Growth of Cultured Sweet Potato Explants

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Plant tissue culture is "the growing of isolated plant parts, aseptically and heterotrophically, as explants on appropriate media (Steward 1983)." It includes basic propagation techniques such as organogenesis, embryogenesis and shoot tip culturing. However, it also includes specialized techniques such as protoplast fusion and genetic transformation used for production of new plant varieties. This is why it has developed into a "valid botanical discipline" (Bottino 1981) and is considered a "key area of biotechnology for the next century" (Fowler et al. 1992). Thus, it is appropriate subject material for a basic biology laboratory (Haldeman & Ellis 1988).

A procedure used in the analyses of these techniques is measurement of growth. Procedures used to measure growth are considered basic; yet, when combined with experiments using plant tissue cultures, they are an exciting way to introduce valuable skills to students.

The plotting of length or weight or some other measurement against time produces a growth curve. These curves can be used to determine the effects of an experimental treatment, such as combinations of growth regulators, on the growth of cells, calluses or other explant material. The goal may be to maximize growth, as with shoot tip cultures used for micropropagation. You may want to minimize growth, as with germ plasm conservation using minimal growth storage. You may simply want to determine the optimum culture period for some other procedure, such as protoplast isolation or the harvesting of secondary metabolites.

Although there are numerous means for determining growth, two of the simplest and most frequently used are fresh weight and dry weight. The procedures presented here are modified from an exercise in Dodds and Roberts (1985). These procedures were used to determine growth curves of sweet potato (*Ipomoea batatas*) root explants grown on Murashige and Skoog medium. Sweet potatoes are commonly sold as 'yams' in your local grocery store; however, sweet potatoes are dicots and the true yams are monocots belonging to the genus *Dioscorea*. We have found that sweet potatoes are useful tools for this demonstration. The roots provide large amounts of sterile tissue that respond well to the culture conditions used.

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Preparation

The experiment can be performed on an open bench top disinfected with 95 percent ethanol. Protective gloves and a lab coat should be worn. Keep foot traffic to a minimum. Instruments needed include a cork borer with an inside diameter of 5 or 6 mm, a cork borer punch, a pair of large forceps, a scalpel and a pair of utility tongs. These are disinfected by placing them in a beaker filled with 95 percent ethanol for at least 15 minutes before use. To use an instrument, remove it from the beaker and shake off excess alcohol. We do not use a flame due to the potential of a fire. When finished with an instrument, replace it in the alcohol.

For the plant material, select sweet potatoes that are not obviously damaged or diseased. Scrub the roots thoroughly under running tap water to remove any soil. Then, with a vegetable scraper, remove the skin. Now cut the potatoes transversely into slices approximately 15 mm in thickness. You will need about six slices for this demonstration. Place these slices in a 600 ml beaker covered with aluminum foil. Disinfect the slices by adding approximately 500 ml of 20 percent (volume/volume) bleach to the beaker. Then swirl the slices every few minutes in the bleach for a total of 10 minutes. Swirling assures that all surfaces of the slices come in contact with the bleach. After 10 minutes, loosen (but do not remove) the foil cover and pour the bleach solution into a sink. Avoid tossing the slices out with the bleach. The inside surfaces of the beaker, with the slices, are considered disinfected at this point and should be treated aseptically.

Now rinse the slices to remove the bleach by carefully lifting the foil cover from the beaker and pouring about 200 ml of sterile distilled water into the beaker. Replace the foil cover and swirl the slices for about 30 seconds. Pour off the rinse water. Repeat this rinsing procedure two more times. Keep the slices in the beaker until they are ready to be used.

Explants

Explants must be removed from the slices. This is difficult for beginning students. We recommend that they work in pairs to make all the necessary manipulations and reduce contamination. Loosen the foil cover on the beaker and remove one of the slices using the utility tongs. Keep the beaker with the rest of the slices covered to maintain sterility. Place the removed slice in the bottom half of a sterile Petri dish (100 mm × 15 mm).

The first partner holds the sweet potato slice steady on the exposed Petri dish using the tongs. The second partner makes borings with the cork borer parallel to the vertical axis of the tissue slice. Stay at least 2 mm inside the slice to avoid including any side surfaces damaged by the bleach during disinfection. Push the cork borer completely through the slice. Since the tissue is firm, the first partner must continue to hold the slice using the tongs while the second partner removes the cork borer. The explant will remain inside the borer. Now the first partner holds open another sterile Petri dish while the second partner plunges the explant into this dish using the cork

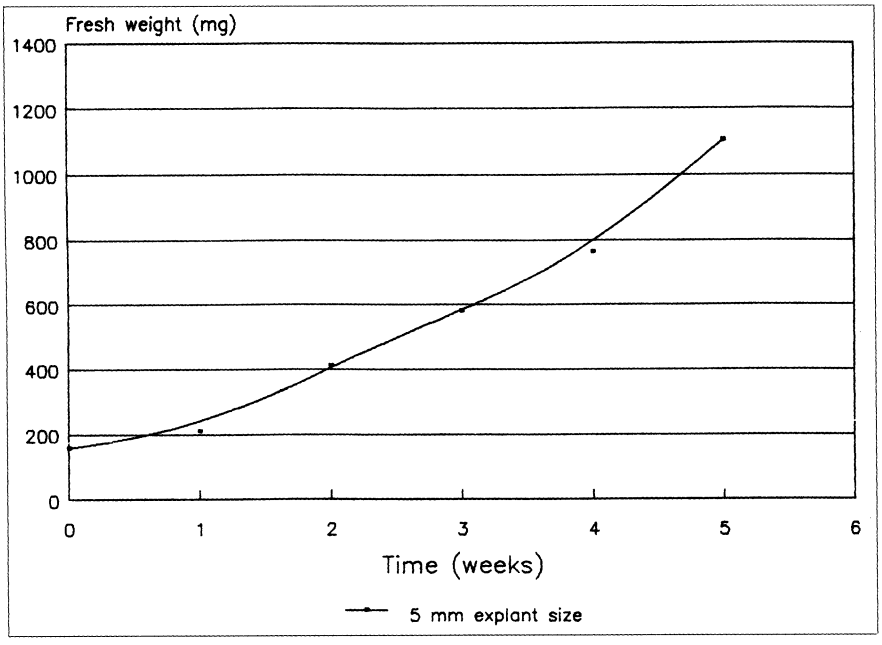

Figure 1. Fresh weight growth curve of cultured sweet potato explants grown on Murashige and Skoog medium at 28°C in the dark.

borer punch. Use gentle pressure to avoid overshooting the Petri dish. Replace the lid on the Petri dish to keep the explants sterile. You should be able to obtain at least five borings from each slice. Repeat this procedure using the other slices to give a total of 30 borings. Use a separate sterile Petri dish for the borings from each slice to reduce the chance of contamination.

For final explant preparation, use a scalpel and forceps to cut and discard about 2 mm of tissue from each end of the borings. You want to remove tissue damaged by the bleach, but leave enough to make a 10 mm long cylinder. Then cut each 10 mm cylinder in half to create two 5 mm explants. Graph paper with 1 mm squares may be placed under the dishes as a cutting guide. This will give a total of 60 explants, and each explant will measure 5 mm in length and 5 or 6 mm in diameter.

Culturing & Sampling

Transfer 50 explants to culture tubes (20 mm × 150 mm) containing 10 ml of Murashige and Skoog salt base with 1 mg/l 2,4-dichlorophenoxyacetic acid, 100 mg/l inositol, 0.5 mg/l nicotinic acid, 0.5 mg/l pyridoxine HCl, 0.4 mg/l thiamine HCl, 2 mg/l glycine, 1 g/l casein hydrolysate, 30 g/l sucrose and 8 g/l agar. This is a modification of tobacco callus medium (Carolina Biological Supply Company 1986). Use forceps to drop each explant into its own tube so it rests flat on the agar surface. You will need 50 tubes of media. The remaining 10 explants will be used for the "baseline" fresh and dry weight data and will not be cultured. This will give you six weeks of data, with the first week, week 0, using the 10 noncultured explants. Place the tubes of explants to be cultured into an incubator at 28°C. No light is necessary. Ten cultured explants will be harvested each week for the next five weeks for fresh and dry weight determinations.

Fresh & Dry Weight Determination

Each week for six weeks the mean fresh weights of 10 explants must be determined to the nearest 0.1 mg. Be sure not to include any of the medium in your weighings.

After fresh weight is determined, the same explants are used for dry weight determination. One procedure for doing this is to dry the explants in an oven at 60°C for 12 to 24 hours and then weigh them. Return them to the oven for an additional 12 to 24 hours and then reweigh them. Repeat this process until two successive weighings are the same. This usually takes from 24 to 48 hours. For a classroom demonstration, this might be difficult to carry out. Thus, it would be more reasonable to dry the explants for a set period, such as 48 hours, and then weigh them.

For the dry weight results of our experiment, the explants were dried for one week at 60°C. We found that at this temperature the actual difference between the 48-hour and seven-day dry weights was less than a tenth of a milligram per explant.

Graphing

Plot the mean fresh weight and mean dry weight for each week as a function of time. The results presented in Figure 1 and Figure 2 are the averages

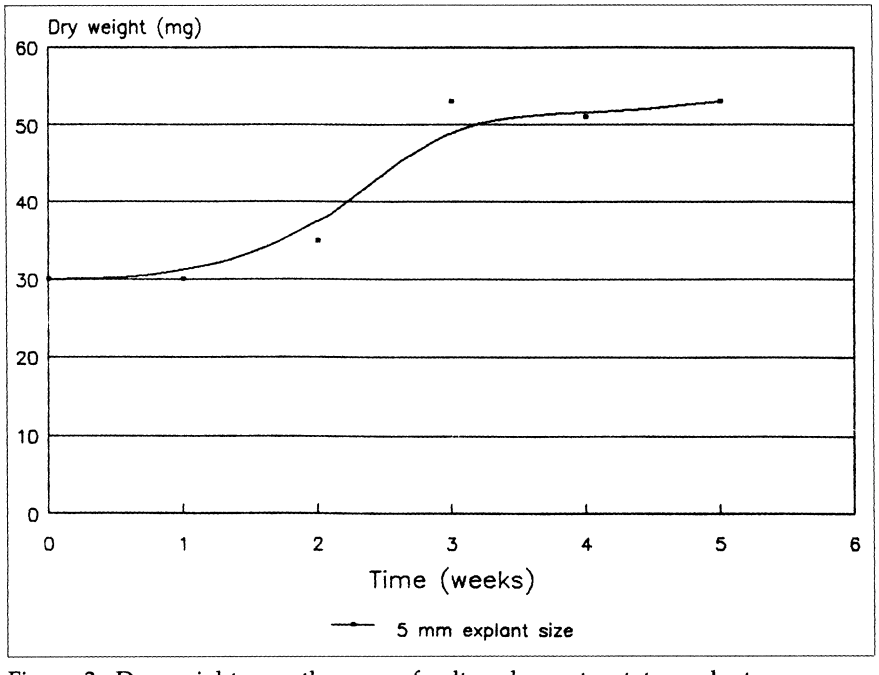

Figure 2. Dry weight growth curve of cultured sweet potato explants grown on Murashige and Skoog medium at 28°C in the dark.

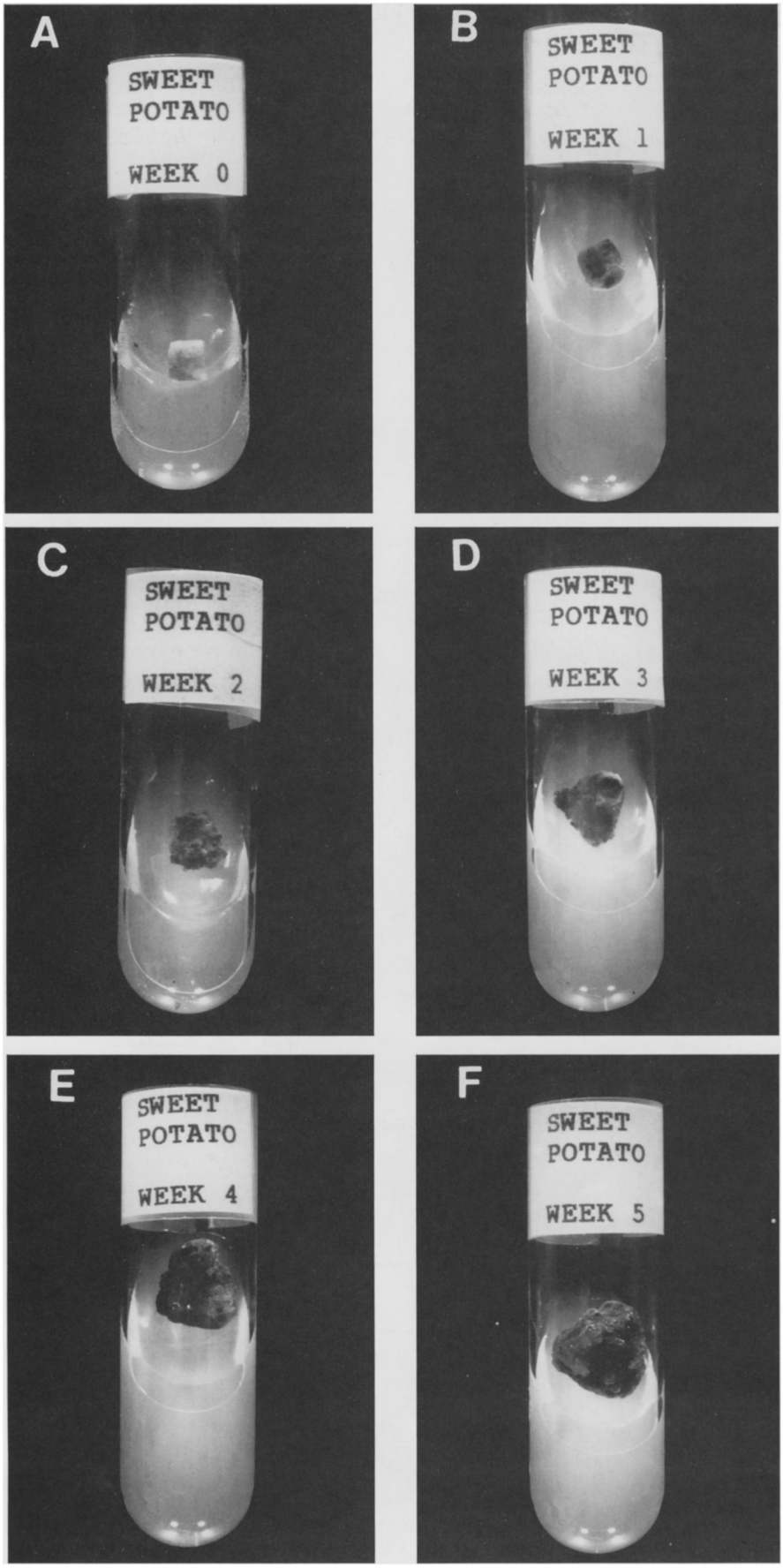

Figure 3. Change in sweet potato explant appearance. At time of preparation (A) and after one (B), two (C), three (D), four (E) and five (F) weeks of growth.

for two experiments. They were performed by students in an introductory plant tissue culture course and were plotted using First Choice, version 3.0.

Results & Discussion

The changes in fresh and dry weights for sweet potato explants over a five-week culture period are presented in Figure 1 and Figure 2, respectively. Under the conditions of this experiment, the dry weight growth curve resembles an idealized S-shaped growth curve, with lag, exponential, linear and stationary phases. However, the fresh weight growth curve does not show the classical sigmoid shape. This curve more closely resembles our visual observations (Figure 3) of the explants. That is, there is continuous growth for five weeks.

This is an exciting exercise for students as they watch their explants grow. Calluses are clearly visible on many explants after just one week of culture, and the calluses increase each week at an almost explosive rate. This is a much faster growth rate than is seen for carrots or tobacco.

Some contamination is inevitable for most students. Growth curves can still be determined using the surviving explants. Once students gain practice in these basic skills, they can conduct experiments of their own design to answer self-generated questions.

Acknowledgment

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References

Bottino, P.J. (1981). *Methods in plant tissue culture*. Kensington, MD: Kemtec Educational Corp.

Carolina Biological Supply Company. (1986). *Carolina plant tissue culture media formulation booklet*. Burlington, NC: Carolina Biological Supply Company.

Dodds, J.H. & Roberts, L.W. (1985). *Experiments in plant tissue culture*. New York: Cambridge University Press.

Fowler, M.W., Warren, G.S. & Moo-Young, M. (Eds.). (1992). *Plant biotechnology*. New York: Pergamon Press.

Haldeman, J.H. & Ellis, J.P. (1988). Using cauliflower to demonstrate plant tissue culture. *The American Biology Teacher, 50*(3), 154–159.

Steward, F.C. (1983). Reflections on aseptic culture. In D.A. Evans, W.R. Sharp, P.V. Ammirato & Y. Yamada (Eds.), *Handbook of plant cell culture, Vol. 1. Techniques for propagation and breeding* (pp. 1–10). New York: Macmillan Publishing Company.