LAB LESSON PLAN
(cover page)

Summer Science Institute For Agriculture Teachers
University of California, Davis

LAB TITLE Plant Development: Leaf disc method **PRESENTER:** Dave Burger

Ag Model Curriculum Standard(s), Learning Outcome(s) & Biological Standard(s) Addressed: Basic Core Standard: Plant Science 3A: Biological Science Standards 1, 2, 9, 10, 12, 13, 17.

Objective: Upon completion of the lab, students should: 1) understand the factors necessary to sustain plant life, 2) describe the role of plant growth hormones in the growth and development of plants, 3) understand the interrelationships among cells, tissues, organs, and systems, 4) appreciate the importance of technological advances in the field of agriculture.

Tools, Equipment & Materials (as necessary): Basic tissue culture lab equipment (see following), pwtunia leaves, growth regulators.

References: See attached references

Procedures (activities) 1) Disinfestation and explanting petunia leaf discs, 2) Osbservation of adventitious shoot formation, 3) Discussion of applications to crop improvement through genetic transformation.

Method(s) of Evaluation) Lab write-ups

FACILITIES NEEDED FOR PLANT TISSUE CULTURE

Equipment	Substitution
1. Autoclave	Pressure cooker, 15 minutes at 212°F
2. Laminar flow hood	squirrel fan, enclosure, respiration filters, UV lamps
3. Distilled or deionized water	Culligan

4. Culture space
   light-dark, room temperature, cool-white, fluorescent lamps, 1000 lux (good reading light)

5. pH meter

6. Stereo microscope

7. Balance

8. Culture vessels
   test tubes, scintillation vials, Mason jars, Magenta boxes

Suppliers

1. Carolina Biological
   Box 187
   Gladstone, Oregon 97027
   800-547-1733

2. Grand Island Biological Co. (GIBCO)
   519 Aldo Avenue
   Santa Clara, CA 95050
   408-988-7611

3. Flow Laboratories
   936 W. Hyde Park Blvd.
   Inglewood, CA 90302
   213-674-2700

GENERAL METHODS
FOR PLANT TISSUE CULTURE

1. Select and collect tissue.
   - leaves
   - nodes containing axillary bud(s)
   - shoot-tips
   - apical meristems
   - flower stalks
   - other tissues

The following work is performed in a laminar flow hood or equivalent equipped with alcohol lamp, dissecting instruments (scalpel, forceps, etc.), and 95% ethyl alcohol (ethanol).

2. Treat tissue in disinfestants (10% Clorox, 70% ethyl alcohol, or hydrogen peroxide). Treatment time varies, but is usually 10-20 minutes. Treat for shorter periods of time or with lower concentrations of disinfestant if tissue is sensitive to the disinfestant used.

3. Wash disinfestant off with repeated washes of autoclaved water (at least 3 washes).

4. Make explants (tissue removed from plant and put into culture) from disinfested tissue. Cut away any tissue damaged by the disinfestant.

5. Place explants on culture media of choice. Culture media variables:
   a. hormone concentrations auxin - cell enlargement, root formation cytokinin - cell division, shoot formation
   b. sugar source - sucrose (table sugar), fructose, glucose
   c. salts - various concentrations or substitutions

6. Place culture vessels under lights or in the dark depending on the plant's requirements.