

EXERCISE 11

Organogenesis and Callus Formation from African Violet Leaves

PURPOSE

To examine the effects of plant growth regulators on organ formation from leaf explants.

The violet leaves already have a ratio of cytokinin to auxin. The cytokinin and auxin added to the growth medium simply add to the endogenous hormones that are already in the tissue. When either a cytokinin or an auxin is added singly to a tissue culture medium, the ratio within the plant tissue shifts to a higher amount of the plant growth regulator (PGR) that was added and a lower amount of the one that was not added. It is the changing of the ratio of cytokinin to auxin that influences whether a cell will become determined to undergo a coordinated series of cell divisions, followed by differentiation to form shoots, roots, or unorganized callus.

These cultures can be incubated on a lab bench with or without grow lights. Light is required, but intensity and duration are not critical. Results should be evident in 2 weeks.

Be careful not to squash or injure the leaf tissue after cutting, or you will just end up with a dead leaf.

Your leaf tissues should be small and include at least 1 major vein.

MATERIALS

4 sterile beakers

70% Ethanol, 20% bleach, sterile ddH₂O, African Violet leaves, sterile forceps, scalpel blades,
4 glass tubes with growth medium

1 tube of shoot-inducing medium (yellow medium) (Contains 10 mg/L IAA; 1 mg/L kinetin)

1 tube of root-inducing medium (pink medium) (Contains 1 mg/L IAA; 1 mg/L kinetin)

1 tube of callus-inducing medium (green medium) (Contains 1 mg/L IAA)

1 tube of differentiation medium (light-blue medium)

PROCEDURE

1. Each person removes 1 leaf from an African Violet plant.
2. Lab partners can place both leaves in a beaker of 70% Ethanol for 5 minutes to surface-sterilize the leaf. Then place the leaves in a beaker of 20% bleach for 5 min. The leaves are rinsed 2 x 5 min. in sterile ddH₂O.

3. Take your leaf and cut off all the outside edges. The remaining section should be 1–1.5 cm wide and include at least 1 major vein. DO NOT use the midrib of the violet leaf, as it is too thick and will result in the leaf edges not touching the growth medium. Place 1 or 2 sections on the surface of the growth medium in 2 of the 4 tubes provided.
4. Your partner will cut his/her leaf the same way and use the other 2 tubes.
5. Label tubes with name, date, and lab section. Place tubes in the tube rack provided. Make observations over the next few weeks. If your leaf section turns grayish brown, it is most likely dead. If it stays green and starts to form bumps on the edges, it is alive. If the contents of the tube turn fuzzy, it is contaminated. Tiny roots will look like white fuzz on the edges of the leaf sections, which should not be confused with contamination.

ANTICIPATED RESULTS

Most contamination will occur in the first week. At this time, the first roots should become visible in the “rooting” (auxin-containing) medium. After about 3 weeks, the roots should be well developed.

After 3 weeks, buds and perhaps small shoots should be visible, especially in the “shooting” medium (cytokinin-containing).

Callus will be visible in 1-2 weeks in all tubes, regardless of treatment. Most callus and a few adventitious roots will form in the “callus” medium (both cytokinin- and auxin-containing).

What do you think is in the 4th tube?

NOTE: Prepared media can be obtained from Ward’s Natural Science.