Plant Tissue Culture and Transformation
February 5 and 7

This laboratory has three parts. The first two parts "*Plant Tissue Culture: Hormones and Organogenesis"* and "Fun with Agrobacterium tumefaciens" plant tissue culture and transformation techniques, and the role of hormones in plant organogenesis. The final part starts plant material that will be used in the laboratory next week.

PART I: PLANT TISSUE CULTURE: HORMONES AND ORGANOGENESIS

Plant tissue culture has contributed greatly to the understanding of the role of hormones in growth and differentiation. Plant tissue culture involves growing plant tissues, organs, and cells *in vitro*. Typically the plant tissue of interest is removed from the plant, surface sterilized and transferred to a defined growth medium. The tissue is often grown on solid agar where, depending on the hormones present, the proliferating cells form callus (a mass of undifferentiated and unorganized plant cells) or undergo organogenesis. Plant tissue culture techniques also have widespread use in the development of new plant varieties, production of pathogen-free plants and the production of transgeneic plants.

In this experiment various combinations of an auxin, naphthalene acetic acid (NAA), and a cytokinin, benzyl adenine (BA), will be tested for their ability to induce shoot and root formation in tobacco pith tissue explants. This experiment is intended to teach you plant tissue culture techniques and strengthen your understanding of the roles of hormones in cell division and differentiation.

Experimental Procedure (work in groups of four):

1. Remove two healthy young well-expanded leaves from a greenhouse-grown tobacco plant.

2. Begin surface-sterilize the leaves by immersing them in 0.5 L of 10% Clorox containing 0.05% (v/v) Tween 20 (a surfactant that helps wet the leaf) in a 1 L beaker.

3. Transfer the beaker to the hood and expose the leave to the sterilization solution for 10 minutes. During this treatment keep the leaves completely immersed and occasionally move them around in the solution using the forceps. Try to avoid damaging the leaves when moving them with the forceps.

4. Wash the leaves 4 times with 300 mL of sterile H_2O.

5. Place one of the sterilized leaves in a large Petri dish with a small amount of sterile water.

6. Using the scalpel cut the blade of the leaf into 1 cm squares. Occasionally sterilize the scalpel.

   Note the scalpel and large forceps can be sterilized by dipping into 70% alcohol followed by removing the ethanol from the tool by incineration. Ignite the ethanol using the flame but do not heat the tool because a hot tool will damage the leaf tissue.

7. Place two squares upside down on the surface of the medium tissue in each of the culture bottles in Table 1. Occasionally sterilize the forceps.

Table 1. Amount of BA and NAA in Each Treatment/Bottle

	No BA	**BA (0.05 mg/L)**	**BA (0.2 mg/L)**	**BA (0.8 mg/L)**
No NAA	Bottle #1	Bottle #2	Bottle #3	Bottle #4
NAA (0.2 mg/L)	Bottle #5	Bottle #6	Bottle #7	Bottle #8
NAA (1.0 mg/L)	Bottle #9	Bottle #10	Bottle #11	Bottle #12
NAA (5.0 mg/L)	Bottle #13	Bottle #14	Bottle #15	Bottle #16

8. Wrap the top of each bottle with surgical tap. This tape allows air exchange into the bottle but prevents contamination by microbes present in the air.

9. Label the bottles with your name and place them in the plant growth space assigned to you by the TA.

WEEKS 3-14

Observe the cultures weekly and, on the following pages and record the growth and appearance of the tissue under the various hormone treatments. Remember to compare the treatments to the controls (bottles #1 -- no added hormone).

Related Chapters in *Biology of Plants*
Chapters 10 and 27

PART II: Fun with *Agrobacterium tumefaciens*

This experiment will explore pathogenesis of by *Agrobacterium tumefaciens*.

Materials: (work in groups of 2)
1) One carrot root
2) Two water agar plates
3) Potato peeler
4) Knife
5) 10% Clorox containing 0.05% (v/v) Tween 20
6) Sterile Water
7) Petri dish
8) 70% ethanol for sterilizing implements
9) Forceps
10) 0.5 mL of sterile bacterial growth medium
11) 0.5 mL of *Agrobacterium tumefaciens* culture
12) P20 pipette and tips
13) Parafilm
14) Scissors

Work in groups of 2. Each group will sterilize a carrot root, cut it into slices (discs), place them on water agar plates and inoculate with control solution or *Agrobacterium tumefaciens*. The carrot root discs are grown on water agar rather than a more complex growth medium because the carrot root is a storage organ and contains stored reserves that can support growth of the cells throughout this experiment.

Experimental:

1. Remove periderm from carrot root with a potato peeler.

2. Immerse carrot root in 10% Clorox containing 0.05% (v/v) Tween 20 (a surfactant that helps wet the leaf) in a beaker.

3. Transfer the beaker to the hood and expose the root to the sterilization solution for 10 minutes. Occasionally move the root around in the solution using the forceps.

4. Rinse the root 4 times with sterile H_2O.

5. Place the root in a large Petri dish.

6. Sterilize the knife by dipping into 70% ethanol and incinerating residual ethanol on the blade. Using the sterile knife cut 10 to 12 0.5 cm thick carrot discs.

7. Place half of the discs on one agar water plate and the other half on the other plate.

8. For one plate, distribute 20 μL of control solution (sterile growth medium) across the top of each disk. Label the plate and seal the edge with Parafilm.

9. For the other plate, distribute 20 μL of *Agrobacterium tumefaciens* culture across the top of each disk. Label the plate and seal the edge with Parafilm.

10. The plates will be grown at room temperature in the dark or in room light.

Related Chapters in *Biology of Plants*
Chapter 10

Experiment is based on:
Klein, R.M. and Tenenbaum, I.L. (1955) A Quantitative Bioassay for Crown-Gall Tumor Formation. Amer. J. Botany 42:709-712.

PART III: PREPARATION FOR THE ANAEROBIC RESPIRATION LAB

Surface sterilized maize seed by:

1. Soak 20 maize seeds for 10 min in 70% (vol/vol) ethanol.

2. Pour off the 70% ethanol.

3. Soak for 10 minutes with 20% (vol/vol) bleach containing surfactant.

4. Rinsing **3 times** with sterile H_2O.

5. Place the sterilized seeds on sterile filter paper that had been wet with sterile 5 mm Tris-HCl (pH 8.0).

6. Grown at 25˚C in the dark.