Plant Tissue Culture: Embryo Isolation and Tissue Culture Initiation

SYNOPSIS FOR CORE EXPERIMENT
Students will excise the embryo of a plant seed to compare the growth of complete and partial embryos in vitro on a nutritionally complete medium. They will form hypotheses and design experiments to analyze the effect of various physical and/or chemical factors on the growth of the embryo.

APPROPRIATE BIOLOGY LEVEL
Advanced or highly motivated introductory level students.

TEACHER PARTNERS
Joyce Dean
Crossland High School
6901 Temple Hills Road
Temple Hills, MD 20706

Lynn Harden
DuVal High School
9880 Good Luck Road
Lanham, MD 20706

SCIENTIST PARTNER
James Saunders, Ph.D.
Research Biochemist
United States Department of Agriculture
CSL, Building 50, Room 100
Beltsville, MD 20705

Note: This investigation has been modified from the following sources, and is reprinted here with permission: Herbert, R. & Thompson, D.J. (1993). Culturing Plants from Embryonic Plant Tissue. Princeton, NJ: 1993 Woodrow Wilson Biology Institute.

Thank you to Paul Bottino (University of Maryland), Lisa Darno (Carolina Biological), and Carol Reiss (Cornell University) for their assistance.

Note to Teachers: Information below is given for the Core Experiment. Additional information needed for each variation of the Core Experiment may be found beginning on page 250. For a specific variation, check the At-A-Glance Map.

GETTING READY
See sidebars for additional information regarding preparation of the lab.

OBJECTIVES FOR CORE EXPERIMENT
At the end of this lab, students will be able to:
• Identify the stages of embryo development.
• Excise an embryo from a plant seed.
• Inoculate a sterile, nutritionally complete growth medium with the excised embryo.
• Observe and record the growth of a plant embryo in vitro.

MATERIALS NEEDED
For teacher preparation, you will need the following for a class of 24:

- 4.0 L sterile, distilled water
- 42.4 g M&S Basal Tissue Culture Medium with sucrose and agar (Sigma M9274) or prepared M&S medium (Carolina #195747)
- 1 hot plate with magnetic stirrer
- 80 15 x 100-mm sterile, disposable petri dishes
- 1 autoclave
- 1 L 10% household bleach solution
- 750 mL rubbing alcohol or 400 mL 95% ethanol
- 1 mL liquid dishwashing detergent with antibacterial agent
- 2 to 4 10-gallon aquaria

LENTH OF LAB
A suggested time allotment follows:
Day 1 (45 minutes)
• Introduce the lab and practice excising embryos on a Monday or Tuesday.
Day 2 (45 minutes)
• Practice excising embryos using aseptic technique.
Day 3 (30 to 45 minutes)
• Inoculate cultures.
Days 4 to 8 (15 to 30 minutes)
• Observe and record data.
Day 9 (45 minutes)
• Analyze data and draw conclusions.

PREPARATION TIME REQUIRED
60 minutes
• Locate and/or gather glassware, fresh supermarket corn, and other equipment.
15 minutes
• Prepare the agar plates.
90 minutes
• Prepare the culture medium, dispensing medium into the culture vessels and autoclaving the vessels.
30 minutes
• Prepare solutions.
TEACHING TIPS

**Seeds**
- If fresh supermarket corn is unavailable year-round in your area, you may want to schedule this laboratory for early fall rather than use dry corn. Alternatively, soak dry corn seeds for 24 to 48 hours before the laboratory.
- Dry seeds must be surface-sterilized by briefly soaking in a 10% bleach solution. The amount of time allowed for surface sterilization of the seeds will vary between 2 to 6 minutes, depending upon the thickness of the seed coat. For example, bean seeds will take less time than corn seeds. Then, soak for an appropriate amount of time to soften the seed coat. Corn should be soaked 36 to 48 hours; pole or lima beans, 3 to 4 hours.
- Lysol® is not recommended for use as a disinfectant on surfaces coming into contact with the embryo, as it can destroy the delicate tissues.
- Students need to practice recognizing and excising the embryo from the seed prior to the actual lab using this procedure.

**Medium**
- Sigma Chemical Company recommends that the entire contents of the plant growth medium bottle be used after opening due to the hygroscopic nature of the dry medium. However, with careful handling and storage of unused medium under refrigeration in an airtight container, dry medium may be kept for up to three months. Prepared and autoclaved medium will keep for a week under refrigeration. Medium may be microwaved to liquefy to pour additional plates.
- If petri dishes are used, pour the medium into plates in a transfer cabinet.
- If test tubes or other narrow-mouthed vessels are used, the surface area for explants may be increased by allowing the medium to solidify at a slant.
- If 1 L of medium or approximately 66 petri dishes is more than you need for the Core Experiment, consider autoclaving 15 mL aliquots in culture tubes. The agar can be melted later in a hot water bath, and students can pour their own individual plates.

**SAFETY PROCEDURES**
- Wear safety goggles and lab coats/aprons at all times.
- Wear protective gloves when wiping surfaces with bleach or alcohol, and while mixing solutions.
- Keep open containers of alcohol away from the same work area as a flame.
- Scalpels/razor blades should be used in a direction away from the body when excising the embryo.
- Dispose of sharp objects, such as razor blades or broken blades, in proper containers.
- Determine if students are allergic to latex, if gloves are used.
- Re-autoclave all medium to destroy possible pathogens introduced by accident during transfer of the embryo before cleaning glass petri dishes or disposing of plastic ones after completion of the experiment.
- If you use flame sterilization, do not put the flame source inside the transfer cabinet. Heat may break the glass or discolor plastics.
- Exercise extreme caution when wearing gloves to flame sterilize.
- Do not place the flaming tools back into the alcohol jar to extinguish flames.

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You will need the following for each group of two to four students in a class of 24:

- 2 to 4 60-cm x 90-cm sheets of heavy-weight, clear plastic
- 1 pair of scissors
- 1 roll of duct tape
- 1 artificial lighting system with 24-hour timer
- 1 250-mL spray bottle of 70% ethanol or 10% household bleach
- 1 sterile petri dish with M&S Basal Medium with sucrose and agar
- 1 sterile petri dish with water agar
- 1 permanent marking pen
- 1 bottle of antibacterial soap
- 1 fresh supermarket ear of yellow corn (*Zea mays*) or dry field corn seeds
- 50 mL sterile water
- 25 mL 10% household bleach-soap solution
- 3 sterile, disposable petri dishes
- 1 sterile film canister or 1 250-mL beaker with aluminum foil cover
- 1 timer
- 1 shallow pan
- 1 sterile surgical scalpel, Bard-Parker No. 3 handle
- 1 sterile No. 10 blade
- 1 sterile No. 11 blade
- 2 sterile forceps, 11-cm fine point
- 1 500-mL beaker
- 2 sterile paper towels
- 2 2.5 x 15-mm Parafilm™ or Petri Seal™ strips
DIRECTIONS FOR SETTING UP THE LAB

Three to five days before
Prepare the tissue culture medium, dispense, and sterilize as described in the following section.

M&S Basal Medium with Sucrose and Agar
1. Stir 1 L of distilled water while adding 42.4 g powdered medium.
2. Heat until the solution is clear, stirring continuously. Do not boil!
3. Autoclave in a container no more than half-full for 15 minutes at 15 pounds per square inch (psi).
4. Allow the medium to cool slightly before pouring approximately 15 mL into each of 66 petri dishes.
Alternatively, if the medium is purchased already prepared, heat in hot water bath until melted.

Water Agar
1. Stir 200 L of distilled water while adding 1.6 g of agar.
2. Heat until the solution is clear, stirring continuously. Do not boil!
3. Autoclave in a container no more than half-full for 15 minutes at 15 pounds per square inch (psi).
4. Allow the medium to cool slightly before pouring approximately 15 mL into each of 66 petri dishes.

One day before
Disinfectant Solutions
10% household bleach (0.5% sodium hypochlorite*) solution
Combine 100 mL household bleach with 900 mL distilled water.

70% Ethanol
Use 70% rubbing alcohol or combine 370 mL 95% ethanol with 130 mL distilled water.

Surface Sterilization Solution
10% Household Bleach-soap (0.5% sodium hypochlorite*) Solution
1. Combine 100 mL household bleach with 900 mL distilled water.
2. Add 2 to 3 drops dishwashing liquid with antibacterial agent.
(*Note: Commercially prepared bleach is usually a 5.25% sodium hypochlorite solution. The dilutions created by each solution above are given in parentheses.)

TEACHER BACKGROUND

Content Information
Plant tissue culture generally is described as the aseptic, in vitro growth of any plant part on or in a nutrient medium. The medium may be solid or liquid, depending upon the application. The underlying concepts of the techniques initially were developed in the late 1800’s and early 1900’s. The German plant physiologist G. Haberlandt refined them in 1902.

In any plant tissue culture experiment:
• A plant section called the explant is removed from the original plant to eliminate any cell, tissue, and/or organ interactions of the intact plant.
• The explant is grown in a chemically defined and physically controlled environment.
• An aseptic environment is maintained to eliminate the effects of external plant pathogens on the development of the explant.

TEACHING TIPS
ml. aliquots for later use.
• Depending upon the level of the students in the class, the teacher may wish to prepare the medium, dispense it into the tissue culture vessels, and autoclave the prepared vessels in advance. Alternatively, if measuring pH adjustment, dispensing of medium, and autoclaving are skills to be taught and reinforced, this may be done as a preparatory lab exercise. This will require an additional 45 minutes and additional time for autoclaving.
• Always use a container twice the size of the final volume of medium being prepared.
• Add the powdered medium while stirring the water.
• If possible, use double distilled water for the medium preparation.
• The medium can be adjusted to a specific pH by using dilute solutions of KOH or HCl.
• After the medium is prepared, pour the medium into the containers. Autoclave any nonsterile, glass containers with medium for 15 minutes at 15 pounds per square inch (psi). Be sure the containers can be autoclaved safely. TAKE CARE NOT TO “OVERCOOK”.

Disinfectant Solutions
• Mix 1 to 2 L quantities of the ethanol and the household bleach solutions in advance to reduce student preparation time.

Transfer Chamber (optional)
• In the absence of a laminar flow hood for excision and implanting, minimize air flow in the work area by using one of the following techniques:
  - Turn an aquarium sideways, wash with warm soapy water, and then wipe down thoroughly with 10% bleach solution. The opening can be covered with a heavy-weight, clear plastic sheet that also has been cleaned and wiped with bleach solution.
  - Staple, sew, or tape together heavy-weight plastic over a hanging file folder support frame and wash with soapy water and bleach.
There have been many practical applications and extensions of Haberlandt’s original work. However, he was not successful particularly at growing explants on nutrient media, as it did not contain auxins, critical plant growth factors. Auxins were unknown at the turn of the century. More recent experiments have used improved methods of plant tissue culture as a way to:

- Maintain pure genetic traits in desirable plants by cloning, the technique of growing whole plants from plant sections.
- Insure development of the embryo in cases where the endosperm inhibits proper growth when the seed is planted in soil. This is true in several plant species that have evolved seeds which germinate only after environmental cues, such as fire, flooding, light exposure, or cold weather.
- Achieve faster growth of the embryo than if it were germinated in soil.

Plants used in tissue culture experiments are selected on the basis of the traits that are desirable to maintain and propagate. Seeds used for embryo studies tend to be readily available and have embryos that are easy to remove. For this introductory exercise in embryo excision, corn and/or bean seeds will be used as they are the easiest to manipulate.

Some facts about tissue culture that may be of interest to students are:

- Whole plants can regenerate from a single, undifferentiated cell.
- Researchers can manipulate explant tissue to produce a genetically altered plant using molecular biology, technology and genetic engineering.
- Plant tissue culture is not confined strictly to research labs in universities, but is an important tool used in agriculture and industry.

Pedagogical Information

The following is a chart of some concepts related to this lab and some student misconceptions of these concepts.

<table>
<thead>
<tr>
<th>Correct Concept</th>
<th>Misconception</th>
</tr>
</thead>
<tbody>
<tr>
<td>A seed is composed of differentiated tissue. Only the embryo develops into a new plant.</td>
<td>The entire seed grows into a plant.</td>
</tr>
<tr>
<td>Cotyledons are immature leaves of the plant embryo. The cotyledons of dicots, such as bean, no longer contain endosperm but still serve as food reserves. In monocots such as corn, endosperm is still present but separate from the cotyledons.</td>
<td>Both monocot and dicot cotyledons are immature leaves of the embryo that contain endosperm.</td>
</tr>
<tr>
<td>Seeds may be germinated in vitro and embryos grown on chemically defined media.</td>
<td>Seeds must be planted in soil in order to grow.</td>
</tr>
<tr>
<td>Specific plant hormones promote differential growth.</td>
<td>Fertilizers are the only chemicals that promote plant growth.</td>
</tr>
<tr>
<td>Seeds are living.</td>
<td>Seeds are nonliving.</td>
</tr>
</tbody>
</table>

INSTRUCTIONAL PROCEDURES FOR THE CORE EXPERIMENT

Introduction

This experiment is a natural extension of a study of plant structure and function. Possible ways to introduce the lab include:

- Introduce the topic of plant tissue culture using the information from the Teacher Background section by demonstrating the techniques of embryo excision and trans-
fer of the excised embryo into the culture vessel. A video camera setup or overhead projection transparencies and/or blackboard diagrams may be used to instruct an entire class in the method of embryo excision. Then, have individual students practice excising the embryos.

• Relate the lab to current topics in genetic engineering. Some questions you might ask are:
  - Cloning animals has been in headline news since the 1980's. Can an animal or a plant be cloned from a single cell to maturity? Guide student discussion of this topic with the following information: A plant can be cloned from a single nucleus of a mature cell. In animals, mammals usually cannot be cloned to maturity from a single cell with the exception of gametic cells. Ask students to research the original paper describing the sheep Dolly's cloning.
  - If so, what are the advantages of cloning plants as compared with propagating them from seeds?
  - Demonstrate the concept that each plant cell in a differentiated organism has all the genetic information for the production of a complete, mature organism. Some possible demonstrations include showing:
    - The growth of a potato plant from a potato “eye.”
    - The Kalanchoe daigremontiana (maternity plant) with plantlets growing from notches in the leaves. Ask students to compare the plantlets with the parent plant and to speculate on what will happen if the plantlets are removed and planted separately in soil.
  - Show students a corn embryo already growing in a nutritionally complete medium. Explain that the embryo was removed from its natural food source before it was placed on this medium. Ask students to:
    - Use their knowledge of the nutritional requirements of a growing plant to predict the components of the growth medium.
    - Predict how the plant would grow if a particular growth ingredient were left out of the medium, or a new ingredient, such as a hormone, were added to the medium.
  - Place two geranium plants side by side at each lab station. One should be grown using tissue culture (e.g. an infertile hybrid variety), and the other should be grown from seed. Have students observe the two plants and record their observations. Then, tell them that the method used to reproduce the two plants is different. Ask them to use their list of observations to determine the method of reproduction for each plant.
  - Introduce students to the influences of hormones on plants using one of the following demonstrations:
    - Show a plant that has grown after having the terminal bud removed and one that has not had the bud removed.
    - Show a cutting from a plant that was treated with a commercial rooting medium and one started at the same time but without the treatment.
  - Assign students to search the Internet for three sites on the World Wide Web (www) that contain information on plant tissue culture.

HYPOTHESIS GENERATION
The following discussion and activities are designed to elicit questions that students can transform into hypotheses. Generate a discussion about plant tissue culture using one or more of the following questions:

• Why fool with Mother Nature? Haven't plants been reproducing successfully on their own for millions of years?
• What are some ways that plant tissue culture could be used in space or in the biosphere?
• What nutritional and environmental conditions are required for plant growth in vitro?
• Can a complete plant grow from a partial embryo?
• How is plant tissue culture used in research?
• How can a plant in tissue culture be protected from pathogens?
• What are some similarities and differences between plant tissue culture and aquaculture?

Sample Hypotheses
• If an embryo is cut longitudinally and cultured on nutritionally complete medium, then each half will develop into a normal plant.
• If an embryo is cut transversely, then one half will develop only as a shoot and the other half will develop only as a root.

On the following pages are a sample hypothesis, procedure, and data analysis set with interpretation that students might develop for the Core Experiment. It is followed by a related test question and answer for teacher evaluation. This example has been included as a potential outcome of the activity and should not be given to the students. Students should develop their own hypotheses and procedures. Make sure they understand that there is not just one correct hypothesis, procedure, or data set. The Variations of the Core Experiment will give each team of students the opportunity to expand on the Core Hypothesis. Additional test questions are found on page 249.

Question
Is the embryo of a seed capable of growing without endosperm?

Core Hypothesis
If a corn embryo is excised from a seed and transferred to a nutritionally complete growth medium, then it will produce shoot and root growth comparable to an embryo excised with its endosperm intact.

Rationale
The culture container should provide the protection offered by the seed coat. If the medium is nutritionally complete, it should substitute for the endosperm.

Procedure
Work Area
1. Wipe the laboratory work bench and transfer chamber with 10% soap-bleach solution. (Note: Some of the following procedures were originally described by Green and Phillips, 1975.)
2. Allow the surfaces to air dry.
3. Obtain 1 sterile petri dish with M&S Basal Medium. Label it “without endosperm.”
4. Obtain 1 sterile petri dish with water agar. Label it “with endosperm.”

Excision of Corn Seeds and Embryo Extraction
1. Cut 3 to 4 corn kernels from a fresh, mature corn cob with a scalpel.
2. Make a small incision beneath 1 kernel very close to the cob in the row adjacent to where the kernels were removed in Step 1. Be careful not to cut the embryo. See Figure 1.
3. Carefully slit the crown of the corn kernel with a scalpel.
4. Gently squeeze the sides of the embryo between the thumb and index finger. The embryo should pop out. It is a cream-colored, oval-shaped hard tissue found in the softer endosperm tissue, approximately 1 to 2 mm in length. See Figure 2.

**Figure 1.** Removing a corn kernel from a fresh, mature cob.

**Figure 2.** Excising the embryo from a fresh corn kernel.
5. Lift up the top half of the petri dish containing the medium just enough to place the embryo directly on the medium while keeping the top half of the dish over the medium. Replace the top half of the dish immediately after placing the embryo.

6. After the first kernel is removed from the cob intact, additional kernels may be removed by simply wriggling them slightly with your fingers. See Figure 3.

![Figure 3. Removing additional kernels with fingers.](image)

7. Repeat Steps 3 to 5 to obtain 4 additional embryos.

**Surface Sterilization if Dry, Not Fresh Seeds Are Used (optional)**

1. After soaking the seeds in sterile water for 2 days, place 5 corn kernels into a film canister containing 70% ethanol or a 250-mL beaker containing 100 mL of 10% bleach solution.

2. Cover the container and gently swirl for 10 minutes.

3. Pour the bleach solution into a 500-mL beaker.

4. Rinse the kernels 3 times in 50 mL of sterile, distilled water. Use aseptic techniques. Pour the rinse water into the 500-mL beaker.

**Incubation and Observation**

1. Seal the edges of the petri dishes with a Parafilm™ strip or Petri Seal™ with the sterile surface next to the dish. Place in an environment with a temperature of 28 to 30°C under a 16-hour light/8-hour dark photoperiod. The cool, white fluorescent bulbs should be 20 to 25 cm away from the petri dishes.

2. Observe the changes in the embryos with and without endosperm for at least one week. Record your results and sketch the embryos. Indicate where most of the growth occurs and the type of growth.

3. Root and shoot growth may be measured aseptically by tracing their shapes with a thread on the surface of the closed petri dish and measuring the thread length.

4. Continue your observations by recording any changes in the developing embryos that are growing on the culture and water agar media.

5. At the end of the observation period, record the numbers of partial embryos showing shoot and/or root growth and compare them with the complete embryos.

6. Observe the changes in the embryos daily for one week. Measure and record root and shoot growth. Measure long, branched roots and estimate their lengths by using a 0.5-inch grid (Marsh, 1971). See Figure 4. Lay the roots in the lid of a petri dish. Add about 5.0 mL of water and tease the roots apart, so that they do not lie on top of one another. Invert the bottom of the petri dish and set it in the top so that it flattens the roots and keeps them from moving. Set the dish on the grid. Count every time a root crosses a vertical grid line, then count every time a root crosses a horizontal grid line. Add the 2 numbers and multiply by 10 to get the root length in millimeters.
SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data

**Table 1.** Growth of excised complete corn embryos on complete, artificial medium and embryos with endosperm on water agar after 5 days.

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Embryos with endosperm</th>
<th>Embryos with artificial medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Length of shoot (mm)</td>
<td>Length of root (mm)</td>
</tr>
<tr>
<td></td>
<td>Length of root (mm)</td>
<td>Length of shoot (mm)</td>
</tr>
<tr>
<td></td>
<td>Length of root (mm)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>28</td>
<td>71</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>59</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>54</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>43</td>
</tr>
<tr>
<td>5</td>
<td>17</td>
<td>57</td>
</tr>
</tbody>
</table>

Appropriate display for these data is a frequency histogram of the total growth for each treatment. It is adequate, however, to describe these data with the mean for each group. The appropriate analytical technique is the statistical t-test. Here, however, the results are so dramatically different, that no analysis is necessary.

Interpretation

There is no overlap in these data. Embryos on artificial medium consistently grew more than embryos with endosperm provided. The overall mean growth for embryos to include shoot and root growth on artificial medium at 231 mm was 5 times that of embryos with endosperm at 45 mm. It appears that fresh endosperm may inhibit the growth of corn embryos.
TEST QUESTION

One group of students recorded the following average growth measurements in the shoot and root of their excised corn embryos with and without endosperm.

Table 2. Growth of excised, complete corn embryos on M&S Basal Medium over 5 days.

<table>
<thead>
<tr>
<th>Day</th>
<th>With endosperm</th>
<th></th>
<th>With artificial medium</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot length (mm)</td>
<td>Root length (mm)</td>
<td>Shoot length (mm)</td>
<td>Root length (mm)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>8</td>
<td>8</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>18</td>
<td>18</td>
<td>53</td>
<td></td>
</tr>
</tbody>
</table>

A valid conclusion from their data would be:
A. The total root growth is greater than the total shoot growth regardless of the nutrient source.
B. The rate of growth was the same for both root and shoot with both nutrient sources.
C. The total shoot growth is greater than the total root growth regardless of the nutrient source.
D. The greatest amount of shoot and root growth occurred on Days 1 and 2.

STUDENT DESIGN OF THE NEXT EXPERIMENT

After the students have collected and analyzed these data from their experiments and shared results and conclusions with the class, encourage them to brainstorm ideas for experiments they could do next. They should think about questions that occurred to them as they conducted the Core Experiment. Ask them what quantifiable experiments could be done based on observations they have made. Have students return to their experimental lab groups to share ideas before writing their proposals. Questions students might consider include the following:
1. What happens if only a partial embryo is grown on nutrient medium?
2. Does the orientation of the embryo or how it is cut determine what tissues develop?
3. Are there home “recipes” used for plant tissue culture?
4. How do variables, such as light and temperature affect plant growth?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

These are possible ways to modify this specific activity for students who have special needs, if they have not already developed their own adaptations. General suggestions for modification of activities for students with disabilities are found in the AAAS Barrier-Free in Brief publications. Refer to page 15 of the introduction of this book for information on ordering FREE copies of these publications. Some of these booklets have addresses of agencies that can provide information about obtaining assistive technology, such as Assistive Listening Devices (ALDs); light probes; and talking thermometers, calculators, and clocks.

Blind or Visually Impaired

Investigations that require sterile technique in growing and transferring plant or animal material are not suitable for students who are visually impaired. This investigation takes considerable time and would be challenging to impossible to keep a
student who is just “sitting by” interested in the work. The blind student and his project may be better located in a greenhouse, preparation room, or some place away from the area where students are using sterile techniques.

**Deaf or Hard-of-Hearing**
Modifications of this experiment for students who are hearing impaired or deaf are not necessary. As for most investigations, the main criterion is the ability of the student to communicate effectively with the instructor and with the laboratory partners.

**Gifted**
- Additional research will be required for students to predict the correlation between the type of hormone present in the growth medium and the differential growth of shoot or root.
- Embryos of other tissues, such as cucumber or melon, will provide a greater challenge for excision with this group of students.

**Mobility Impaired**
This is an investigation not suitable for students who are manually impaired.
- Invite other students who are mobility impaired to hold a conference with the instructor to determine the suitability of this investigation.

**ADDITIONAL TEST QUESTIONS**
Test questions for the Core Experiment also may include the following:
1. What part of the complete seed performs the same function as the growth medium in the culture vessel?
   A. seed coat  
   B. endosperm  
   C. embryo  
   D. cotyledons
2. Not all embryos that were transplanted grew equally well. What are some factors that might explain this observation?
3. You are planning to grow embryos using agar cultures in the greenhouse. What are some factors that you must deal with to be successful?

**REFERENCES AND SUGGESTED READINGS**
VARIATION 1
The Effect of Orientation of Embryos on Development of Roots and Shoots on Nutritionally Complete Growth Medium

SYNOPSIS
Students will compare the types and numbers of roots and/or shoots developed in fresh corn embryos placed vertically and horizontally on nutritionally complete growth medium.

ADDITIONAL MATERIALS
- 10 fresh corn embryos

HYPOTHESIS GENERATION

Question
Does the orientation of the embryo affect the amount of time required for development?
Sample Hypothesis
If corn embryos are oriented vertically with the radical end of the embryo in the medium rather than horizontally, then the roots and shoots will develop more quickly.

Rationale
Vertical orientation places the corn embryo in the position that it is expected to grow. No time is necessary to reorient.

Sample Experimental Procedure
Transfer to culture vessels
1. Obtain 2 sterile petri dishes. Label one “horizontal” and the other “vertical.”
2. Follow the procedure of the Core Experiment for sterilizing the surface of the corn embryos.
3. Aseptically, orient 5 corn embryos horizontally on the petri dish of M&S Basal Medium labeled “horizontal.” See Figure 5.

![Figure 5. Corn embryos oriented horizontally.](image1)

4. Prepare the petri dishes and incubate the embryos with the light regime of the Core Experiment.
5. Aseptically, orient 5 corn embryos on the petri dish of M&S Basal Medium labeled “vertical.” As you transfer these embryos, slit the agar with the scalpel and gently place each embryo vertically in a slit.

![Figure 6. Corn embryos oriented vertically.](image2)

6. Prepare the petri dishes and incubate the embryos with the light regime of the Core Experiment.
7. Observe the changes in the “horizontal” and “vertical” embryos at room temperature for at least 1 week. Record your results and sketch the embryos, indicating where most of the growth takes place, and the type of growth that occurs.
8. Continue your observations by recording any changes in the developing embryos that are growing on the culture medium.
9. At the end of the observation period, record the numbers of “vertical” embryos showing normal shoot and/or root growth, as compared to the “horizontal” embryos.
10. Display and analyze these data.
SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 3. Effect of embryo orientation on growth of excised embryos on nutritionally complete medium. Values reported are total lengths after 7 days of growth.

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Vertical</th>
<th></th>
<th>Horizontal</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Root</td>
<td>Shoot</td>
<td>Root</td>
</tr>
<tr>
<td></td>
<td>length (mm)</td>
<td>length (mm)</td>
<td>length (mm)</td>
<td>length (mm)</td>
</tr>
<tr>
<td>1</td>
<td>340</td>
<td>70</td>
<td>650</td>
<td>91</td>
</tr>
<tr>
<td>2</td>
<td>740</td>
<td>106</td>
<td>1210</td>
<td>105</td>
</tr>
<tr>
<td>3</td>
<td>310</td>
<td>108</td>
<td>520</td>
<td>79</td>
</tr>
<tr>
<td>4</td>
<td>230</td>
<td>60</td>
<td>560</td>
<td>107</td>
</tr>
<tr>
<td>5</td>
<td>350</td>
<td>81</td>
<td>520</td>
<td>97</td>
</tr>
<tr>
<td>6</td>
<td>470</td>
<td>94</td>
<td>630</td>
<td>81</td>
</tr>
<tr>
<td>7</td>
<td>210</td>
<td>93</td>
<td>860</td>
<td>102</td>
</tr>
<tr>
<td>8</td>
<td>250</td>
<td>62</td>
<td>520</td>
<td>97</td>
</tr>
<tr>
<td>9</td>
<td>930</td>
<td>113</td>
<td>1240</td>
<td>95</td>
</tr>
<tr>
<td>10</td>
<td>290</td>
<td>100</td>
<td>590</td>
<td>93</td>
</tr>
</tbody>
</table>

Total plant growth for each replicate and analyze the totals with a statistical t-test. An appropriate display of these data would be a frequency histogram.

TEST QUESTION

1. A group of students tested the effect of orientation on growth of excised corn embryos. They collected growth measurements daily. Their results are presented in Graph A. Write a conclusion for their experiment.

2. Which grows faster in tissue culture: corn embryos laid on the surface of the medium, or corn embryos inserted vertically into the medium? Give two possible reasons for your answer.

Graph A. Average growth of 5 corn embryos with different initial orientations.

Answer to Test Question

1. Embryos oriented horizontally consistently grew better than embryos oriented vertically.
2. Horizontally. Possible reasons include: gravity influences embryo growth and embryos are damaged by vertical implantation.
SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL Blind or Visually Impaired

- Have students compare roots and shoots on young plants grown in plain and hydroponic solutions. This could be done by sighted students as well.

VARIATION 2
The Effect of Temperature on the Growth of Corn Seeds on a Nutritionally Complete Medium

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 2.

SYNOPSIS
Students will compare the growth of corn embryos in vitro at different temperatures.

ADDITIONAL MATERIALS NEEDED
You will need the following:
- 1 refrigerator at 4°C
- 15 corn kernels
- 3 lights with timers
- 3 controlled temperature locations
- 3 petri dishes of M&S Basal Medium

HYPOTHESIS GENERATION

Question
How does temperature affect the growth of the embryo?

Sample Hypothesis
If corn seed embryos are grown at 28 to 30°C on a nutritionally complete growth medium, they will develop more quickly than embryos grown at 4°C on a nutritionally complete growth medium.

Rationale
Most plants begin to grow as the temperature increases in the spring even if they received adequate moisture when the temperature was decreasing in the fall.

Sample Experimental Procedure
1. Prepare 15 fresh corn embryos as directed in the Core Experiment.
2. Aseptically, transfer 5 embryos to each of 3 petri dishes containing M&S Basal Medium.
3. Incubate the embryos with the light regime of the Core Experiment at three different temperatures between 4°C (refrigerator) and 30°C.
4. Store dishes and make observations as directed in the Core Experiment.

TEACHING TIP
The temperature related change in growth resembles a response curve expected as a result of temperature effects on enzyme activity. Students can demonstrate that the cold, incubated plants have been stunted because their enzymes are inactive by bringing the petri dishes into the warm location and continuing the incubation. They should find that the growth rate increases in the new conditions.
### SAMPLE DATA ANALYSIS AND INTERPRETATION

#### Sample Data

**Table 4.** Effect of room temperature on growth of isolated corn embryos. Embryos were grown for 7 days.

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Total production (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4°C</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

Use the means to describe these data. Display the results as a bar graph with the temperature as the independent variable on the x-axis and the mean length for each treatment as the dependent variable on the y-axis. Use standard deviation for error bars.

#### TEST QUESTION

1. How does temperature affect the growth of the excised embryo?
2. Design an experiment that would test whether light or temperature variations have the greatest effect on the total growth of excised embryos.

#### SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

**Blind or Visually Impaired**

- Have students study the effect of temperature on the growth of seedlings in a nutritionally complete hydroponic solution. This may be done by sighted students as well.

#### VARIATION 3

**The Effect of Kinetin and Adenine on the Growth of Corn Embryos in Vitro**

**Note to Teachers:** In addition to the information found in the Core Experiment, the following material has been provided for Variation 3.

**SYNOPSIS**

Students will compare the growth of corn embryos on a nutritionally complete medium with and without kinetin plus adenine added.

**ADDITIONAL MATERIALS NEEDED**

For the teacher preparation you will need the following for a class of 24:

- 10 mL 1N NaOH
- 200 mL distilled H$_2$O
- 2 mL 1N HCl
- 1 pH meter
- 6 sterile petri dishes
You will need the following for each group of two to four students in a class of 24:

- 1 sterile petri dish of Adenine Amended M&S Basal Medium
- 1 sterile petri dish of M&S Basal Medium
- 10 fresh corn kernels

**DIRECTIONS FOR SETTING UP THE LAB**

**Kinetin Stock Solution**
1. Dissolve 0.01 g of kinetin in 10 mL of 1N NaOH.
2. Add 8 mL distilled H₂O.
3. Store at 0°C.

**Adenine Stock Solution**
1. Dissolve 0.08 g of adenine in 2 mL of 1N HCl.
2. Add 8 mL distilled H₂O.
3. Store at 0 to 5°C.

**Adenine-Kinetin Amended Medium**
1. Add 4.24 g of M&S Basal Medium with sucrose and agar to 98 mL of room temperature distilled H₂O while gently stirring the water.
2. Stir continuously and heat until the solution becomes clear. Do not boil!
3. Add 1 mL Kinetin Stock Solution.
4. Add 1 mL Adenine Stock Solution.
5. Adjust the pH to 5.7 with 1N NaOH or 1N HCl.
6. Autoclave for 15 minutes at 15 pounds per square inch (psi).
7. Dispense to 6 sterile petri dishes.

**HYPOTHESIS GENERATION**

**Question**
What effect does the combination of the hormones kinetin and adenine have on embryo growth?

**Sample Hypothesis**
Fresh corn embryos grown in a nutritionally complete medium with kinetin plus adenine added will develop more shoots, as compared with embryos grown in a nutritionally complete medium without kinetin plus adenine added.

**Rationale**
A differential development favoring shoots has been shown for this kind of amendment with tobacco.

**Sample Experimental Procedure**
1. Prepare 10 corn embryos for tissue culture as in the Core Experiment.
2. Aseptically, transfer 5 embryos to M&S Basal Medium and 5 embryos to Adenine-Kinetin Amended Medium.
3. Incubate the embryos as in the Core Experiment.
4. After several days, measure and record the shoot and root growth.
SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 5. Root and shoot production by excised corn embryos. Embryos were grown for 7 days.

<table>
<thead>
<tr>
<th>Replicate</th>
<th>M &amp; S Basal Medium</th>
<th>Adenine-Kinetin Amended Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot length (mm)</td>
<td>Root length (mm)</td>
</tr>
<tr>
<td>1</td>
<td>26</td>
<td>28</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>26</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>18</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>42</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>35</td>
</tr>
<tr>
<td>Mean</td>
<td>27</td>
<td>30</td>
</tr>
</tbody>
</table>

Interpretation

The embryos were consistently smaller when they grew on Adenine-Kinetin Amended Medium. The ratio between roots and shoots for plants on M&S Basal Medium (1.13) is far greater than that for plants on Adenine Amended Medium (0.07). These data support the hypothesis.

Graph B. Effect of an adenine-kinetin addition to the medium is shown in the change in the relationship between roots and shoots.

TEST QUESTION

One group of students extended this exercise by testing to determine whether the Adenine-Kinetin Amended Medium could be used to clone corn from isolated roots of 4-day-old plants. Over 6 days, their roots on Adenine-Kinetin Amended Medium showed no increase in root length and no development of shoots. On the average, their roots on M&S Basal Medium grew 72% longer than their initial length, but no shoots developed. A second group wanted to know whether they could clone corn by using these media with isolated shoots from 4-day-old plants. They found that shoots on Adenine-Kinetin Amended Medium increased in length an average of 52%, but formed no roots. Shoots on M&S Basal Medium increased an average of 70% in length and 50% of these shoots developed single roots longer than 10 mm. Write a conclusion for these results.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

• Have students study the growth of large plant embryos, such as avocado, with and without kinetin plus adenine. Sighted students may do this study as well.
• Provide molecular model kits to show the difference in chemical structure of adenine and kinetin.

VARIATION 4
The Effect of Kinetin-Indole-3-Acetic Acid (IAA) on the Growth of Corn Embryos in Vitro

Note to Teachers In addition to the information found in the Core Experiment, the following material has been provided for Variation 4.

SYNOPSIS
Students will compare the growth of corn embryos on a nutritionally complete medium with and without the addition of kinetin and IAA.

ADDITIONAL MATERIALS NEEDED
For the teacher preparation you will need the following:
- 5 mL 1N NaOH
- 110 mL distilled H₂O
- 5 mL 1N HCl
- 1 pH meter
- 6 sterile petri dishes
- 1 balance
- 0.03 g Indole-3-Acetic Acid (IAA) (Carolina #F6 198250)
- 1 mL Kinetin Solution
- 1 autoclave
- 4.24 g M&S Basal Medium with sucrose and agar

You will need the following for each group of four students in a class of 24:
- 1 sterile petri dish of Kinetin-IAA Amended Medium
- 1 sterile petri dish of M&S Basal Medium with sucrose and agar
- 10 fresh corn kernels

SAFETY PROCEDURE
Use the liquid form of IAA where possible. If using powdered form of IAA, exercise caution when preparing solutions.

DIRECTIONS FOR SETTING UP THE EXPERIMENT
Kinetin Stock Solution
See Variation 3.

IAA Stock Solution (if not purchased)
1. Dissolve 0.03 g of IAA in 2 mL of 1N NaOH.
2. Add 8 mL distilled H₂O.
3. Store at -0°C.

Kinetin-IAA Amended Medium
1. Add 4.24 g of M&S Basal Medium with sucrose and agar to 98 mL of room temperature distilled H₂O, while gently stirring the water.
2. Stir continuously and heat until the solution becomes clear. Do not boil.

3. Add 1 mL Kinetin Stock Solution.

4. Add 10 mL IAA Stock Solution.

5. Adjust the pH to 5.7 with 1N NaOH or 1N HCl.

6. Autoclave for 15 minutes at 15 pounds per square inch (psi).

7. Dispense to 6 petri dishes labeled Kinetin-IAA Amended Medium.

HYPOTHESIS GENERATION

Question

How does the addition of IAA to kinetin affect embryo growth?

Sample Hypothesis

Fresh corn embryos grown in a nutritionally complete medium with kinetin plus IAA added will develop more roots, as compared with embryos grown in a nutritionally complete medium without kinetin and IAA.

Rationale

Students should provide their own rationale.

Sample Experimental Procedure

1. Prepare 10 fresh corn embryos as directed in the Core Experiment.

2. Aseptically, transfer 5 of the embryos to the dish containing the Basal Medium, as directed in the Core Experiment.

3. Aseptically, transfer 5 embryos to the dish containing the Kinetin-IAA Amended Medium.

4. Store dishes, observe and record data as directed to in the Core Experiment.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 6. Effect of Kinetin-IAA Amended Medium on shoot and root lengths.

<table>
<thead>
<tr>
<th>Replicate</th>
<th>M &amp; S Basal Medium</th>
<th>Kinetin-IAA Amended Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot length (mm)</td>
<td>Root length (mm)</td>
</tr>
<tr>
<td>1</td>
<td>81</td>
<td>630</td>
</tr>
<tr>
<td>2</td>
<td>102</td>
<td>860</td>
</tr>
<tr>
<td>3</td>
<td>97</td>
<td>520</td>
</tr>
<tr>
<td>4</td>
<td>95</td>
<td>1240</td>
</tr>
<tr>
<td>5</td>
<td>93</td>
<td>590</td>
</tr>
<tr>
<td>Mean</td>
<td>94</td>
<td>768</td>
</tr>
</tbody>
</table>

Interpretation

The embryos were consistently smaller when grown on Kinetin-IAA Amended Medium. The ratio between roots and shoots for plants on M&S Basal Medium (8.19) is far greater than that for plants on Kinetin-IAA Amended Medium (1.48). These data do not support the hypothesis.

Graph C. Effect of a Kinetin-IAA Amended Medium is shown in the change in the relationship between roots and shoots.

TEST QUESTION
One group of students extended this exercise by testing whether Kinetin-IAA Amended Medium could be used to clone corn from the isolated roots of 4-day-old plants. Over 6 days, their roots on Kinetin-IAA Amended Medium showed no increase in root length and no development of shoots. On the average, their roots on M&S Basal Medium grew 72% longer than their initial length, but no shoots developed. A second group wanted to know whether they could clone corn from isolated shoots of 4-day-old corn plants by using these media. They found that on the average shoots on Kinetin-IAA Amended Medium increased in length 83%, but formed no roots. Shoots on M&S Basal Medium increased an average of 70% in length and 50% of these shoots developed single roots longer than 10 mm. Write a conclusion for these results.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL Blind or Visually Impaired
• See Variations 1, 2, and 3.

VARIATION 5
The Effect of Photoperiod on the Growth of Corn Embryos on a Nutritionally Complete Growth Medium

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 5.

SYNOPSIS
Students will compare the growth of corn embryos on nutritionally complete medium when exposed to different photoperiods.

ADDITIONAL MATERIALS NEEDED
You will need the following for a class of 24:
- 2 identical temperature and humidity levels:
  - 1 24-hour per day light source
  - 1 12-hour per day light source
  - 1 24-hour timer

You will need the following for each group of four students in a class of 24:
- 2 petri dishes of M&S Basal Medium
- 10 fresh corn kernels

HYPOTHESIS GENERATION
Question
What is the effect of a continuous light period on embryo growth?

Sample Hypothesis
If corn embryos are exposed to a continuous light period, then their total growth will be greater.

Rationale
Continuous light provides continuous opportunity for the plant to manufacture energy-rich compounds needed for growth.

Sample Experimental Procedure
1. Prepare 10 fresh corn embryos as directed in the Core Experiment.
2. Aseptically, transfer 5 embryos oriented horizontally to each of 2 petri dishes
containing nutritionally complete medium. Seal dishes with Parafilm™, as directed in the Core Experiment.

3. Store 1 dish in an area that will receive 24 hours of continuous artificial light.
4. Store the other 3 dishes in an area that will receive 12 hours of continuous artificial light followed by 12 hours of darkness.
5. Make sure all dishes are kept at the same temperature and receive the same type of artificial light.
6. Make observations as directed in the Core Experiment.
7. Analyze these data.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 7. Total root and shoot length produced by corn embryos grown on M&S Basal Medium under different photoperiods for 7 days.

<table>
<thead>
<tr>
<th>Replicate</th>
<th>24-hour light (mm)</th>
<th>12-hour light (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>72</td>
<td>53</td>
</tr>
<tr>
<td>2</td>
<td>80</td>
<td>54</td>
</tr>
<tr>
<td>3</td>
<td>74</td>
<td>60</td>
</tr>
<tr>
<td>4</td>
<td>55</td>
<td>29</td>
</tr>
<tr>
<td>5</td>
<td>71</td>
<td>55</td>
</tr>
<tr>
<td>Mean</td>
<td>70</td>
<td>50</td>
</tr>
</tbody>
</table>

Appropriate analysis for these data is a statistical t-test. Appropriate display for these data is a frequency histogram, but because the sample size is small, an alternate display is acceptable.

TEST QUESTIONS

1. Compare the amount of growth in a corn embryo to the number of hours of light it receives each day.
2. The growth of each plant under the Core Experiment photoperiod was greater than that for any plant under these conditions. Provide a reasonable explanation for this observation.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL

Blind or Visually Impaired

• See Variations 1, 2, and 3.

VARIATION 6

Growth of Dicots as Compared to Monocots on a Nutritionally Complete Growth Medium

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 6.

SYNOPSIS

Students will compare growth of an isolated dicot embryo without its seed-food source with that of an isolated monocot embryo without its seed-food source.

ADDITIONAL MATERIALS NEEDED

You will need the following for each group of four students in a class of 24:

10 fresh or soaked pea, squash, or bean seeds
1 sterile foil-covered 250-mL beaker
100 mL 10.0% bleach-soap solution
2 sterile forceps
2 sterile scalpels with blades
5 fresh corn kernels
2 sterile petri dishes of M&S Basal Medium

HYPOTHESIS GENERATION

Question
How does the removal of the seed-food source of monocot and dicot affect their growth?

Sample Hypothesis
If the seed-food source of a dicot is removed, then the dicot will grow less vigorously than a monocot with its seed-food source removed.

Rationale
The cotyledons (dicot seed-food source) provide a continuous food supply for the plant because they photosynthesize.

Sample Experimental Procedure
1. Prepare corn embryos for tissue culture as in the Core Experiment.
2. Aseptically, transfer 5 embryos to petri dishes of M&S Basal Medium labeled “corn.”
3. Prepare the petri dish and incubate the embryos with the light regime of the Core Experiment.
4. Use the soiled scalpel and forceps to practice excising dicot embryos from 5 pea seeds.
   a. Cut and remove the seed coat.
   b. Separate the cotyledons by slipping the scalpel blade between them and twisting it.
   c. Slide the scalpel blade along the cotyledon to where the remainder of the embryo is attached and cut the embryo from the cotyledon.
5. Sterilize the surface of the dicot seeds in bleach solution for 10 to 50 minutes if they are fresh and cleaned. If you are using dried seeds, soak them in water for 1 hour to overnight before surface sterilizing them.
6. Use the procedures you perfected in practice and aseptic techniques to extract the dicot embryos. Transfer 5 embryos to a second petri dish of M&S Basal Medium.
7. Seal the petri dishes with Parafilm™ or PetriSeal™ and incubate the pea embryos with the corn embryos.
8. After several days, measure and record the growth of shoots and roots on the embryos.
9. Display and analyze these data.

DATA ANALYSIS AND INTERPRETATION

Sample Data

Table 8. Growth of excised dicot and monocot embryos with only tissue culture nutrients grown for 13 days.

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Corn</th>
<th></th>
<th></th>
<th>Peas</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root length (mm)</td>
<td>Shoot length (mm)</td>
<td>Total plant (mm)</td>
<td>Root length (mm)</td>
<td>Shoot length (mm)</td>
<td>Total plant length (mm)</td>
</tr>
<tr>
<td>1</td>
<td>720</td>
<td>106</td>
<td>730</td>
<td>15</td>
<td>3</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>310</td>
<td>108</td>
<td>418</td>
<td>15</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>260</td>
<td>60</td>
<td>320</td>
<td>12</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td>250</td>
<td>81</td>
<td>331</td>
<td>11</td>
<td>6</td>
<td>17</td>
</tr>
<tr>
<td>5</td>
<td>470</td>
<td>94</td>
<td>564</td>
<td>14</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>473</td>
<td>17</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Interpretation
Pea embryos grew very poorly when tissue culture medium was substituted for cotyledons. Perhaps another medium formulation would produce good growth, but this medium does not appear to replace the contribution of the cotyledons. Additional testing to determine how well the peas would grow in these conditions when the embryo was intact should be used to compare the growth of embryos on tissue-culture medium. Corn grew much better than peas, so our hypothesis is supported by these data.
The appropriate display for these data is a frequency histogram. The display here is not a frequency histogram because the data sample is small. Appropriate analysis here would be the statistical t-test, but since there is no overlap in these data, a test is not necessary.

TEST QUESTION
Another group of students grew winter squash embryos and compared the growth of complete embryos on 7% water agar with embryos without cotyledons on M&S Basal Medium. Their data are presented in Table 9. Is M&S Basal Medium a good substitute for the cotyledons? How do you know?

Table 9. Winter squash growth with nutrients supplied by the cotyledons or by M&S Basal Medium after 6 days. Shoot growth is measured to the tip of the base of the cotyledons or to the base of the unexpanded first true leaves.

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Complete embryos</th>
<th>Incomplete embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root growth (mm)</td>
<td>Shoot growth (mm)</td>
</tr>
<tr>
<td>1</td>
<td>137</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>97</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>267</td>
<td>16</td>
</tr>
<tr>
<td>4</td>
<td>126</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>70</td>
<td>8</td>
</tr>
</tbody>
</table>

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL
Blind or Visually Impaired
• See Variations 1, 2, and 3.

VARIATION 7
The Effect on Embryo Development of Cloning by Longitudinally Dividing an Excised Fresh Corn Embryo

Note to Teachers In addition to the information found in the Core Experiment, the following material has been provided for Variation 7.

SYNOPSIS
Students will compare the growth produced by complete fresh corn embryos on a nutritionally complete growth medium with the growth produced by longitudinally divided embryos.

ADDITIONAL MATERIALS NEEDED
You will need the following for each group of two to three students:

- 4 sterile petri dishes of M&S Basal Medium
- 10 fresh corn kernels

HYPOTHESIS GENERATION
Question
What effect does dividing the embryo longitudinally have upon its growth?
Sample Hypothesis
If an embryo is divided longitudinally, then it will develop more slowly than a complete embryo.

Rationale
The part of the embryo that would have developed a shoot is ready to do so, and the part that will develop a root is ready to do so. However, the longitudinal half-embryo has only half as many cells of each kind as the complete embryo.

Sample Experimental Procedure
1. Label 2 sterile petri dishes with M&S Basal Medium “complete” and 2 sterile petri dishes “longitudinal.”
2. Prepare 10 corn embryos for tissue culture as in the Core Experiment.
3. Aseptically, transfer 5 complete embryos to each of 2 petri dishes of M&S Basal Medium.
4. Seal the petri dishes and incubate the embryos with the light regime of the Core Experiment.
5. Aseptically, cut the remaining corn embryos in half longitudinally.
6. Aseptically, transfer 5 of the longitudinal half-embryos to each of 2 sterile petri dishes of M&S Basal Medium.
7. Seal the petri dishes and incubate the embryos with the light regime of the Core Experiment.
8. After several days, measure and record the shoot and root lengths produced by each embryo and half-embryo.
9. Display and analyze these data.

DATA ANALYSIS AND INTERPRETATION
Sample Data

Table 10. Growth measurements of excised corn embryos after 13 days on tissue culture medium.

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Longitudinal half-embryos</th>
<th>Complete embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root length (mm)</td>
<td>Shoot length (mm)</td>
</tr>
<tr>
<td>-----------</td>
<td>-------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>1</td>
<td>420</td>
<td>89</td>
</tr>
<tr>
<td>2</td>
<td>440</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>340</td>
<td>80</td>
</tr>
<tr>
<td>4</td>
<td>160</td>
<td>66</td>
</tr>
<tr>
<td>5</td>
<td>230</td>
<td>83</td>
</tr>
<tr>
<td>6</td>
<td>430</td>
<td>111</td>
</tr>
<tr>
<td>7</td>
<td>300</td>
<td>101</td>
</tr>
<tr>
<td>8</td>
<td>510</td>
<td>100</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>6</td>
</tr>
</tbody>
</table>
**Table 11.** Growth of partial and complete corn embryos over 3 days.

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Partial embryos</th>
<th>Complete embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average shoot length (mm)</td>
<td>Average root length (mm)</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>6</td>
</tr>
</tbody>
</table>

A valid conclusion from their data would be:
A. Total root growth is greater than total shoot growth for both partial and complete embryos.
B. The rate of growth was the same for both root and shoot for partial and complete embryos.
C. Total root growth is greater than total shoot growth for both partial and complete embryos.
D. The greatest amount of growth occurred on the second day.
SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL
Blind or Visually Impaired
• See Variations 1, 2, and 3.

VARIATION 8
The Effect on Embryo Development of Cloning by Transversely Dividing an Excised Fresh Corn Embryo

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 8.

SYNOPSIS
Students will compare the shoot to root ratio developed by complete fresh corn embryos on a nutritionally complete growth medium with the ratio developed by transversely divided embryos.

ADDITIONAL MATERIALS NEEDED
You will need the following for each group of four students:
- 4 sterile petri dishes of M&S Basal Medium
- 20 corn embryos

HYPOTHESIS GENERATION
Question
What is the effect on embryo growth if the embryo is divided transversely?

Sample Hypothesis
If an embryo is divided transversely, then it will develop roots or shoots differently than an undivided embryo.

Rationale
The part of the embryo that would have developed a shoot is ready to do so, but new cells need to be formed and oriented for producing a root. Therefore, the shoot half should develop shoot more quickly than root. The reverse might be expected for the root half.

Sample Experimental Procedure
1. Label 2 sterile petri dishes “complete” and 2 additional sterile dishes “transverse.”
2. Prepare corn embryos for tissue culture as in the Core Experiment.
3. Aseptically, transfer 5 complete embryos to each of 2 petri dishes of M&S Basal Medium labeled “complete.”
4. Seal the petri dishes and incubate the embryos with the light regime of the Core Experiment.
5. Remove 5 corn embryos and cut them in half transversely.
6. Aseptically, transfer 5 of the transverse half-embryos to each of 2 petri dishes of M&S Basal Medium labeled “transverse.”
7. Seal the petri dishes and incubate the embryos with the light regime of the Core Experiment.
8. After several days, measure and record the shoot and root lengths produced by each embryo and half-embryo.
9. Display and analyze these data.

TEACHING TIPS
• Students may want to compare total growth. They certainly should expect to see a difference since they have reduced the number of starting cells by approximately half.
• Here the expectation should be focused on qualitative difference (how it grew) rather than on quantitative difference (how much it grew).
DATA ANALYSIS AND INTERPRETATION

Sample Data

**Table 12.** Growth measurements of excised corn embryos after 13 days on a tissue culture medium.

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Transverse half-embryos</th>
<th>Complete embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root length (mm)</td>
<td>Shoot length (mm)</td>
</tr>
<tr>
<td>1</td>
<td>610</td>
<td>89</td>
</tr>
<tr>
<td>2</td>
<td>730</td>
<td>76</td>
</tr>
<tr>
<td>3</td>
<td>950</td>
<td>93</td>
</tr>
<tr>
<td>4</td>
<td>410</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>680</td>
<td>104</td>
</tr>
<tr>
<td>6</td>
<td>690</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>170</td>
<td>50</td>
</tr>
<tr>
<td>8</td>
<td>380</td>
<td>75</td>
</tr>
<tr>
<td>9</td>
<td>550</td>
<td>70</td>
</tr>
<tr>
<td>10</td>
<td>210</td>
<td>75</td>
</tr>
</tbody>
</table>

**Graph E.** Distribution of growth among complete (a) and transverse half (b) embryos.

**Answer to Test Question**

Each cell in a corn embryo must have the ability to develop into either shoot or root tissues, because each transverse half was able to make a complete embryo.

**TEST QUESTION**

Although the complete embryos and half embryos grew at different rates. They did not produce statistically different shoot to root ratios. What does this tell you about the genetic potential of embryonic corn cells?
SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL
Blind or Visually Impaired
• This variation is not suitable to copy in larger size.

VARIATION 9
The Effect of Endosperm to Embryo Ratio on the Growth of Grass Embryos on a Nutritionally Complete Medium

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 9.

SYNOPSIS
Students will determine if grass species with different amounts of endosperm provided relative to the embryo size will produce different growth responses in tissue culture.

APPROPRIATE BIOLOGY LEVEL
Advanced

ADDITIONAL MATERIALS NEEDED
You will need the following for each group of three students in a class of 24:
- 300 mL of 10% bleach-soap solution
- 3 sterile, foil-covered 250-mL beakers
- 3 petri dishes of M&S Basal Medium
- 30 seeds of each of 3 or more kinds of grass

HYPOTHESIS GENERATION
Question
What is the effect of endosperm to embryo ratio on embryo growth?

Sample Hypothesis
If a grass has a small endosperm to embryo ratio, then it will grow long more quickly than if it has a large endosperm to embryo ratio.

Rationale
An embryo provided with little endosperm needs to produce its own food promptly. The faster the shoot reaches the light and leaves expand, the sooner the plant can produce its own food.

Figure 8. An unmilled grass seed will look like this diagram. The embryo is on the far side of the seed, opposite the surface showing in this diagram.

• All of the grasses used here have a groove opposite the side where the embryo is located.
Sample Experimental Procedure
1. Soak seeds for about 1 hour in 30°C tap water to soften the accessory floral parts before removing them. Discard any seeds that have begun to germinate.
2. Sterilize the surface of seeds as in the Core Experiment.
3. Excise the embryos by cutting them away from the endosperm.
4. Transfer 5 embryos of each kind to a separate petri dish of M&S Basal Medium.
5. Incubate the excised embryos as in the Core Experiment.
6. After several days, measure and record the shoot and root lengths produced.

DATA ANALYSIS AND INTERPRETATION
Sample Data

Table 13. Growth of various grasses in tissue culture. Plants were grown for 7 days. The average endosperm/embryo ratios were obtained by drying several excised embryos and their endosperms. The pericarp was included with the endosperm except for corn. The sample size was 20 seeds except in the case of wheatgrass, where 45 seeds were used.

<table>
<thead>
<tr>
<th>Grass genus</th>
<th>Replicate</th>
<th>Shoot length (mm)</th>
<th>Root length (mm)</th>
<th>Endosperm/embryo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zea (corn)</td>
<td>1</td>
<td>71</td>
<td>210</td>
<td>0.128</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>59</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>54</td>
<td>113</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>43</td>
<td>290</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>57</td>
<td>165</td>
<td></td>
</tr>
<tr>
<td>Agropyron (wheatgrass)</td>
<td>1</td>
<td>27</td>
<td>32</td>
<td>0.033</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>25</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>17</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>20</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>11</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Triticum (wheat)</td>
<td>1</td>
<td>23</td>
<td>25</td>
<td>0.038</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>12</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>15</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>23</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>12</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Hordeum (barley)</td>
<td>1</td>
<td>53</td>
<td>27</td>
<td>0.039</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>51</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>50</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>28</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>40</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>
Graph F. The relationship between shoot growth and endosperm/embryo ratio. All plants were grown for 7 days.

TEST QUESTION
Assuming that the relationship developed in your graphical presentation is correct, how long would you expect the shoot of a grass with an embryo to endosperm ratio of 0.085 to be in 7 days?

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL Blind or Visually Impaired
• See Variation 8.

VARIATION 10
The Effect of Alternate Basic Tissue Culture Medium on the Growth of Complete Corn Embryos

Note to Teachers: In addition to the information found in the Core Experiment, the following material has been provided for Variation 10.

SYNOPSIS
Students will compare the growth of isolated corn embryos on a commercial medium with their growth on a simplified medium.

ADDITIONAL MATERIALS NEEDED
You will need the following for a class of 24:
- 6 mL 10-10-10 water soluble fertilizer
- 1.5 L distilled H₂O
- 720 mL table sugar
- 2 500-g inositol tablets
- 2 multivitamin tablets containing thiamine
- 60 g agar flakes
- citric acid
- bicarbonate of soda

Interpretation
It was predicted that the wheatgrass, wheat, and barley, with low embryo to endosperm ratios, would have more rapid shoot growth than corn when all of the embryos were supplied with the same nutrient source. These data do not support the hypothesis. Of the embryos tested, corn has the most rapid shoot growth. The correlation (R² = 0.7413) between shoot length and the embryo to endosperm ratio is not strong. Additional data points would be useful to further support this hypothesis or to refute it.

Answer to Test Question
The shoot of this mystery grass grown from an isolated embryo should be 43 mm long in 6 days.

TEACHING TIPS
• This recipe for tissue culture is from the Internet Site: http://www.une.edu.au/~agronomy/AgSSrHortTCinfo.html. It calls for rain water, but distilled water is available in supermarkets and has been substituted here. Multivitamins come with a wide variety of thiamine amendments. Those used to obtain these data contained 10 mg and minerals not specified in the recipe. The original recipe also called for fertilizer with a 10:10:10 formulation not commonly available here. The volume of solutions was adjusted to arrive at the correct concentrations.
• In the early 1940’s coconut milk was introduced to promote embryo growth in tissue culture. Amending this formulation with coconut milk could provide interesting results. Encourage your students to speculate on the cause of the growth increase, particularly if they have investigated effects of specific hormones. To make the amendment, add 1 cup of coconut milk and 1 teaspoon of malt.
You will need the following for each group of three students in a class of 24:

- 1 sterile petri dish of Market Tissue Culture Medium
- 1 sterile petri dish of M&S Basal Medium
- 10 fresh corn kernels

**DIRECTIONS FOR SETTING UP THE LAB**

**Fertilizer Stock**

Add 6 mL of 10:10:10 (N:P:K) water soluble fertilizer to 1 L distilled water.

**Market Tissue Culture Medium**

1. Combine
   - 480 mL distilled water
   - 240 mL Fertilizer Stock
   - 720 mL table sugar
   - 2 500-mg inositol tablet, crushed
   - 2 thiamine-containing multivitamin tablets, crushed
   - 4 tablespoons agar flakes
2. Boil gently, stirring constantly, until the agar dissolves. Use citric acid or bicarbonate of soda to adjust the pH to between 5 and 6. Autoclave and dispense this agar the same way you autoclaved and dispensed the M&S Basal Medium.

**HYPOTHESIS GENERATION**

**Question**

Do isolated embryos have specific nutrient requirements for growth?

**Sample Hypothesis**

If isolated corn embryos are grown on medium other than M&S Basal Medium, they will not grow as quickly.

**Rationale**

Commercial media prepared with purified ingredients is usually formulated for culturing specific species. General-purpose medium of common ingredients is unlikely to support vigorous corn growth because corn is not a species hobby horticulturists are likely to culture.

**Sample Experimental Procedure**

1. Surface sterilize seeds following directions of the Core Experiment.
2. Using the procedures of the Core Experiment, extract embryos and place 5 embryos horizontally on M&S Basal Medium and 5 embryos on Market Tissue Culture Medium.
3. Seal the petri dishes with Parafilm™ or PetriSeal™ and place the dishes under lights as done in the Core Experiment.
4. Calculate the total length of roots and shoots over a period of several days.
DATA ANALYSIS AND INTERPRETATION

Sample Data

**Table 14.** Total shoot and root growth produced by whole corn embryos during 13 days incubation with a photoperiod of 16 hours light/8 hours dark.

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Medium type</th>
<th>Market</th>
<th>M &amp; S</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>155</td>
<td>599</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>280</td>
<td>617</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>288</td>
<td>667</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>450</td>
<td>741</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>490</td>
<td>1315</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>333</td>
<td>788</td>
</tr>
</tbody>
</table>

**Graph G.** The production of corn embryos on different media after 13 days is represented by the total length of roots and shoots. Each bar represents the average growth for 5 plants.

**TEST QUESTION**

Another group of students decided that they were concerned about the ability of corn grown on Market Medium to produce a root/shoot ratio that was typical of plants nourished by endosperm. They obtained the following data. Determine the root/shoot ratios and compare these values.

**Table 15.** Root and shoot growth produced by corn embryos with different nutrient sources.

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Market Medium</th>
<th>Endosperm present</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root length(mm)</td>
<td>Shoot length</td>
</tr>
<tr>
<td>1</td>
<td>240</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>130</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>250</td>
<td>38</td>
</tr>
<tr>
<td>4</td>
<td>400</td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>430</td>
<td>60</td>
</tr>
</tbody>
</table>

Interpretation

The hypothesis is supported by these data. Plants grown on the commercially formulated medium (M&S) produced more total growth than did plants grown on the Market Tissue Culture Medium (Market). The average growth on M&S medium was more than twice that on the Market Medium.
Table 16. A comparison of the root to shoot ratio produced by corn grown with different nutrient sources.

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Market Medium</th>
<th>Endosperm present</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root length(mm)</td>
<td>Shoot length</td>
</tr>
<tr>
<td>1</td>
<td>240</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>130</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>250</td>
<td>38</td>
</tr>
<tr>
<td>4</td>
<td>400</td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>430</td>
<td>60</td>
</tr>
</tbody>
</table>

These results clearly show that root growth is retarded relative to shoot growth by fresh endosperm. The average root/shoot ratio in market medium (6.6) is 4.7 times greater than the average ratio (1.4) with endosperm present.

SUGGESTED MODIFICATIONS FOR STUDENTS WHO ARE EXCEPTIONAL
Blind or Visually Impaired
• See Variation 8.
Plant Tissue Culture: Embryo Isolation and Tissue Culture Initiation

Directions for Students

INTRODUCTION

Imagine that you are chosen to be a member of the food production team aboard the first Spacelab and that your responsibility is to manage the growth of plants to support life in the self-contained environment. What technique(s) could you use to produce food quickly and without soil?

The commercial use of plant tissue culture is a relatively recent technique for growing plants quickly. Several types of culture techniques have been developed for the specific purposes of micropropagation, cloning of plants with desirable characteristics, and nutritional studies in a controlled environment. One technique that is easily demonstrated in the lab is excising or “cutting” an embryo from the seed and growing it on a sterile growth medium. See Figure 1. The advantages to this technique are:

- more rapid growth of the embryo
- more plants can be grown in a small space, and
- specific nutrient requirements and physical conditions affecting the plant can be easily controlled and modified.

Figure 1. Excising the embryo from a corn kernel.

By removing the embryo from the seed and growing it in a culture vessel (in vitro), you are altering its natural environment. What conditions must be provided for the embryo to grow successfully in vitro?

OBJECTIVES

At the end of this lab you should be able to:

- Excise a plant embryo from a seed.
- Inoculate a sterile, nutritionally complete growth medium with the excised embryo.
- Observe the growth of a plant embryo in vitro.
- Describe the effects of specific hormones on plant embryo growth in vitro.
SAFETY NOTES

Safety goggles and lab aprons/coats are to be worn at all times when working with chemicals, heat, and glassware.

Use care when working with sharp instruments. Cut away from your body when using scalpels.

Maintain sterile conditions at all times in the work area.

Wash hands with antibacterial soap as instructed throughout the lab procedure and before leaving the lab.

At the conclusion of the lab exercise, all living materials must be autoclaved before disposing to prevent exposure to accidentally introduced pathogens.

Keep open containers of alcohol away from flames.

MATERIALS NEEDED

You will need the following for each group of two to four students in a class of 24:

- 1 250-mL spray bottle of 70% ethanol or 10% household bleach
- 1 sterile petri dish with M&S Basal Medium with sucrose and agar
- 1 sterile petri dish with water agar
- 1 permanent marking pen
- 1 bottle of antibacterial soap
- 1 fresh supermarket ear of yellow corn (Zea mays) or dry field corn seeds
- 50 mL sterile water
- 25 mL 10% household bleach-soap solution
- 3 sterile, disposable petri dishes
- 1 sterile film canister or 1 250-mL beaker with aluminum foil cover
- 1 timer
- 1 shallow pan
- 1 sterile surgical scalpel, Bard-Parker No. 3 handle
- 1 sterile No. 10 blade
- 1 sterile No. 11 blade
- 2 sterile forceps, 11-cm fine point
- 1 500-mL beaker
- 2 sterile paper towels
- 2 2.5 x 15-mm Parafilm™ or Petri Seal™ strips

STUDENT LITERATURE SEARCH SUMMARY WITH REFERENCES

Do a literature or web search on the topic of plant tissue culture. Summarize your findings and cite your references. Your teacher may be able to suggest some journals.

HYPOTHESIS GENERATION

From the information you have on this topic develop a hypothesis that could be tested in a controlled experiment that gathers quantitative data. Explain the reasoning behind your hypothesis.

Answer the following questions:
1. What is the question you are investigating?
2. What makes this question an interesting or important topic for investigation?
3. What additional variables need to be controlled for? How will you accomplish this? Why is it important to control for these variables?
PLAN OF INVESTIGATION

Design an experiment to test your hypothesis. Be sure that you include an experimental control and enough replicates to provide reliable data. Consider how you will analyze and present your results. Write the procedure you will follow. Make a numbered list of the steps you will use to investigate your topic. Answer the following questions:
1. How many samples have you included?
2. What will you measure? How will you make these measurements?
3. How can you show your results in a graph?

QUESTIONS AND ANALYSIS

1. Can just an embryo grow without the rest of the seed?
2. Usually, corn left on the ground in fall does not begin growing until the following spring. How can you overcome the delay in the ability of the corn to grow?
3. What factors does tissue culture provide for the embryo’s survival?
4. Even differentiated tissues like leaves and roots can be induced to produce whole plants. How might this ability be beneficial in a space station?
5. What procedures are necessary or helpful to maintain aseptic cultures?

DESIGN OF VARIATIONS OF CORE EXPERIMENT

• Does the orientation of the embryo affect the amount of time required for development?
• How does temperature affect the growth of the embryo?
• What effect does the combination of the hormones kinetin and adenine have on embryo growth?
• How does the addition of IAA to kinetin affect embryo growth?
• What is the effect of a continuous light period on embryo growth?
• How does the removal of the seed-food source of monocot and dicot affect their growth?
• What effect does dividing the embryo longitudinally have upon its growth?
• What is the effect on embryo growth if the embryo is divided transversely?
• What is the effect of endosperm to embryo ratio on embryo growth?
• Do isolated embryos have specific nutrient requirements for growth?