Dodds J.H., Roberts L.W. 1985. Experiments in Plant Tissue Culture. Cambridge University Press.

Organogenesis

Shoot initiation or caulogenesis in cultured plant tissues can be induced in many systems by an appropriate balance of exogenous auxin and cytokinin, and, in some cases, either one or the other of these growth regulators must be omitted from the medium in order to produce buds (Street, 1977). Buds are initiated in many dicot callus cultures by transferring the callus to a medium containing a cytokinin:auxin ratio in the range of 10 to 100, whereas callus production is favored by an auxin:cytokinin ratio of approximately 10 to 100. The term "reversal transfer" has been applied to the transfer of a culture from a callus-supporting medium to a shoot-inducing medium (Murashige and Nakano, 1965; Gresshoff, 1978). Unlike the conventional tobacco system, monocot callus derived from alfalfa (*Medicago sativa*) produces roots after a 4-day exposure to a high kinetin:2,4-D ratio. Shoots are formed by a similar treatment involving the same exposure to a relatively high 2,4-D:kinetin ratio. Organogenesis does not occur on the induction medium, but is initiated only after transfer of the callus to a medium devoid of growth regulator (Walker, Wendeln, and Jaworski, 1979). With other monocot cultures exogenous cytokinin may be unnecessary for the initiation of buds. The omission of auxin from the medium may be sufficient to induce shoot formation in these cultures, and two successive transfers on auxin-free media are recommended (Gresshoff, 1978). In addition to cytokinins, a variety of related compounds are capable of replacing cytokinins for shoot induction. These chemicals include substituted pur-

ines, pyrimidines, and ureas (Thorpe, 1980). In addition, adenine sulfate is capable of functioning as a cytokinin in the induction of buds (Skoog and Tsui, 1948; Sterling, 1951).

Auxin:cytokinin supplements or omissions from the medium have failed to induce shoots in the culture of many species, and Street (1977) has offered some possible reasons for these failures: (1) Additional hormones may be required; (2) endogenous hormones may accumulate, and their inhibitory effect on organogenesis is not reversed by the exogenous hormones; and (3) cultural conditions involving nutritional and physical factors may block the onset of the process.

The relative concentration and spectrum of endogenous gibberellin-like compounds change during shoot initiation, and this group of hormones plays a role in organogenesis (Thorpe, 1980). Gibberellins, in general, tend to suppress shoot and root formation. Shoot-forming callus accumulates starch, and this biosynthetic process is thought to be required for bud initiation. The inhibitory effect of GA_3 on caulogenesis has been related to the lowering of the starch content of the bud-forming cells (Thorpe, 1978; Maeda and Thorpe, 1979).

Endogenous ethylene may be a factor in caulogenesis. Early in culture this gaseous hormone blocks organogenesis, but during primordia formation ethylene enhances their development (Huxter, Reid, and Thorpe, 1979). Endogenous ethylene was identified as a factor in the induction of buds from cultured tobacco cotyledons (Everett, 1982). Indirect evidence suggests a similar role for ethylene in cultured *Lilium* bulb tissue (Aartrijk and Blom-Barnhoorn, 1983). Additional information on the organogenetic effects of growth-active substances can be found in the review by Thorpe (1980).

Carbohydrate metabolism is another factor to be considered in caulogenesis (Thorpe, 1982b). In addition to serving as a respiratory energy source, the exogenous carbohydrate may act as an osmotic agent. Mild osmotic stress apparently causes biochemical alterations that are reflected in changes in callus growth and morphology. The partial replacement of the sucrose requirement for bud formation in tobacco callus with mannitol supports the view that at least part of the carbohydrate in the system is performing an osmoregulatory function (Brown, Leung, and Thorpe, 1979). Murashige (1977) also stressed the importance of high osmolarity, as well as light and chelating agents, in shoot regeneration.

In some cultured tissues an error occurs in the developmental programming for organogenesis, and an anomalous structure is formed. In-

ternodal stem segments of *Quercus rubra* L. seedlings produced "organoids" on callus arising from the primary explant and the subcultured callus (Seckinger, McCown, and Struckmeyer, 1979). Although these structures contained the dermal, vascular, and ground tissues present in plant organs (Esau, 1977), they differed from true organs in that the organoids were formed directly from the periphery of the callus and not from organized meristemoids. The organoids proliferated in culture, and adventitious roots were formed from them (Seckinger et al., 1979). Similar types of anomalous structures have been observed on the callus of a different genus in the Fagaceae (Keys and Cech, 1978), on bacteria-free cultures of crown gall of *Nicotiana* (Braun, 1959), and on normal tissues of *Taraxacum* roots (Bowes, 1971).

In the present experiment the student will attempt to induce the formation of plantlets from leaf explants of *Saintpaulia ionantha* Wendl. (African violet). This plant has been propagated in vitro from explants of leaf lamina (Start and Cummings, 1976; Cooke, 1977; Vasquez, Davey, and Short, 1977), petioles (Bilkey, McCown, and Hildebrandt, 1978; Harney and Knap, 1979), and floral organs (Hughes, 1977; Vasquez et al., 1977). Harney (1982) has given a résumé of the in vitro propagation procedures for the regeneration of African violets.

PROCEDURE

Prepare 1 liter of MS medium as outlined in Chapter 4, and supplement with *myo*-inositol (100 mg/l), nicotinic acid (0.5 mg/l), pyridoxine-HCl (0.5 mg/l), thiamine-HCl (0.4 mg/l), NAA (0.1 mg/l), BAP (5.0 mg/l), and adenine sulfate (80 mg/l). Adjust the pH of the final medium to 5.7. The experiment as outlined requires 200 cm^3 of the medium, and this is prepared in two 100-cm^3 aliquots. Each aliquot will contain sucrose (3.0% w/v) and agar (0.7% w/v). The autoclaved medium is poured into 20 culture tubes (10 cm^3 each).

Culture procedure

1. Excise several healthy leaves, both young and old, and discard the petioles. Wash the blades or laminas briefly in cool soapy water. Rinse them in running tap water, and prepare for aseptic procedures. Because the steps in the preparation of the plant material for organogenesis follow the same basic procedure as outlined in Chapter 5, the following instructions will be given in abbreviated form.

2. Dip the blades in ethanol (70% v/v) and rinse in sterile DDH_2O in a 250-cm^3 beaker. Complete the surface sterilization by immersing the blades in the hypochlorite solution for 10 min. This two-step disinfestation is considered necessary because of the abundance of epidermal hairs on the surface of the blade. Rinse the blades in 3 successive baths of DDH_2O.

3. Each blade is transferred to a sterile Petri dish containing filter paper, and explants are prepared with forceps and scalpel. The filter paper will remove the excess moisture from the final DDH_2O rinse. The blade tissue most effective in organogenesis is located in the central part, and the outer margins and leaf tip are relatively unproductive. Slice the blade into rectangles approximately 10–12 mm on a side, ensuring that each explant contains a portion of the midvein of the leaf. Place the explants individually in culture tubes in an upright position with one-quarter of the explant embedded in the agar medium (Start and Cummings, 1976).

4. Place the cultures in a plant growth chamber maintained at 25°C

RESULTS

Shoots will appear within 2 to 4 weeks, and the initial shoots are usually associated with the severed veins on the adaxial surface of the blade. After 6 to 8 weeks of culture the proliferated shoots can be aseptically subdivided and subcultured for the initiation of roots. Rooting is promoted by transferring the shoots to a medium that is devoid of plant hormones and has a sucrose concentration of approximately 1.6% (w/v) (Start and Cummings, 1976). Some research workers believe that this subculture step is unnecessary, since the subdivided shoots will readily establish a root system in a sterile potting soil mixture (Harney and Knap, 1979).

QUESTIONS FOR DISCUSSION

1. Can you think of any observations made on the production of shoots by the cultured leaf explants of African violet that suggest endogenous factors may play a role in the initiation of bud primordia?
2. An unsuccessful attempt was made to induce shoot formation in a dicot callus culture by supplementing the medium with a high cytokinin:auxin ratio. What are some alternative techniques that might induce shoot formation in this culture?
3. What is a meristemoid? Is a meristemoid considered totipotent?
4. In regard to organogenesis, what unusual characteristic was shown by an alfalfa callus culture?
5. What possible relationship exists between caulogenesis and the inhibition of this developmental process by gibberellins?