods and constant agitation as described in this paper results in rapid growth of tissue from the shoot meristem. The constant shaking apparently checks any polarity that might develop in the tissue and thus, inhibits shoot formation. This results in a multiplication of the protocorm-like tissue. Hence, within 4 to 6 months from the original isolation, the meristem tissue can give rise to numerous large tissue masses. This tissue when cut up and transplanted to solid media will yield several hundred plants.

Currently experiments are under way to extend this technique to other genera of orchids.

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[2] Morel, G. M. 1960. Amer. Orchid Soc. Bull. 29: 495-497.

Biology Department
Brookhaven National Laboratory
Upton, L.I., New York

FIGURE LEGENDS

Fig. 1. A tissue culture of Cymbidium shoot tissue after 1.5 month's growth. The original fragment was approximately 4 mm in diameter and taken from the second transfer from isolation; it is now about 2 cm. Agitation was for 1 month, followed by 2 weeks of rest. The shoots developed during the latter 2 weeks.

Fig. 2. Aseptic clonal propagation of a Cymbidium from tissue culture of a shoot apical meristem. Fragments of tissue were placed on nutrient agar in this flask 2 1/2 months prior to the picture. The tissue was taken from a tissue mass at the end of the third transfer from the original isolation.

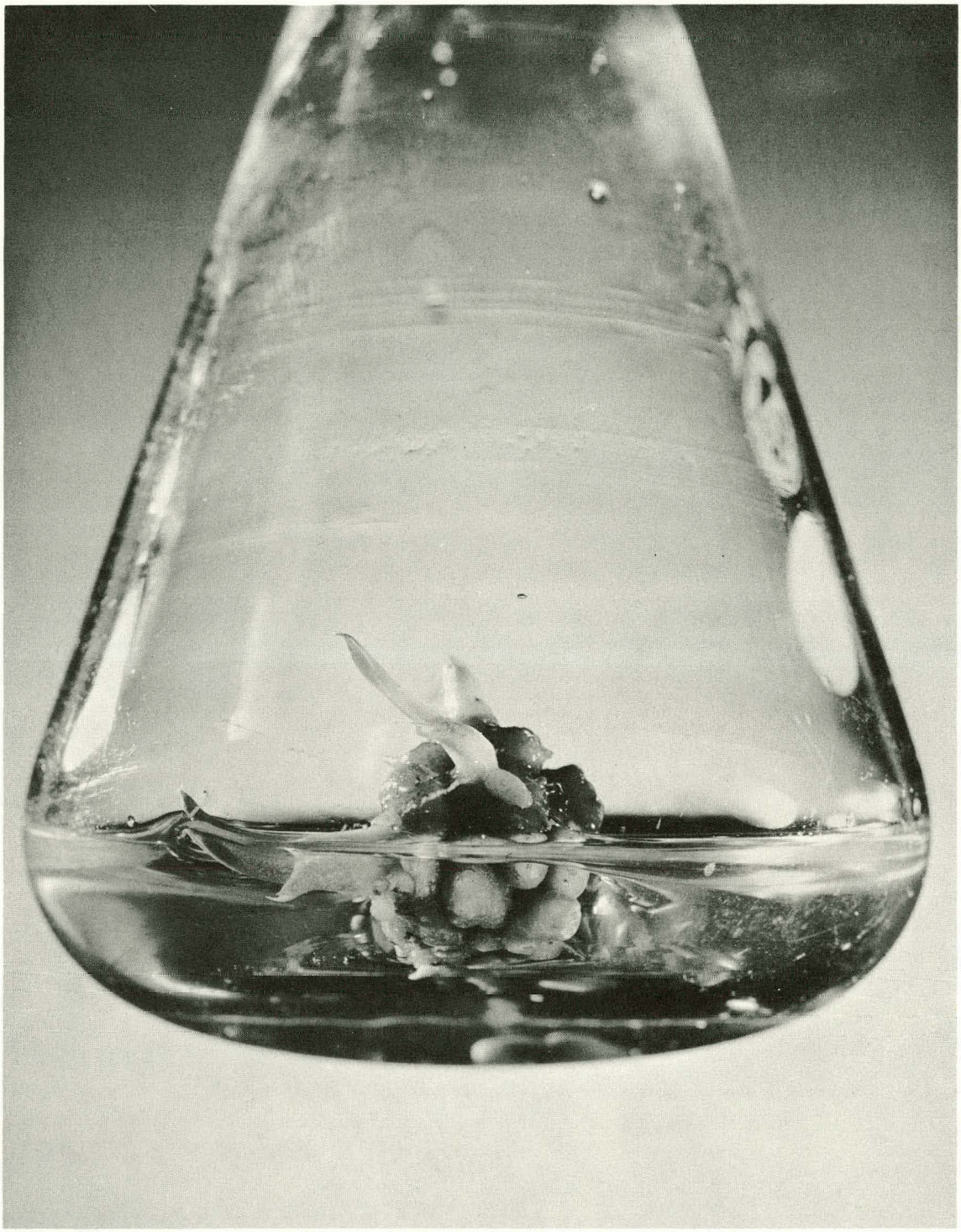

Fig. 1

Fig. 2