

Lesson A3–6

Propagating Plants by Tissue Culture

Unit A. Horticultural Science

Problem Area 3. Plant Propagation

Lesson 6. Propagating Plants by Tissue Culture

New Mexico Content Standard:

Pathway Strand: Plant Systems

Standard: I: Apply principles of anatomy and physiology to produce and manage plants in both a domesticated and natural environment.

Benchmark: I-C: Explain and use basic methods for reproducing and propagating plants.

Performance Standard: 4. Describe the principles of plant micro-propagation. **5.** Apply principles and practices of biotechnology to plant propagation.

Student Learning Objectives. Instruction in this lesson should result in students achieving the following objectives:

1. Discuss the importance of tissue culture.
2. Discuss a tissue culture method of propagation used in the greenhouse industry.

List of Resources. The following resources may be useful in teaching this lesson:

Recommended Resources. One of the following resources should be selected to accompany the lesson:

Osborne, Edward W. *Biological Science Applications in Agriculture*. Danville, Illinois: Interstate Publishers, Inc., 1994.

Schroeder, Charles B., et al. *Introduction to Horticulture*, Third Edition. Danville, Illinois: Interstate Publishers, Inc., 2000.

Biondo, Ronald J. and Dianne A. Noland. *Floriculture: From Greenhouse Production to Floral Design*. Danville, Illinois: Interstate Publishers, Inc., 2000.

Other Resources. The following resources will be useful to students and teachers:

Osborne, Edward and Jeff Moss. *Biological Science Applications in Agriculture: Plant Science*. Urbana-Champaign, Illinois. Agricultural Education University of Illinois, 1990.

Skirvin, Robert M. and Ken D. McPheeters. *Plant Tissue Culture: Sterile Technique*. Urbana, Illinois: Vocational Agriculture Service Publication, AU 8030, 1997.

Plant Tissue Culture Techniques Video. San Luis Obispo, California: Vocational Education Productions, 1990.

Carolina Biological Supply Company, 2700 York Road, Burlington, North Carolina 27215. Phone: (800) 227-1150.

List of Equipment, Tools, Supplies, and Facilities

Writing surface

Overhead projector

Terms. The following terms are presented in this lesson (shown in bold italics):

Auxins

Callus

Cytokinins

Explants

Plantlet

Sterile agar medium

Sterile technique

Tissue culture

Interest Approach. Use an interest approach that will prepare the students for the lesson. Teachers often develop approaches for their unique class and student situations. A possible approach is included here.

Bring an African violet plant into class. Discuss with students how a commercial grower of violets would fill an order for 1000 plants identical to the plant in the classroom. What methods could be used to produce this number of plants? What problems or challenges for the grower would this present? Discuss how tissue culture can play a part in solving this propagation problem.

Summary of Content and Teaching Strategies

Objective I: Discuss the importance of tissue culture.

Anticipated Problem: What is tissue culture and why is it important?

- I. **Tissue culture** is the practice of growing plant cells on artificial media.
 - A. Tissue culture involves the culture or growing of small pieces of plant tissue. It is performed on an artificial medium under sterile conditions. Foliage plants, pot plants, and cut flowers are propagated by tissue culture methods.
 - B. There are several advantages to tissue culture over other methods of propagation. Tissue culture techniques allow:
 1. Large numbers of plants to be produced from a single plant in a relatively small space in a short period of time. This reduces growing space, labor and plant maintenance requirements.
 2. Viruses and other systemic diseases are eliminated by propagating the quickly dividing cells of the shoot tip.
 3. The grower is able to produce plants with identical flowers.
 4. Horticultural cultivars to be improved by selecting plants, which vary slightly from the mother plant. Examples are leaf shape, disease resistance, growth habit and flower color.
 5. The growth of genetically engineered plant cells.

Discuss the importance of tissue culture to the commercial grower. Show students as many examples as you can find of plants that are propagated by tissue culture. Have students read about tissue culture in the Biological Science Applications in Agriculture textbook.

Objective 2: Describe a tissue culture method of propagation used in the greenhouse industry.

Anticipated Problem: What process is used for tissue culture propagation?

II. The tissue culture propagation process can be defined in four main stages.

A. In the first stage, small pieces of plant material, called **explants**, are carefully removed from the parent plant. Explants are obtained from the actively growing part (shoot tips, sections of leaves, stems and roots, embryos etc.) of a desired plant. The explants are cleaned and placed on sterile agar medium in glass bottles or test tubes. The **sterile agar medium** is a gel that contains water, sugars, nutrients, and plant hormones to support and promote plant growth. Plantlets grown on the sterile agar medium have unusually small parts. Their tiny leaves, stems, and roots make tissue culture possible.

In stage two, the cells of the explants multiply in one of two ways:

1. The cells may form a **callus**, which is a group of cells with no particular function. Supplied with the correct hormones in the medium these callus cells can develop into a normal plant.
2. The explant may produce many new explants if **cytokinins**, hormones responsible for cell division and differentiation, are placed in the medium. Cytokinins encourage the increase in the number of buds on the explants to six or more per shoot. Each bud is capable of becoming a plant and producing more buds. Branching occurs as these buds develop into plant shoots or **plantlets**. These plantlets are divided and transferred to new containers. In this way, a single explant can produce millions of plantlets in a year.

When the plantlets have developed they are ready for the third stage of during which they form roots. The shoots are transplanted to another medium that contains auxins. **Auxins** are plant growth hormones that promote root formation.

In stage four the plantlet is prepared for normal growing conditions. The plantlet is removed from the glass container. They are divided, planted in sterile growing medium, and placed in a greenhouse. Care must be taken to acclimatize the plants to their new environment.

B. One of the most important aspects of tissue culture is sterile technique. **Sterile technique** is the maintenance of an environment that is free of bacteria, fungi and viruses. Sterilization of agar media is essential. In addition, the slightest air movement can stir spores of bacteria and fungi. Special sterile workstations are used when possible however, plastic bags which are sterile if unused may be substituted with some success.

Cleaning of the plant before removal of the explant is usually accomplished by a brief soaking in a bleach solution, followed by a rinse with sterile water. The tissue culture agar medium and other materials used to prepare and place the explant must be sterilized.

Cultures are transferred from one container to another at various stages in their development. This transfer must occur under sterile conditions to prevent contamination by microorganisms. Sterilized equipment must be used for these transfers.

Several science supply companies have excellent plant material, tissue culture equipment, and agar growth media available. The BSAA curriculum and textbook also include activities on propagating plants through tissue culture. The Activity Manual for Introduction to Horticulture is another source for activities.

Review/Summary. Use the student learning objectives to summarize lesson. Ask the students to explain the response to the anticipated problem of each objective. Student responses can be used to determine which objectives need further review.

Application. Application may involve the following student activity. Students may propagate African violet, fern, Hosta, miniature rose or lily plants. Commercial tissue culture propagation media is readily available for classroom use.

Evaluation. Evaluation should focus on student achievement of the objectives of this lesson. Various techniques can be used, such as student performance on the application activity. The self-check or review section at the end of each chapter in the suggested references will be helpful. A sample written test is attached.

Answers to Sample Test:

Part One: Matching

1. h 2. e 3. b 4. g 5. d 6. a 7. c 8. f

Part Two: Completion

1. auxins, cytokinins
2. sterile
3. two
4. glass containers
5. answers will vary: violets, daylilies, Hostas, chrysanthemums, and orchids

Part Three: Short Answer

1. Plant tissue culture involves the culture or growing of small pieces of plant tissue. It is performed on an artificial medium under sterile conditions.
2. Plant growth hormones have a dramatic effect on plant cell growth and differentiation. Plant hormones determine whether the explant will develop root and/or shoots.

Test

Lesson A3–6: Propagating Plants by Tissue Culture

Part One: Matching

Instructions. Match the term with the correct response. Write the letter of the term by the definition.

- | | | |
|----------------------|------------------------|-------------------|
| a. auxin | d. callus | g. cytokinin |
| b. explant | e. plantlet | h. tissue culture |
| c. sterile technique | f. sterile agar medium | |

Part Two: Completion

- _____ 1. The method of asexual propagation that involves the growing of small pieces of plant tissue.
- _____ 2. Shoots that develop from buds in tissue culture.
- _____ 3. Small pieces of plant material used in tissue culture.
- _____ 4. Plant hormone responsible for cell division and differentiation.
- _____ 5. A group of plant cells with no particular function.
- _____ 6. A plant hormone that promotes cell elongation and root formation.
- _____ 7. Maintenance of an environment that is free of bacteria and fungi during propagation.
- _____ 8. Sterile gel that may contain minerals, sugar, vitamins and growth regulators that are necessary for plant and cell growth.

Part Two: Completion

Instructions. Provide the word or words to complete the following statements.

- Two growth regulators used with tissue culture techniques are _____, and _____.
- _____ environment is free from disease causing bacteria and fungi.
- Plantlets are divided and encouraged to multiply in stage _____.
- In stage four, plantlets are removed from the _____ and planted into sterile growing medium.

5. Two examples of plants being commercially propagated through tissue culture are _____ and _____.

Part Three: Short Answer

Instructions. Provide information to answer the following questions.

1. What is plant tissue culture?

2. Why are growth regulators used with tissue culture technique?