his doctorate at the University of California at Davis. Wes, we'd like to hear what you have to tell us about what is happening and what is going to happen in this field.

APPLICATION OF TISSUE CULTURE TO PLANT PROPAGATION

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Some very interesting and exciting experiments have been carried on in the past 15 years to show that shoots and roots can be caused to form on masses of undifferentiated tobacco callus tissue cultured under aseptic conditions (13). It has also been shown with cultures of carrot callus tissue that embryos which ultimately will become normal carrot plants can be obtained from single cells (2, 14). These are examples of organogenesis and embryogenesis from undifferentiated tissue (tissue not recognizable as normal plant organs such as leaves, stems, or roots). In both cases relatively large numbers of new plants can be produced in a relatively small space under controlled conditions.

It is interesting to speculate on possible potential uses of such tissue culture techniques in plant propagation. Several possibilities come to mind:

1. The use of tissue culture as a means of rapid propagation of new cultivars, especially hybrids which require a complicated seed production system.
2. Tissue culture techniques may allow us to propagate vegetatively species which resist conventional vegetative (asexual) methods of propagation.
3. The use of tissue cultures to maintain pathogen-free plant material for long periods of time in a small amount of space.
4. Establishment of a tissue culture bank as an economical means of long-term storage and maintenance of germ plasm (breeding and propagating stock).

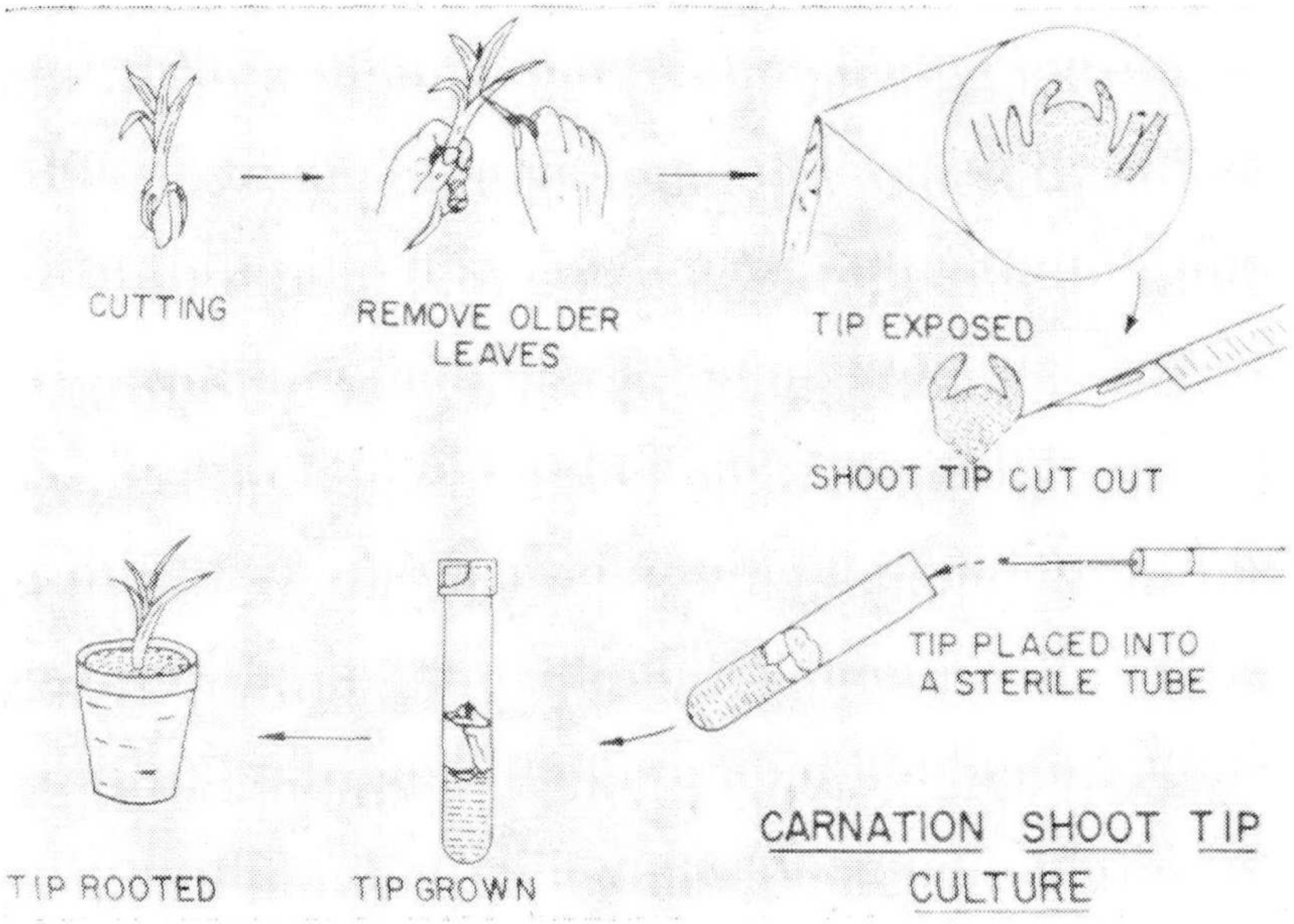
Unfortunately very little is known about the control of organogenesis and embryogenesis and the examples given above seem to be rather isolated cases of success in obtaining new plants from undifferentiated tissue. Even if regeneration can be controlled in a wide variety of species, there may be problems such as gene mutations or changes in the chromosome number. Much work is needed before complete regeneration of plants from undifferentiated tissues can be used as a propagation technique but some day such techniques will probably be used.

If tissue culture is defined broadly to include the culture of plant organs, segments of organs, and embryos on controlled media under aseptic conditions, several applications of tissue culture to plant propagation can be enumerated.

The aseptic culture of embryos or seeds has been used as a method of propagation for many years. This has been the usual method for germinating orchid seeds which have a very simple undifferentiated embryo that won't germinate under normal seed germinating conditions (4). It has also been used to obtain hybrids of some plants whose seeds fail if left to develop within the fruit (16) and to obtain prompt germination of certain dormant seeds (5).

More recently, interest has arisen in the aseptic culture of shoot apices as a method of obtaining virus-free plants from clones of vegetatively propagated species which are wholly infected with viruses. As compared to other parts of the plant, many viruses are not found in high concentration in the shoot apex. This technique has been called shoot-tip culture or meristem culture and has been used with Dahlia (8), potatoes (9), carnations (1, 12) and orchids (6, 7). In most cases the shoot tip or meristem technique has been used in conjunction with a prior high temperature treatment of the plants which inhibits virus multiplication while the shoot is able to carry on growth (3).

In carnation, the technique (Figure 1) involves growing a mature plant from the shoot apex (0.2-0.5 mm. in length) of a heat-treated cutting (15). This is done by stripping off the older leaves macroscopically and then taking off the small young leaves under a dissecting microscope with a sterile dissecting needle or scalpel. The exposed shoot apex or tip consists of about four primordial leaves and the apical meristem. This tip is sterile and can be cut off and transferred aseptically to a suitable nutrient medium. Several media are suitable



CARNATION SHOOT TIP CULTURE

but the one formulated by Murashige and Skoog (10) is particularly good. With carnation, each tip implanted yields only one mature plant.

The technique for orchids (6, 7) is much the same as for carnations but instead of forming leaves and roots very quickly as with the carnation, the orchid shoot apex first forms a small, round body with root hairs. This body is identical with the protocorm derived from orchid seed (11). In time a plant with leaves and roots will develop from the protocorm. However, it has also been found that this protocorm divides or produces lateral protuberances and that this phenomenon can be induced by agitation in a liquid culture medium or by wounding (quatrering of the protocorm) on a solid medium (7, 18). This process provides a means of vegetative (clonal) propagation at a very high rate. Morel (7) states that if each protocorm gives only four new ones per month, it is possible to obtain more than 4,000,000 plants in a year from a single shoot apex. Modifications of Knudsen's media (4) are used with orchids.

Recently, at the University of California, we have developed an aseptic tissue culture procedure for multiplying and maintaining carnation shoot apices. The starting material (the shoot apex) is very similar to that used for the shoot tip culture method of obtaining pathogen-free carnation plants, where one plant is obtained from each implanted shoot apex. The results of the procedure are comparable to those obtained with Cymbidium orchids where one shoot apex is implanted and in a very short time with proper manipulations a large number of potential plants are obtained.

In this procedure a shoot apex which is about 0.5 mm. high and which has about four primordial leaves is used. In the axils of these four-leaf primordia are primordial buds (primordial shoot apices) which are potential plants. By mutilating the leaf primordia at the time of excision and by placing the excised shoot apex on a medium high in inorganic nutrient salts and high in naphthaleneacetic acid (NAA) further development of the terminal meristem is inhibited and the primordial axillary buds are forced into growth. However, these lateral bud axes are also soon inhibited and their axillary buds are forced into growth. The result is a rapid proliferation of very short shoots which are potential plants. Once established, this proliferation tissue can be cut up into small pieces (subcultured) and placed on fresh medium and be maintained and increased many fold. Such proliferation cultures have been carried for 18 months.

These proliferation cultures have little resemblance to carnation plants. However, by cutting them into small pieces and placing them on a medium suitable for shoot development and elongation (lower in total inorganic nutrients and devoid of NAA), normal carnation shoots, some with roots, develop. These normal shoots can be taken out of the culture vials and

planted in a light soil mix. If they are not rooted, they can be treated with a root-promoting compound to induce rooting. These plants, or potential plants, are rather fragile and so they must be treated with care for a time.

It should be pointed out that the White Sim carnation cultivar (which is a periclinal chimera) sometimes reverts to a red flower color when cultured by this technique. This indicates that the outer layer of cells of the meristem has broken down, allowing cells of the core layers to break through and take over. Perhaps by changing the procedure to reduce the possibility of injury to the meristematic areas, this problem could be overcome.

This procedure, and the concept of utilizing the primordial axillary buds as sources of new plants, may have practical application as a method of propagation and as a method of maintenance of pathogen-free plant materials. With this procedure it is possible to hold pathogen-free carnation tissue for many months in a small space without exposing it to possible re-infection in a greenhouse or screenhouse. However, more work is needed to perfect the procedure.

The applications of tissue culture to plant propagation are few in number and relatively unimportant from an economic standpoint. It is not known whether such procedures are generally applicable to many species but work to determine this is in progress. Also, these methods and procedures have not been evaluated over a long period of time so their potentials and problems are not known.

Tissue culture has many potential applications for the field of plant propagation and disease control and deserves investigation by universities and experiment stations. There is a need for fundamental information concerning control of organogenesis and embryogenesis and a need for research to develop procedures for the utilization of tissue culture techniques in plant propagation and disease control.

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MODERATOR MAIRE: Now I'm sure there are some questions so who would like to start?

BRUCE BRIGGS: Dr. Murishige: What is the strength of the Clorox solution you used?

DR. MURISHIGE: Normally we use a ten percent solution. We take the commercial preparation and dilute it one to ten. We expose the tissue anywhere from 10 to 20 minutes. You can also use Purex or any other similar commercial preparation.

BRUCE BRIGGS: The reason I asked is because we also use it as a pre-dip on cuttings. I wondered how strong we could go without injuring the cuttings.

RON HUROV: Have you determined why you have so much polyploidy appearing in tissue culture?

DR. MURISHIGE: No. This is a real problem; most people working with tissue culture have experienced this. We know that this occurs as a normal process in the plant, only it occurs slowly. If you look at any mature tissue in the plant you are bound to run into a few polyploid cells. In tissue culture this occurs more rapidly. Why, we don't know. This is a problem we'd like to have resolved as soon as possible.

RON HUROV: What about apical meristem culture as opposed to callus culture?

DR. MURISHIGE: Well, apical meristem culture is a different situation since you're working with very young cells. In tissue culture we tend to take mature cells; in the case of carrots from the storage root — the part that you eat — things of that sort. These are non-growing cells, normally. We reactivate them. We find that when this is done there is a greater tendency to run into polyploids. Now, with the meristem itself it's very difficult to get callus formation.