ASEPTIC CULTURE OF CHRYSANTHEMUMS IN THE PLANT PROPAGATION CLASS

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Aseptic culture of herbaceous plants is becoming an important commercial method of plant propagation. For some plants aseptic culture provides virus-free plants and the greatest number of plants produced per year. We developed aseptic culture of chrysanthemums for our herbaceous propagation laboratory. The aseptic culture laboratory illustrates the rapid rate of multiplication possible.

Aseptic culture involves several steps: 1) selection of plant species and plant parts, 2) selection and preparation of a growing medium, 3) development of aseptic isolation procedures, 4) growth and division of plantlets for continued multiplication or rooting, 5) establishment of rooted plantlets into soil.

PLANT MATERIAL

We used chrysanthemums because they are easily propagated by cuttings and propagation procedures using aseptic culture have been reported (1).

MEDIUM

Our medium was similar to that described by Murashige et al. (3). We used a 2.7 g/l agar because Romberger et al. (4) showed that decreasing agar concentration yielded increasing growth. Instead of kinetin, we substituted 4 mg/l of N^6-benzyladenine which proved satisfactory in preliminary trials and 2 mg/l of indoleacetic acid (IAA). We chose these concentrations based upon preliminary studies, but a 50% change in the concentrations still gave acceptable growth. An excellent reference dealing with the preparation of the medium is the article by Romberger, et al. (4). Hartmann and Kester also introduce this subject (2). The stock solution of auxin, whether IAA or IBA, should be clear and colorless.

The pH was adjusted to 5.6 using HCl or KOH, and autoclaved for 15 minutes at 121°C (250°F). We transferred 12 ml of the medium to each sterile, disposable, 60 X 20 mm plastic petri dish. Petri dishes have a large open area that makes division and transfer of the cultures easy for students. We incubated dishes containing the medium for at least 2 days at room temperature before using them to be sure the medium was uncontaminated.

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EQUIPMENT

Procedures that required sterile conditions were done in a laminar flow bench (Pure Aire Corporation of America, Van Nuys, CA) which has a fan which forces air through a filter to remove 99.9% or more of all particles 0.3 microns or greater in diameter. This removes all spores and other contaminants from the air. The clean air flow sweeps contaminants out of the work area and minimizes contamination, but contaminated objects placed upwind of aseptic materials can contaminate those materials. The laminar flow bench allowed students to work in a common laboratory instead of a special tissue culture room.

Other equipment needed was: a good analytical balance capable of weighing accurately to 1.0 mg, a pH meter or accurate pH paper, a source of distilled water, a dissecting microscope (convenient but not necessary) and an autoclave. Flasks and pipettes were needed for mixing chemicals and storing stock solutions.

STOCK PLANTS AND MERISTEM ISOLATION

We grew stock chrysanthemum plants in the greenhouse with supplemental night lighting to ensure vegetative growth. Romberger et al. (4) reported that for aseptic culture no surface sterilization of the stock material was needed if overhead watering was avoided. Therefore, we prohibited overhead watering of the stock plants.

We cut terminal stems from stock plants and isolated the meristems (shoot-tips) under a dissecting microscope in the laminar flow bench. The base of the cuttings were held in the fingers without contaminating the meristem. We removed one or two leaves from the cuttings with a sterile scalpel, then dipped the scalpel in 95% ethanol, and flamed to re-sterilize. This exposed the meristem (Fig. 1) and maintained sterility. We transferred about 1 mm of the stem tip to the medium.

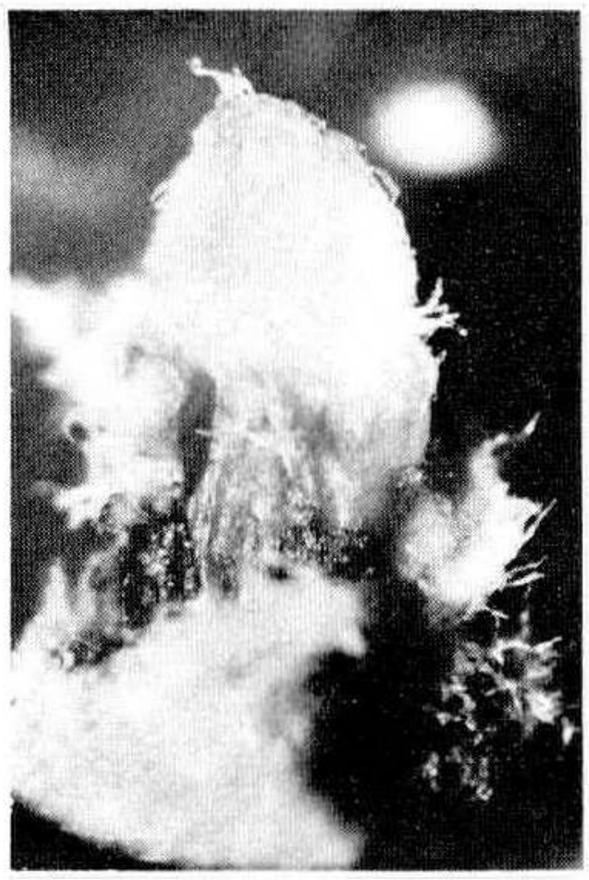

Figure 1. Chrysanthemum shoot-tip ready for separation. Cut was made in smooth area at base of shoot-tip. Distance from tip to base was approximately 1 mm.

We isolated meristems 10 to 12 weeks before the students needed the cultures for division. The cultures were divided to yield the maximum number of new cultures and maximize the time between divisions. When growth was most rapid the number of cultures doubled each week.

STUDENT ACTIVITIES

Students practiced meristem isolation in the classroom. Then they divided and transferred sterile meristem cultures in the laminar flow bench. Students came in one at a time, washed down the inside of the bench with 95% ethanol and ran the fan for 5 minutes to eliminate contamination. With a sterilized scalpel they split the plantlets (Fig. 2) into three or four pieces, and transferred these pieces to new petri dishes (Fig. 3).

Figure 2. Multiple plantlet of chrysanthemum ready for division.

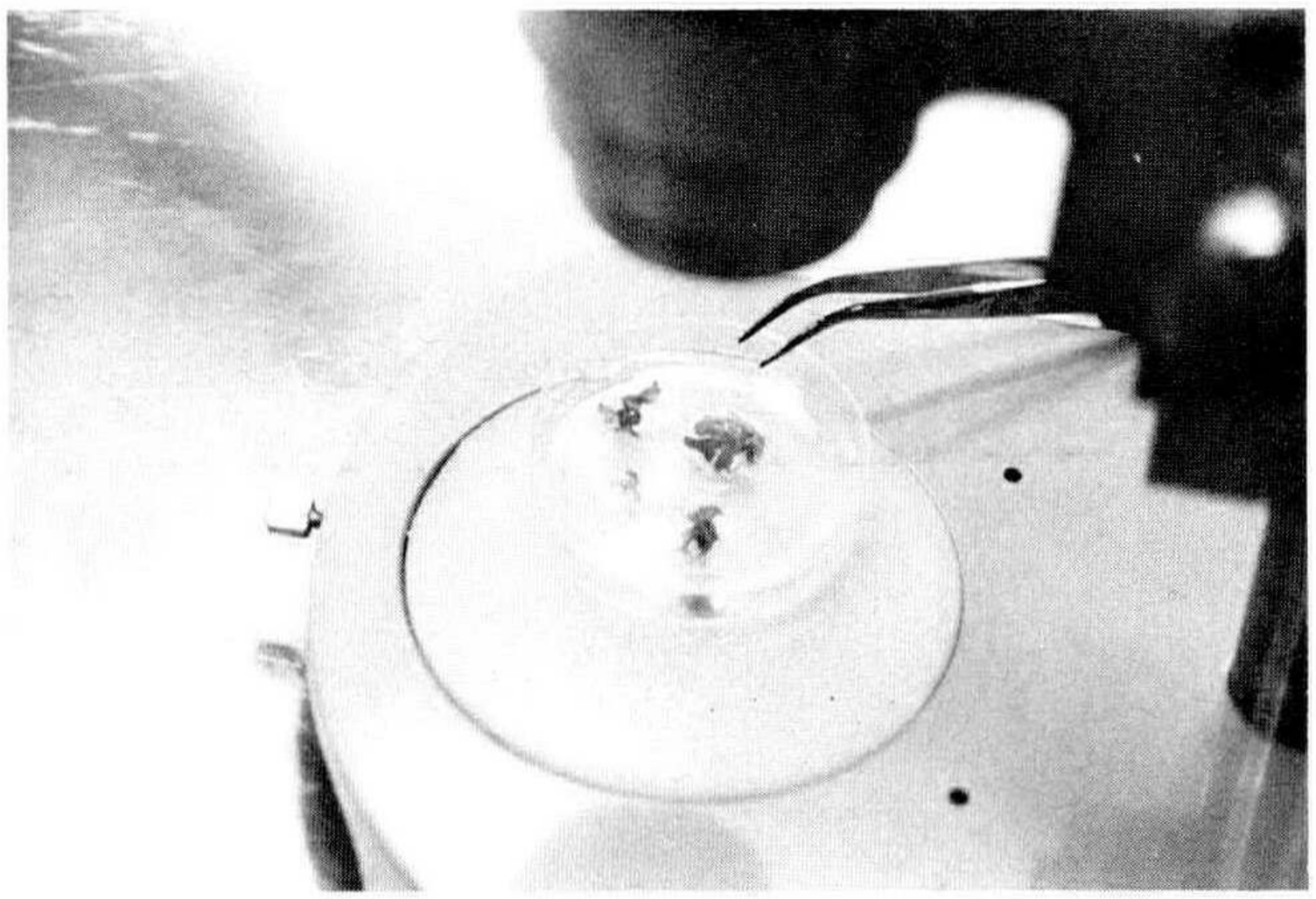

Figure 3. Chrysanthemum plantlets after division ready for transfer to new medium.

The scalpel and other tools were dipped in 95% ethanol and flamed as needed to sterilize. Caution, never put the flaming scalpel back into the beaker·of ethanol. Usually one student, technician, or teaches does this each year, so we keep a fire extinguisher nearby to put out the fire.

One or two students each semester did a special project in aseptic culture in addition to the aseptic culture laboratory. When possible we incorporated parts of their successful projects in next year's aseptic culture laboratory.

SPACE AND ENVIRONMENTAL REQUIREMENTS

The cultures were placed 50 to a clear plastic box (12x18x6 inches) with a beaker of distilled water inside to maintain high humidity, and a second beaker containing some charcoal to absorb gases produced by the cultures. Before using charcoal, we smelled a sweet aroma in the boxes. The boxes were kept at room temperature with continuous fluorescent light. We did not investigate the effects of different light periods, sources, or intensities.

ROOTING

Students achieved successful rooting by treating plantlets with Rootone F and sticking in vermiculite on an intermittent mist bench with bottom heat. Rooting occurred in 1 to 2 weeks with a 60 to 70% success in rooting. Transferring the plantlets to a rooting medium before they are treated with auxin should improve the rooting success.

CONCLUSION

Our plant propagation class introduced students to aseptic culture as a propagation technique. With proper choice of plant material, equipment, and technique the majority of students should be able to successfully propagate plants using aseptic culture.

REFERENCES

1. Earle, E. D. and R. W. Langhans. 1974. Propagation of *Chrysanthemum in vitro*. I. Multiple plantlets from shoot tips and the establishment of tissue cultures. *J. Amer. Soc. Hort. Sci.* 9:128-132.
2. Hartmann, H. T. and D. E. Kester. 1975. Plant Propagation: Principles and Practices, 3rd ed. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.
3. Murashige, T., M. Serpa, and J. B. Jones. 1974. Clonal multiplication of *Gerbera* through tissue culture. HortScience 9:175-180.
4. Romberger, J. A., R. J. Varnell, and C. A. Tabor. 1970. Culture of apical meristems and embryonic shoots of *Picea abies*. USDA Forest Service Tech. Bull. No. 1409.