Using Plant Tissue Culture to Investigate Plant Cell Differentiation and Dedifferentiation

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One of the challenges in teaching developmental biology is designing laboratory exercises that introduce important concepts and techniques, provide some latitude for independent study, and which are not prohibitively expensive. Described here is an experimental project that uses plant tissue culture techniques to examine cell differentiation in carrot. The specific questions asked in this experiment are: (1) Does the ability of carrot tissue to form callus depend upon the type of tissue examined?, and 2) Does the ability of carrot tissue to form callus depend upon the age of the tissue?

The techniques used in this project are relatively straightforward: students prepare sterile seedlings, prepare callus cultures, monitor, measure, and record callus growth, and analyze data. Seeds are sterilized by soaking them for 15 seconds in 15% silver nitrate. The seed suspension is then quickly poured through a funnel lined with a cone of filter paper. After seeds had dried for 2–4 hours, they are sprinkled onto a petri dish containing suitable medium (see below). Seedling cultures are incubated in the light. After germination, the sterile seedlings are used to start callus cultures. It is essential that sterile technique be maintained throughout these procedures. For our tissue type experiment, students separate root, shoot, and leaf tissue. The tissues are cut into pieces approximately 5 mm in length. Such tissues are placed on media suitable for callus formation (see below). Incubation was in the dark at 28°C. Cultures are examined once or twice per week. The percentage of tissues forming callus is recorded as are the lengths, widths, and general appearance of each tissue piece.

This project is very successful both in terms of experimental results and student enthusiasm. In carrot, both the age and the types of tissue determined how well callus was able to form. Students were pleased about learning tissue culture techniques and their group work and record keeping skills were improved. Class discussion of the experimental results generated several insights about similarities and differences between plant and animal development and as well as ideas for further extensions to this experiment. The project can be adapted for use in introductory courses or in upper-division offerings including cell biology, developmental biology, botany, or plant physiology.

Standard culture media for growing plants from seed: MS salts (Murashige-Skoog salt base, can be purchased from Carolina Biological Co. and other suppliers), 100 mg of myo-inositol, 30 g of sucrose, and 1 liter of distilled or deionized water. Adjust pH to 5.8, add 9 g of agar, and autoclave for 20 minutes with a slow exhaust. (Pre-made media can also be purchased from Carolina Biological Co. and other suppliers.)

Carrot callus induction medium: Same recipe and instructions as above except add 0.5 mg/liter of 2,4-dichlorophenoxyacetic acid (2,4-D). (Pre-made carrot callus induction medium can be purchased from Carolina Biological Co. or other suppliers.)