ORCHID PROPAGATION BY IN VITRO CULTURE TECHNIQUES

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The orchid industry has been a pioneer in commercially applying *in vitro* culturing of plants as a means of rapid propagation. Since Knudson's (1) work in the 1920's nearly all new hybrids are products of laboratory culturing of rudimentary embryos. Hundreds of millions of orchid seeds are germinated aseptically in growing flasks each year in our laboratory alone. Continual selection of seedlings throughout the years to flowering eliminates plants with undesirable growth or flowering characteristics.

Rapid clonal propagation was impossible until George Morel (2), while attempting to free a cymbidium of virus by culturing the shoot tip, noticed that the isolated piece of tissue seemed to revert to the seedling protocorm stage and proceeded to divide into a clump of 3 or 4 identical structures which eventually became plants. He continued his research on the so-called method of "meristem culture," applying it to many other orchid genera, so that during the 1960's many orchid labs around the world were clonally propagating orchids by his techniques.

In this paper I shall attempt to explain the seedling and meristem techniques used in my lab for the propagation of orchids.

SEXUAL PROPAGATION

A ripe orchid pod may contain up to 500,000 seed. Under natural conditions the pod splits and the seed are dispersed by the wind, lodging on trees or on the soil. Most seed die for lack of proper germinating conditions or are killed by pathogens, so that perhaps only 1 to 10 in a million seed survive to become mature plants. Under favorable circumstances a mycorrhizal relationship develops between the young seed and a host fungus.

The seed consists of a thin, nonliving seed coat and a rudimentary embryo of from 50 to a couple hundred cells. There is no endosperm. Relatively few fat and protein globules within the cells represent the extent of stored food reserves. Mineral and carbohydrate reserves are nil.

During germination the embryo swells into a rotund structure called a protocorm, developing hair-like absorbing organs called papillae. The first leaf emerges from the top of the protocorm and the first root soon follows. New shoots and roots are produced and eventually the bloom spike 3 to 7 years later.

Commercially, all crosses are controlled by manual cross-pollination, and the seed is collected rather than let fly in wind currents. Pollination is effected by smearing pollen on the stigmatic surface of an emasculated flower. If the pollen is compatible the flower collapses within 72 hours. Generally, fertilization of the ovules takes place about 90 days after pollination. In 9 to 12 months the pod is mature. It is split open and the seed collected on onion skin paper A microscopic examination for embryos gives us an estimate of the amount of viable seed. Wrapped in thin paper, the seed can be stored up to 6 years in a refrigerated dessicator.

Preparatory to seed sowing, sterile formula must be made in culturing bottles. Orchid literature abounds with seed germinating formulas, but all have sugar, minerals, water and a gelling agent such as agar. In addition, depending upon the requirements of the genus, other substances may be added, such as vitamins, hormones, protein hydrolysates and fruit homogenates Formula is distributed into 32 oz. French square bottles, capped with mercury-treated cotton and a metal cap Bottles of formula are sterilized in large pressure cookers for 15 minutes at 15 psi and then allowed to cool on their sides

A portion of the seed is decontaminated for 10-20 minutes in a vial of filtered calcium hypochlorite (10 gm / 150 ml) to which has been added a few drops of diluted wetting agent. A sterile cotton plug drenched in 5% Clorox helps to collect the seed in the bottom of the vial by filtering off the sterilizing solution. In a sterile transfer chamber, the seed is scooped out of the vial with a sterile spatula and is plated in a bottle of formula. By another method of seed sowing, called green-podding, a ripe pod is surface decontaminated in 5% Clorox, cut open with a sterile scalpel and the seed is spread directly onto the sterile formula Germination is apparent in a couple months, and by the fourth to sixth month after sowing, the small plants must be thinned out and reflasked to another bottle.

A year after sowing, the seedlings are large enough to be removed from the bottles and are flatted in 0 to ⅛'' grade white fir bark. They remain in the flat about one year and then are potted into 3'' pots with ⅛ to ½'' grade bark After another year they are shifted into 4'' pots with ¼ to ½'' grade bark and the plants are staked and tied. Some *Cattleya* orchids bloom in the 4'' pot stage, in their fourth year The potting mixture and procedures of growing seedlings outside bottles varies for the different types of orchids.

VEGETATIVE PROPAGATION

Prior to the last decade the only way of increasing a clone was by division of the parent plant This was very slow because a plant produces only one or a few new growths every year. People desiring divisions of valuable plants had their names on long waiting lists

With the advent of "meristeming" the hobbyist has access to quality clones at a cost a fraction of the value of the original plant. The flower grower benefits by being able to crop color and quality in quantity to meet peak seasonal demands.

In our lab we are currently meristeming *Cymbidium, Cattleya, Miltonia, Oncidiums, Phalaenopsis* and a few other genera. Formula preparation involves the same sterile procedures as with seed sowing, except that most of our cultures are proliferated in flasks of liquid formula rather than on gelled formula.

I shall use *Cymbidium* to typify the procedures. An actively growing vegetative shoot is excised with a sterile knife to avoid virus infections. The outer leaves are stripped away to reveal the bulb-like growth at the base of the shoot. This portion, having large axillary buds and a shoot tip, is soaked in 5% Clorox to decontaminate it In a sterile case under a stereoscope, the axillary buds and shoot tip are cut off and dipped in 1% Clorox, then into liquid formula in culturing flasks. Tissue swells at its base in 4 to 6 weeks of culturing on rotating wheels. These protuberances, which resemble seedling protocorms, are cut off and subcultured. Every 3 to 4 weeks the pieces have enlarged enough, 4 to 8 fold, to be resliced We can produce 10,000 pieces from one shoot in 6 to 8 months

When production figures are met, cutting stops and the pieces are arranged on solid formula just as seedlings are reflasked. In 8 months leaves and roots are well developed and the young mericlones are removed from the bottles and flatted.

Mericloning has been greatly simplified here. There are methods of subculturing the tissue, such as slicing techniques, timing and special formula, which are beyond the scope of this paper Certain orchids, such as *Paphiopedilum,* have been recalcitrant in culture so far. Many other problems remain to be solved in successful *in vitro* culturing of orchids.

LITERATURE CITED

1 Knudson, L. 1922. Non-symbiotic germination of orchid seeds. Bot. Gaz 73:1-25.

2. Morel, G. M 1964. Tissue culture — a new means of clonal propagation of orchids. *Amer. Orchid Soc. Bul.* 33:473-478.

DAVID ADAMS: I suppose you could hear the wheels turning out there while Dick was talking. Some of these very difficult-to-produce rhododendrons and so forth that are bringing six, eight, ten, and 25 dollars per plant. If you had a set-up of this type where you could grow many, many thousands a year, what might happen to the market?

RICHARD SMITH: You may be interested in the cost figures on this. From the time of meristeming, they cost us about 20 cents a plant The major cost of growing the orchid is then the years after that we spent bringing them to flower. A typical seedling costs us about five cents a plant to produce.

DAVID ADAMS: Thank you. There are a lot of crops in which we could take a real long look at this method of propagation. To start a new daffodil, for example; once you have one flowering, it can take as many as 10, maybe even 15 years before it can be put on the market with sufficient stock to expect a large volume of sale. What could we do with type of culture. They've done it with asparagus, they've done it with orchids, there are many crops for which we know it's possible, it's just a matter of working out the techniques We want to thank you very much, Mr. Smith, for an excellent presentation

Our next speaker today is going to be talking to us about grapes. As many of you have heard, grapes have become more and more popular here in the Willamette Valley. Mr. Charles Coury from the Coury Nursery near Forest Grove, Oregon is going to talk to us about grape production, both in the field and in greenhouse culture. Mr Coury [1]

DAVID ADAMS: Next we have Jiro Matsuyama with us Mr Matsuyama has grown up in the nursery business They grow trees and shrubs primarily at their nursery and today he's going to talk to us about a plant that we don't normally think of growing in this area but again it could be something we could move up along the Pacific Coast maybe on up into Seattle. I don't know if it would be hardy that far north or not. Jiro Matsuyama is going to speak to us today about the propagation of *Bignonia*. Jiro:

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[1]Charles Koury described the propagation methods now being used for grapes in the Willamette Valley, Oregon.