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HOUCHANG KHATAMIAN: Strawberries are small plants and can be heat-treated in a growth chamber. Can we heat-treat the explants or do we have to grow small plants?

DAN MILIKAN: I think the thing to do is to heat-treat to remove the virus diseases. If you run into a virus, such as the stem grooving virus, which resists the heat treatment we have been successful in the grafting of the meristems on to a seed-ling.

RON GIROUARD: Are you limited to only one season for your buds?

DAN MILIKAN: We have taken apple buds year round. Terminal buds grow best. I feel that the bud scales are the source of the inhibiting factors, not the meristem.

## THE LABORATORY OF MICROPROPAGATION AT CESENA, ITALY

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In 1976, a growers' cooperative (Centrale Ortofrutticola alla Produzione) in Cesena, Italy, began planning a tissue culture laboratory for large-scale production of several horticultural crops. Dr. Carmine Damiano, of the Istituto Sperimentale per la Frutticoltura in Rome, was instrumental in designing and organizing the laboratory and in consulting on problems once tissue culturing got under way. P. Boxus, of Gembloux, Belgium, also served as a consultant on the project.

The laboratory was built in the basement of the main building of a Technical Agricultural Institute in Cesena. The laboratory consists of a media preparation room, a dishwashing and autoclave room, a transfer room, a large growth room, and office and storage space. The rooms are large, well-lit and constructed so that maintaining a clean work environment is facilitated. The capital cost for the laboratory was about \$200,000.

The laboratory facilities are well designed with a good work flow from one section to another. Six large laminar flow hoods are located in a large transfer room which is separate from, but with good access to, the other parts of the laboratory. Up to 10 technicians can be transferring cultures simultaneously. The growth room has the capacity of nearly 20,000 half-liter canning jars which are used for growing the cultures.

Starting with 250 to 300 strawberry meristems in culture provided by Dr. Damiano in August, 1977, the laboratory had some 36,000 strawberry explants growing by the end of December, 1977. In the first full year of operation (1978), about 800,000 plants were produced; this was nearly doubled in the second year, with a further increase to 3 million plants planned for the third year. Strawberries accounted for most of the first year's production, slightly more than half of the second year's, and will be about one-third of the third year's. Cultivars propagated this way include 'Aliso', 'Gorella', 'Pocahontas', 'Tioga', 'Belrubi', 'Sequoia', 'Primella', and 'Redgauntlet'. Other plants being propagated are rootstocks of apple ('M26'), cherry, and peach ('GF 677', 'GF 43', 'St. Julien 655-2', 'Damasco 1869'), 'Calita' Japanese plum, and broccoli and cauliflower types. Techniques for mass production are being developed, or possibilities being explored, for a wide range of other crops including apple, pear, cherry and Chinese gooseberry or kiwifruit (Actinidia) cultivars and a number of vegetable and floricultural crops.

The medium used for growing strawberries is that of Boxus whereas a modified Murashige and Skoog medium is used for the other fruit crops. The medium composition is varied according to the crop and the cultivar. Technical grade agar is used at 4 to 5 g/liter to give a very soft gel. They also now use 2 g of agar supplemented with 8 to 10 g/liter of pectin, the type used for making jam. They have tried 5 brands of pectin but only 2 gel satisfactorily. When pectin is added to the medium, the pH is adjusted after adding the pectin and agar; otherwise, the pH is adjusted before adding the agar.

For the peach rootstocks, an elongation stage is added after the shoot multiplication stage to produce longer shoots which are easier to handle for rooting. The medium used for elongation has BA reduced from 0.6 to 0.1 mg/liter and  $GA_3$  increased from 0.1 to 0.5 mg/liter. This stage lasts about 2 weeks and the rooting stage which follows lasts 2 to 3 weeks.

The laboratory has 9 plastic greenhouses with approximately 1800 m² (18,000 ft²) of growing space for acclimating plants produced in tissue culture. Rooted strawberry plants are removed from the agar medium and planted in ground beds filled with peat in the plastic greenhouses. These beds are covered with a sheet of plastic film for several days until the plants are established and then this extra plastic is removed. After 30 to 40 days, the plants are dug and shipped bareroot. Rooted understocks of apple, cherry, peach and plum are planted in a peat-perlite (4:1 or 5:1) mix in a variety of containers including paper pots and Styrofoam trays. The proper peat is extremely important to get good growth of the plants; only TKS-1 peat is used now. After planting, the beds are covered with a plastic sheet which is lifted gradually after several days to harden the plants.

About 70% of the rooted explants are sold before acclimation. The peach rootstocks sell for 18 to 36 cents (150 to 300 lire) per rooted plant directly from the tissue culture container or 35 to 70 cents (285 to 575 lire) for a well-rooted plant in a paper pot ready to go to the field. Strawberry plants sell for 13 cents (110 lire) per rooted plant directly from culture and 20 cents (170 lire) for a well-rooted, acclimated plant to be used as a mother plant in the nursery.

The technical aspects of the work at the Laboratory for Micropropagation are under the direction of G. Zuccherelli, V. Venturi and G. di Paoli, while S. Barducci serves as business manager for the cooperative and laboratory. There are about 6 other employees in the laboratory and several additional ones in the greenhouse. Additional workers are brought in as needed; for example, during a busy period in the spring, the transfer hoods are used 14 hours a day, necessitating 2 shifts of workers.

Unquestionably, this laboratory has the most efficient design of any that I have yet seen. Further, it has been very well managed, as evidenced by their rapid progress in just 2 years. Anyone planning to set up a large commercial laboratory would be well-advised to visit Cesena.

## Friday Morning, December 14, 1979

Dr. Harold Davidson, served as moderator of the morning session.