LAB LESSON PLAN
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California Agriscience Institute for Agriculture Teachers
California Department of Education

LAB TITLE: APPLE CULTURE

Ag Model Curriculum Standard(s), Learning Outcomes(s) & Biological Standard(s) Addressed: Biological Standards 1,2,9,10,12,13,17

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Objective(s): Upon completion of the lab activity, the learner will be able to: Introduce students to the basic concepts of and techniques of tissue culture and micropropagation. To enable students to understand the relationship of plant tissue culture to plant biotechnology. Students appreciate the importance of technological advances in the field of agriculture.

Teacher Preparation: More than one day less than one week. Order supplies.

How many class periods will lab take? One to three days to setup. One to two weeks to observe.

Procedures (activities):

Method(s) of Evaluation: Laboratory writeup and teacher observation.

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Time required for experimental effects: 5-10 days

LABORATORY #1 - APPLE TISSUE CULTURE

AGRICULTURAL APPLICATIONS AND PRACTICES

Plant tissue culture is one form of biotechnology that has already had a dramatic impact on agricultural practice. Theoretically, one piece of plant tissue can produce an infinite number of new plants. Because tissue culture requires a minimum amount of plant material to start with, significant savings can be realized by reducing investments in stock plants and growing facilities. With tissue culture, growers can produce large numbers of stock plants in months instead of years. Development of new crop varieties usually take four to six years, due to the time required to produce test plants in actual field or growing conditions. Certain trees take up to 10 years to begin producing seed that can then be used in plant breeding efforts.

Plant tissue culture has become an important part of plant breeding programs. As a supplement to traditional plant breeding programs, tissue culture allows scientist to clone the most desirable plants, set these plants in growing areas, and breed them, conventionally with the complementary parent. The result is a more cost-effective hybrid ready for marketing much sooner. For many years, plants have been cloned by rooting cuttings, layering and grafting. Tissue culture produces the same result with one major advantage: multiplication in tissue culture is much more rapid. Regenerating plants from cells by tissue culture makes it possible to manipulate millions of cells in the laboratory instead of growing millions of plants in the field. Scientists can select superior plants by viewing the genetic materials in a cell instead of growing the whole plant. Tissue culture, unlike some other advances in biotechnology, has already become important commercially. For example, tissue culture has been used to eliminate a wide variety of viruses from lilies, carnations, citrus, potatoes, and berries.

INTEREST APPROACH

Bring a beautiful African violet into class. Have students assume they are managing or working in a growing operation. Tell them that you received an order for 1000 plants like the one in front of them. What methods could be used to produce this number of plants? What problems/challenges for the grower would this present? Can biotechnology play a part in helping us respond to this request? How?

SCIENCE CONNECTIONS - QUESTIONS FOR INVESTIGATION

1. What is plant tissue culture?

2. How is it possible for tissue culture to produce large numbers of new plants?

3. Why are growth regulators used with tissue culture techniques?

4. Why is a sterile environment critical when performing tissue culture?

5. Why do certain parts of plants produce better tissue culture results than other parts of plants?

PURPOSE OF LAB AND STUDENT PERFORMANCE OBJECTIVES

The purpose of this experiment is to demonstrate the plant tissue culture process and compare the effects of growth regulators on the establishment of a new plant via tissue culture. Through this lab students should be able to:

1. Explain, from a plant science standpoint, how tissue culture works.

2. Describe the effects of growth hormones on tissue culture success.

3. Successfully perform plant tissue culture.

MATERIALS AND EQUIPMENT

- Alcohol (rubbing)
- Apple seeds
- Cheesecloth
- Bleach solution (10 percent chlorox)
- Liquid dish detergent
- Clear plastic bags (large)
- Sterile water (can be made by boiling 5-10 minutes)
- Razor blades or scalpels
- Prepared tissue culture salts medium (available from science supply stores, some contain growth regulators)
- Test tubes
- Petri dishes

PROCEDURES

1. All equipment used in this experiment must be sterilized by autoclaving. This process can be accomplished by using a pressure cooker at the same rate used for cooking meat or taken to a place that as an autoclave. This should be done by the teacher prior to the lab.

2. Prepare tissue culture medium (agar) according to directions and place in test tubes (at least six are needed).

3. Wash hands thoroughly to the elbows with soap and rinse, but do not dry. Swab hands and workplace with 70 percent alcohol (ethanol).

4. Extract six seeds from an apple, wrap in cheesecloth, place in a plastic bag and soak for five minutes in 10 percent Chlorox solution.

5. Rinse the seeds for five minutes in sterile water to wash away the bleach.

6. Scrape the seed coat from three apple seeds using a single edge razor blade. Be careful not to cut the pointed end (embryo) of the seed).

7. Soak the scraped seeds in ten percent Chlorox solution for five minutes and rinse in sterile water for five minutes.

8. Transplant the seeds onto sterile agar, putting one seed in each test tube.

9. Observe the test tubes for five days and record your observations.

10. Prepare tissue culture medium according to directions.

11. Remove one of the shoots from the agar and place in the rooting culture medium.

12. After roots are formed, transfer the plant to sterile soil.

13. Put transplanted plant into plastic bag to keep the shoots from drying out.

14. Observe growth of the plant.

DATA SUMMARY AND ANALYSIS

Have students observe growth in each of the six test tubes for one to two weeks. Data observations taken every two days should be recorded. The focus should be on both qualitative and quantitative data.

ANTICIPATED FINDINGS

Apple seed coats contain compounds which inhibit seed germination until the seed is stored in the cold. This mechanism prevents seeds from sprouting in early autumn and being killed by the winter cold. The dormancy of seeds is broken by the cold and seeds germinate in the spring. If the apple used in this experiment has been exposed to sufficient cold temperatures to break dormancy, then the seeds with coats will not germinate while the seeds without coats should germinate in a few days. If the apple was cold stored for more than a few weeks, then both seeds should germinate at about the same rate.

IDEAS FOR ADDITIONAL EXPERIMENTS

1. Tissue culture can be achieved using other plant parts besides shoots which have been started from seed. You may wish to replicate steps 10 - 14 of the experiment using shoot tips or root tips.

2. Compare the success of the tissue culture as the culture medium is varied.

3. Examine the success of tissue culture using a variety of plant species.

UNDERLYING SCIENCE CONCEPTS

A. <u>Relevant Science Concepts</u>

Biotechnology
Cell differentiation
Cloning
Propagation
Reproduction
Totipotence

B. Key Terms

1. Advantitious growth - growth of new shoots, roots, buds, or leaves from unusual locations.

2. Agar - a gel high in sugar concentration derived from certain algae.

3. Callus - an unorganized, proliferating mass of cells.

4. Clone - plants produced asexually from a single plant.

5. Explant - the part of the plant that is removed and placed in tissue culture.

6. In vitro - in glass.

7. Micropropagation - plant propagation by tissue culture.

8. Plantlets - small plants developed from tissue culture that are capable of developing into complete plants.

9. Sterile - a bacteria and fungus-free condition.

10. Subculture - a group of cultured cells or tissues that is transferred to a fresh medium.

11. Tissue culture - the aseptic growth of cells, tissues, or organs in artificial media.

12. Totipotence - the capability of a single cell to develop into an entire plant under proper conditions.

C. Relevant Principles of Plant Tissue Culture

1. Each plant cell has all the genetic information it needs to reproduce and envelop into an entire plant. Plants regenerated through tissue culture are called clones, because they have the same origin and are identical to the parent plant.

2. Actively, growing plant parts, such as root and shoot tips, developing leaves, lateral shoots, and seed embryos, work the best for tissue culture. Although almost any plant tissue can be used, cells from young tissue have been found to proliferate better.

3. The explant should be small to ensure fairly homogenous cells. The first form of growth of the tissue culture is called a callus, which is generally formed by placing the explant in contact with a selected culture medium. Important characteristics of the growing

medium include salt mixture, solidity, pH, and hormone concentration. Optimum growth occurs at 25 to 28 C. Growth and differentiation of the explant depend primarily upon the culture medium.

4. Cytokinin is a plant growth hormone that promotes cell division. The proportion of the growth hormones IAA (an auxin) and cytokinin determine whether roots and/or shoots develop from the callus. A high cytokinin to auxin ratio promotes shoot development, while a high auxin to cytokinin ratio promotes root development.

5. The steps involved in tissue culture are (a) establishment of an aseptic culture (explantation), (b) multiplication of cells in the explant tissue (proliferation), and (c) preparation of the new plant for existence outside of the culture (acclimation and establishment).

6. Plants possess a unique capability to regenerate not only tissues and organs, but also entire plants.

7. New growth is usually initiated in meristematic tissue. Cells in this tissue differentiate into leaves, stems, roots, etc.

8. Some differentiated cells (usually parenchyma cells) are able to revert to a meristematic or undifferentiated state and to initiate growth of new and different tissue. Plant tissues normally respond to a wound by producing a mass of parenchyma cells, called a callus. These dedifferentiated cells often account for adventitious growth.

9. New plant material started from tissue culture develops smaller than normal leaves, a process not well understood. However, after the tissue culture plantlets are rooted and have grown in soil for a short time, they produce normal size leaves.