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

Directions for Teachers

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 Darmo (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

LENGTH 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.

- 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

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

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.

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. Or, autoclave the medium in 100-

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.

TEACHING TIPS

- Use a commercial transfer cabinet or build one from a sheet of shower paneling and a piece of Plexiglas™. The bottom can be open if you will be working on a counter that can be wiped clean. If you build cabinets, you may want to hinge the sides and set the clear cover on with a lip across the back to hold it in place. A cabinet made this way can be collapsed for storage.
- If the experiment must be performed on open lab benches, cardboard "walls" can be constructed to reduce air flow through the work area. This method is considerably less effective in preventing contamination than the previous ones.

• Wipe down the inside of the box after each use with 10% bleach solution if using one of the alternatives for a laminar hood.

• Clear, heavy-weight plastic may be purchased in a fabric store by the yard.

Culture Vessels

• Culture vessels should be wide-mouth and short enough in height to use standard (10-cm) forceps. Longer forceps are prohibitively expensive. Baby food jars with reclosable caps are an excellent alternative to expensive laboratory vessels.

• If narrow-mouthed vessels such as test tubes or culture tubes are used, allow the sterilized vessels to cool and the medium to solidify on a slant to increase surface area for embryo implants.

Sterile Technique

• Sterile procedures are a "must" and include sterilizing:
  - the work area with a 10% bleach solution.
  - instruments by autoclaving in autoclave paper or soaking for 20 minutes in a covered container with 70% ethanol.

• Sterile plastic or glass petri dishes should be used for storing and manipulating the seeds.

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.

Correct Concept	Misconception
• A seed is composed of differentiated tissue. Only the embryo develops into a new plant.	• The entire seed grows into a plant.
• 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.	• Both monocot and dicot cotyledons are immature leaves of the embryo that contain endosperm.
• Seeds may be germinated in vitro and embryos grown on chemically defined media.	• Seeds must be planted in soil in order to grow.
• Specific plant hormones promote differential growth.	• Fertilizers are the only chemicals that promote plant growth.
• Seeds are living.	• Seeds are nonliving.

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?

TEACHING TIPS

- Minimize contamination by stressing that students wash their hands thoroughly with an anti-bacterial soap.
- Use only sterile instruments to hold or cut seeds and to transfer embryos to culture vessels.
- Wearing sterile gloves may be necessary for students who are allergic to disinfectant solutions, or when steadying seeds for embryo removal.
- Students should practice excising embryos in petri dishes. A supervising partner can watch for asepsis.
- Sterilize the medium in the culture vessels that will be used for the experiment.
- Sterilize water and glass petri dishes unless disposable plastic dishes are being used.
- The surface of the Parafilm™ that is next to the paper is sterile. If sealable plastic bags are used, the inside of the bag is sterile.
- If your students will flame sterilize tools, demonstrate holding the tools with the tips down so that burning alcohol does not flow onto their hands.
- Data analysis can be done outside of class. Most of the manipulations are simple math or can be done on personal calculators.
- Schedule lab setup so that weekend data collection is not necessary.

TEACHER'S NOTES

- 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 aqua culture?

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.

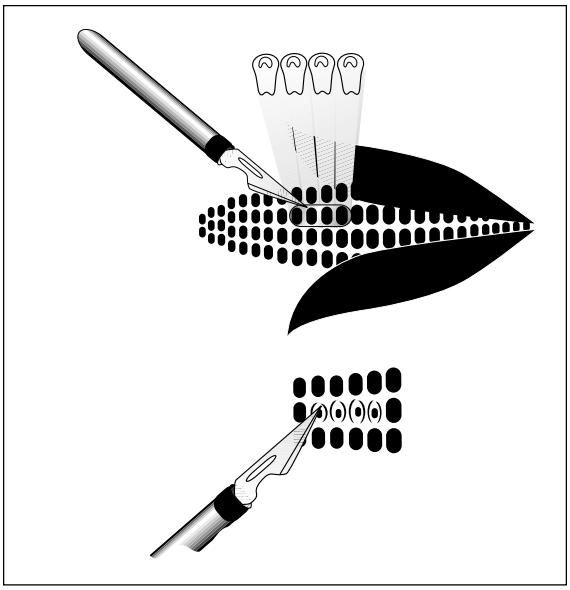

Figure 1. Removing a corn kernel from a fresh, mature cob.

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.

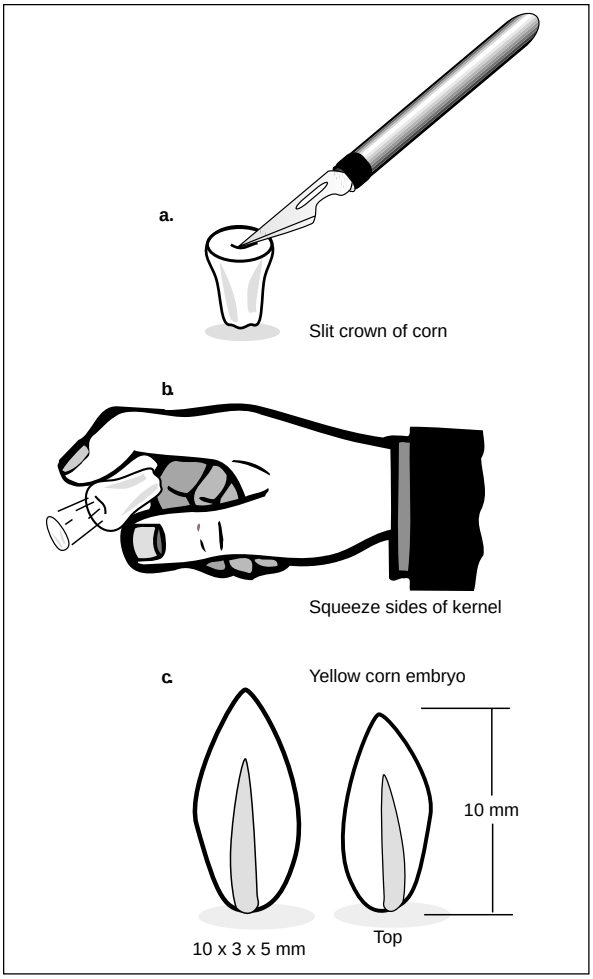

Figure 2. Excising the embryo from a fresh corn kernel.

TEACHER'S NOTES

TEACHER'S NOTES

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.

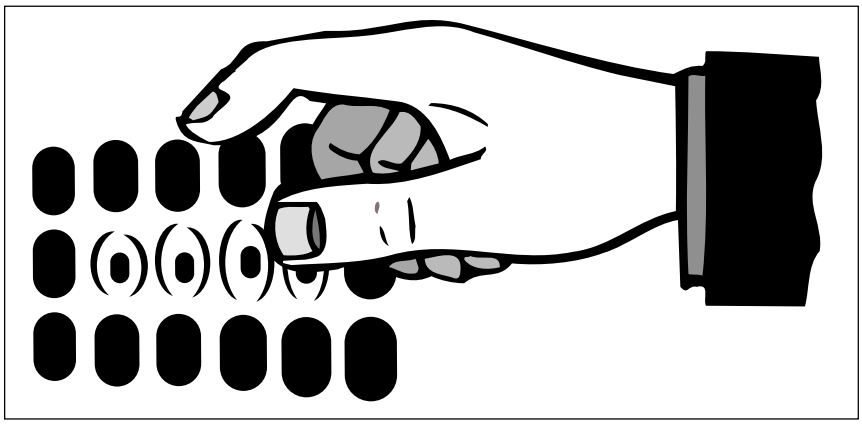

Figure 3. Removing additional kernels with fingers.

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.

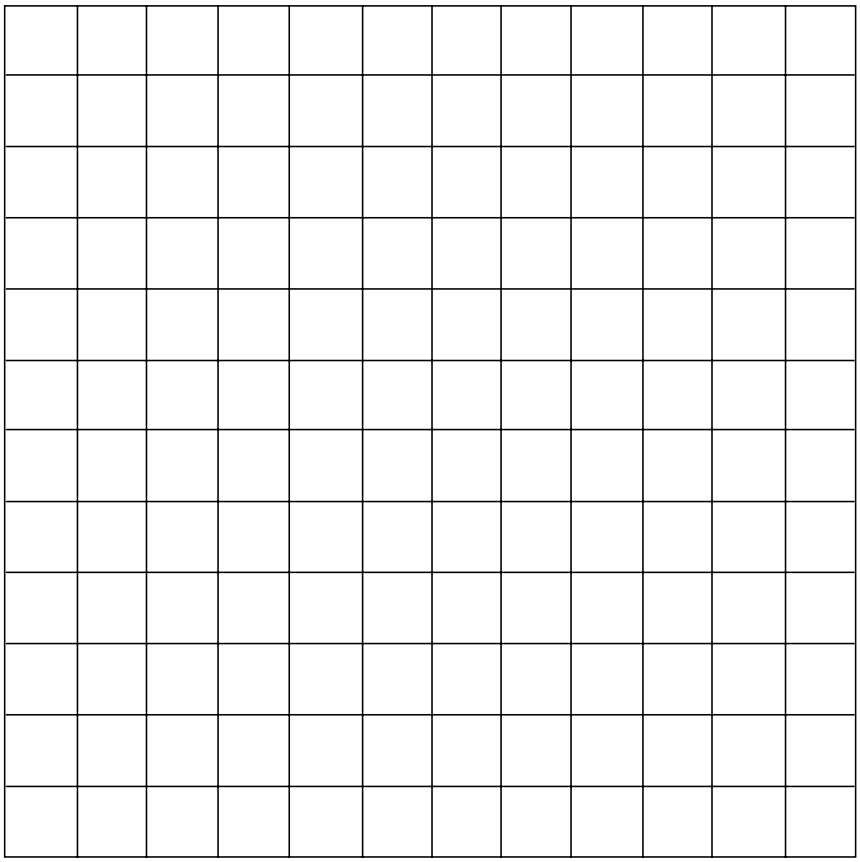

Figure 4. Grid.

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.

Replicate	Embryos with endosperm		Embryos with artificial medium	
	Length of shoot (mm)	Length of root (mm)	Length of shoot(mm)	Length of root (mm)
1	28	64	71	210
2	24	25	59	92
3	8	9	54	113
4	10	8	43	290
5	17	33	57	165

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.

TEACHER'S NOTES

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.

Answer to Test Question

A

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.

Day	With endosperm		With artificial medium	
	Shoot length (mm)	Root length (mm)	Shoot length (mm)	Root length (mm)
1	1	2	1	3
2	2	3	2	6
3	4	4	4	12
4	6	8	8	27
5	8	18	18	53

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

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Reinert, J. & Yeoman, M. M. (1982). *Plant Cell and Tissue Culture: A Laboratory Manual*. New York: Springer-Verlag. Ch. VII.

Skoog, F. & Miller, C. O. (1957). Chemical regulation of growth and organ formation in plant tissues cultured in vitro. *Symposium of Society of Experimental Biology, 11*, 118-130.

TEACHER'S NOTES

Answers to Additional Test Questions

1. C
2. Cell damage during handling of the embryos could have resulted in poorer growth of some embryos. In addition, it is possible that both detected and undetected contamination could have inhibited or enhanced growth of some transplanted embryos.
3. To culture tissues in a greenhouse, the light period, shade, or heat will need to be extended to maintain a constant temperature, and some clean laboratory conditions will be needed to prepare the explants for culture.

Answers to Questions and Analysis on Student Page

1. Corn embryos can grow without the rest of the seed. Excised embryos grew better than embryos kept with the rest of the seed.
2. Corn does not germinate immediately after harvest in the fall. It may be protected by something in the endosperm from germinating at a time when seedlings are unlikely to survive. This inhibition would be removed through maturation of the grain or by changing weather conditions in the spring.
3. Tissue culture provides constant moisture and nutrients in needed quantities and the necessary growth promoting temperature and photoperiod.
4. If entire plants can be grown from pieces of leaf, one would not have to wait for fruits to mature before starting another crop and all fruits that mature can be eaten, rather than saving some or replanting.
5. Conditions useful to establish and maintain aseptic cultures include still air, disinfecting of all surfaces, sealing culture containers, and good personal hygiene with hair tied back, no bracelets, and no long sleeves.

Skoog, F. & Tsui, C. (1948). Chemical control of growth and bud formation in tobacco stem segments and callus culture in vitro. *American Journal of Botany, 35*, 782-787.

Stribling, J. M. (1983). Pioneers in plant tissue culture. *Carolina Tips, 46*(9), 33-35.

Vasil, V. & Vasil, I. K. (1982). Characterization of an embryogenic cell suspension culture derived from cultured inflorescences of *Pennisetum americanum* (Pearl millet, Gramineae). *American Journal of Botany*, *69*, 1441-1449.

Vasil, V. & Vasil, I. K. (1982b). The ontogeny of somatic embryos of Pennisetum americanum, L.) K. Schum I. In cultured immature embryos. *Botany Gazette*, *143*, 454-465.

POSSIBLE SOURCES OF MENTORS

- Local college or university botany professor
- Local research lab or industry specializing in plant tissue culture
- Cooperating research scientist: James A. Saunders, Ph.D., United States Department of Agriculture; Biotechnology Unit; Building 50, Room 100; Beltsville, MD 20705. Phone 301.504.7477; Fax 301.504.6478; E-mail: saund10449@aol.com.

VARIATIONS ON THE CORE EXPERIMENT

After completing the Core Experiment, students should use the results to develop a variation of that experiment. The following directions are intended only as a guide for the teacher. They suggest possible hypotheses students may develop and data that may result.

Note to Teachers: Only information that is unique to each Variation of the Core Experiment is found in this section. Unless otherwise noted, teacher information not listed for each variation is the same as that found in the Core Experiment. Materials listed in this section are needed in addition to the materials listed for the Core Experiment.

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.

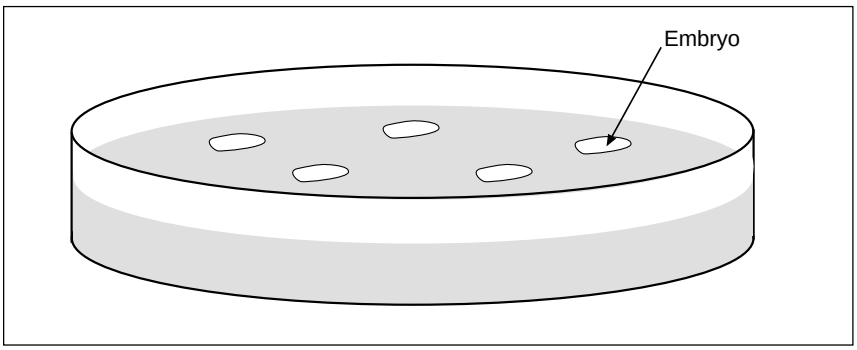


Figure 5. Corn embryos oriented horizontally.

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.

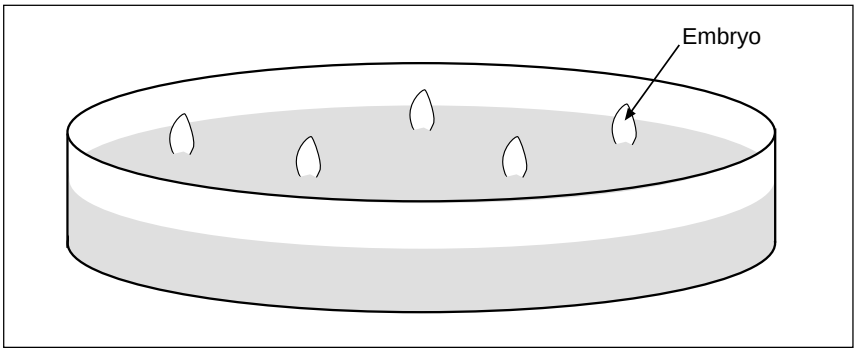


Figure 6. Corn embryos oriented vertically.

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.

TEACHER'S NOTES

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.

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

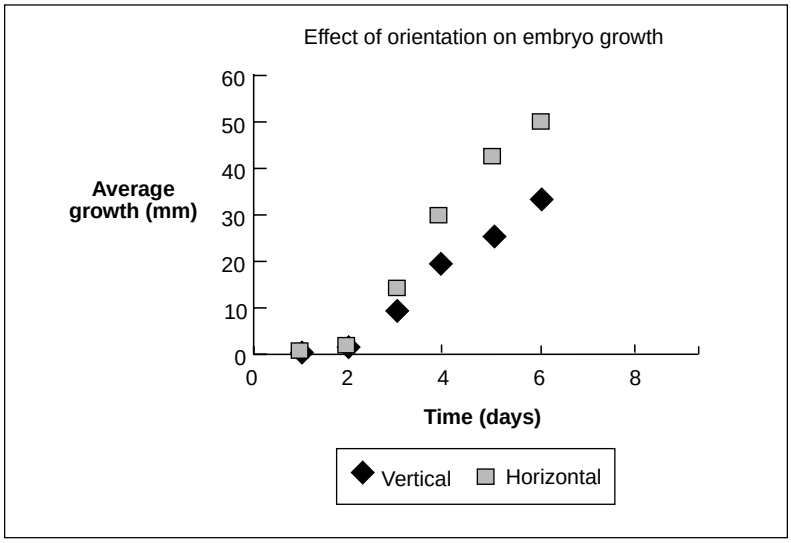
Total plant growth for each replicate and analyze the totals with a statistical t-test. An appropriate display of these data would be a frequency histogram.

Interpretation

Variations in the average total growth as great as found between horizontally and vertically oriented embryos are unlikely to be the result of chance alone. Reject the hypothesis and by inspection conclude that horizontally oriented embryos grew more than vertically oriented embryos.

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.



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

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.

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.

Replicate	Total production (mm)		
	4°C	19°C	28°C
1	0	9	55
2	0	17	49
3	0	11	55
4	0	15	48
5	0	11	28

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.

Interpretation

These data support the hypothesis. There was no growth at 4°C. As the temperature was increased to 28°C, growth increased.

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.

Answer to Test Question

1. Temperature extremes inhibit plant growth. At temperatures above 35°C enzyme function in cells will be adversely affected. At temperatures below 10°C enzyme function is significantly slowed, but the damage is not irreversible if the plant is brought back into a room temperature environment (20 to 25°C).
2. Using a nutritionally complete medium and one type of seed (embryo), set up culture vessels in various temperature conditions with normal light and in various light conditions with normal temperature. Record the growth (shoot + root) of the embryos daily. Average the growth for embryos in each of the various settings and compare them by plotting the average growth versus time (days) for each of the various settings on the same graph.

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_2O
- 2 mL 1N HCl
- 1 pH meter
- 6 sterile petri dishes

- 1 balance
- 5 g M&S Basal Medium with sucrose and agar (Carolina #195701)
- 0.01 g Kinetin (Carolina #F6 198353)
- 0.08 g Adenine (Carolina #F6 198331)

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 H_2O.
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 H_2O.
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 H_2O 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.

TEACHING TIPS

- Purchase medium containing the hormones for Variations 3 and 4. Adjusting the pH so that the agar sets up correctly is very time intensive and not always successful.
- Prepare stock enzyme solutions or purchase through a biological supply house: Kinetin (Carolina #F6 198353), Adenine (Carolina #F6 198331).
- The concept that a balance of common substances in plants controls differentiation is an economical mechanism. It is not necessary to postulate a different regulatory substance for each morphogenetic change in plants. Roots, shoots, sepals, petals, stamens, and pistils can be induced by regulating ratios of hormones rather than by developing different regulating substances.
- Some loss of kinetin activity occurs when it is autoclaved with other medium components, but that is only a concern in technical work.
- Adenine is structurally similar to kinetin and has kinetin activity. See Figure 7. The effect of adding adenine and kinetin should be similar to that of increasing kinetin concentrations.

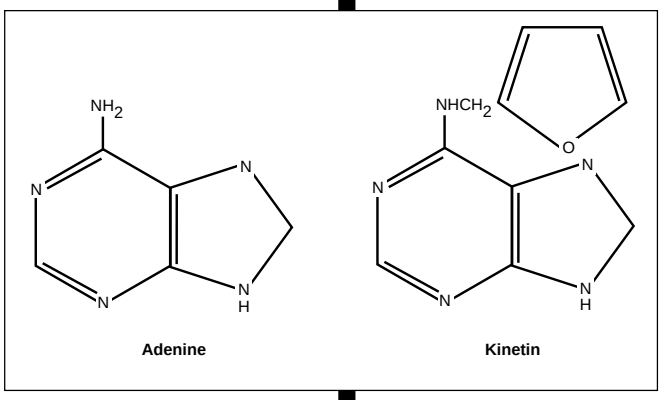


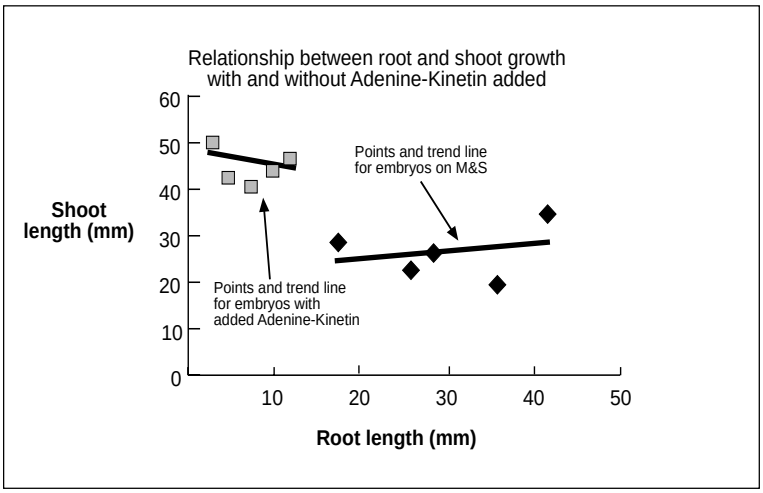
Figure 7. The structures of adenine and kinetin are similar.

SAMPLE DATA ANALYSIS AND INTERPRETATION

Sample Data

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

Replicate	M&S Basal Medium		Adenine-Kinetin Amended Medium	
	Shoot length (mm)	Root length (mm)	Shoot length (mm)	Root length (mm)
1	26	28	41	3
2	25	26	39	5
3	30	18	42	8
4	35	42	49	2
5	20	35	43	11
Mean	27	30	43	6



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

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.

Answer to Test Question

Both groups attempted to clone differentiated corn tissues. They found that the Adenine-Kinetin Amended Medium is not useful for cloning corn shoots or roots, at least at the concentrations used in a 6-day time frame. Although they found shoot growth and root development with their shoots of the M&S Basal Medium, they did not succeed in producing more plants. If this and the Core Experiment are the only information they have to draw on, they should conclude that cloning corn plants from 4-day-old plant tissue is not possible with these media.

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.

TEACHER'S NOTES

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 H_2O
- 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 H_2O.
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 H_2O, while gently stirring the water.

TEACHING TIPS

- Nearly 50 years ago, Skoog and Tsui (1948, in Leopold, 1964) demonstrated that a balance between adenine and auxin determined which organs were formed from tobacco callus. Buds were produced when adenine was abundant and roots were produced when the auxin IAA was abundant. This medium should enhance the growth of shoots.
- Kinetin induces cell division in tobacco pith when IAA is also present. The optimum production of new cells occurs at kinetin concentrations less than 10 ppm (Skoog & Miller, 1957 in Leopold, 1964).

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

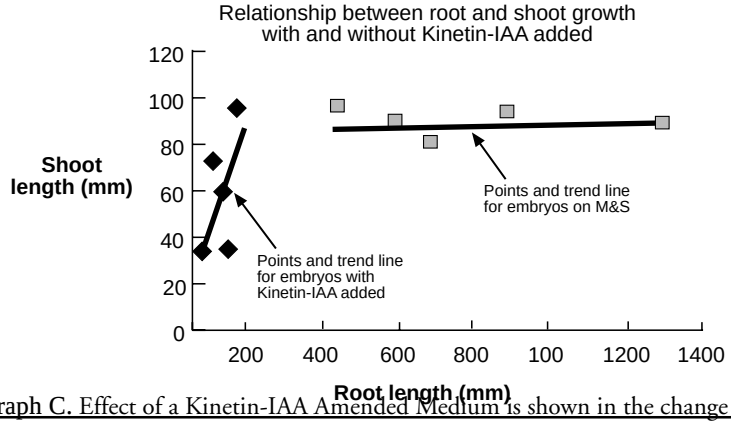
Sample Data

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

Replicate	M&S Basal Medium		Kinetin-IAA Amended Medium	
	Shoot length (mm)	Root length (mm)	Shoot length (mm)	Root length (mm)
1	81	630	96	140
2	102	860	73	68
3	97	520	33	19
4	95	1240	61	82
5	93	590	37	115
Mean	94	768	60	85

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

(

Answer to Test Question

Both groups attempted to clone differentiated corn tissues. They found that the Kinetin-IAA Amended Medium is not useful for cloning corn shoots or roots, at least at the concentrations used in a 6-day time period. Although they found shoot growth and root development with their shoots of the M&S Basal Medium, they did not succeed in producing more plants. If this and the Core Experiment are the only information they have to draw on, they should conclude that cloning corn plants from sections of 4-day-old embryos is not possible with these media.

TEACHING TIPS

- The light source will also provide a heat source.
- An increase in root hair production, sometimes seen in this variation, is an indication of increased transpirational demand. If you can use the same light source for both photoperiods and provide a cover for the shorter exposures, you can avoid differences in temperature.
- The production of some plant growth regulators requires a dark period.
- Students may have collected data on growth with the Core Experiment photoperiod. They could use these data here as well.
- Plant growth chambers may be used to simulate the 12- and 24-hour photoperiods.

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.

Replicate	Total root and shoot production (mm)	
	24-hour light	12-hour light
1	72	53
2	80	54
3	74	60
4	55	29
5	71	55
Mean	70	50

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.

Interpretation

We conclude that results like ours are likely to occur by chance alone. There is no significant difference in the growth response of corn embryos under these two conditions. These data do not support the hypothesis.

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.

Answers to Test Questions

1. The more hours of light an embryo gets each day, the longer it grows.
2. Answers will vary. Possible explanations include: The lab temperature this week was probably less than it was the week we performed the Core Experiment or the variety of corn used this week was different than the variety used for the Core Experiment. Some critical growth-regulating hormone is only produced during long days, and requires a dark period for production.

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.

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

TEACHING TIPS

- You can use dried seeds or fresh seeds, but fresh seeds are easier to work with because they are already soft. Dried seeds should be soaked for 2 days with a change of sterile water after the first day before they are as soft as fresh seeds.
- Produce from the supermarket is a good source of these materials.
- Squash seeds should be exposed to the bleach for 20 to 50 minutes rather than for only 10. To remove the seed coat from squash seeds, cut through it inside the ridge around the edge, except at the pointed end. Use the scalpel blade to lift the seed coat and push out the embryo. A squash embryo can be separated from the cotyledons by simply cutting off 1 to 2 mm at the pointed end.
- It is easier to dissect and locate the embryo in bean seeds than in either corn or cucumber seeds. If you choose to use bean seeds, have students surface sterilize them as they did the corn seeds in the Core Experiment. Students will need to remove the seed coat from the dicot seed and to separate the embryo from the cotyledons. Be careful to excise both the radicle (root) and the shoot of the bean seed. You will find it "sandwiched" between the cotyledons. Forceps can be used for this dissection.

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.

Replicate	Complete embryos		Incomplete embryos	
	Root growth (mm)	Shoot growth (mm)	Root growth (mm)	Shoot growth (mm)
1	137	11	60	5
2	97	12	22	5
3	267	16	30	5
4	126	8	28	5
5	70	8	20	5

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?

Answer to Test Question

The M&S Basal Medium is not an adequate substitute for cotyledons for winter squash. The embryos that retained their cotyledons grew an average of 4 times larger than those that had their cotyledons removed.

TEACHING TIPS

- Students may want to compare shoot to root ratios. There is no intrinsic reason to expect a difference. Collecting root and shoot lengths rather than just total growth will help them see this.
- Here the expectation should be focused on quantitative difference (how much it grew) rather than on qualitative (how it grew).
- Although the hypothesis relates to the rate of growth, it is not necessary to make daily measurements. If all embryos are grown for the same length of time, total production is an adequate measure of growth rate.

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.

Replicate	Longitudinal half-embryos			Complete embryos		
	Root length (mm)	Shoot length (mm)	Total growth (mm)	Root length (mm)	Shoot length (mm)	Total growth (mm)
1	420	89	519	660	91	751
2	440	100	540	1210	105	1315
3	340	80	420	520	79	599
4	160	66	226	560	107	667
5	230	83	313	520	97	617
6	430	111	541	630	81	711
7	300	101	401	860	102	962
8	510	100	610	520	97	617
9	0	9	9	1240	96	1336
10	0	6	6	590	93	680

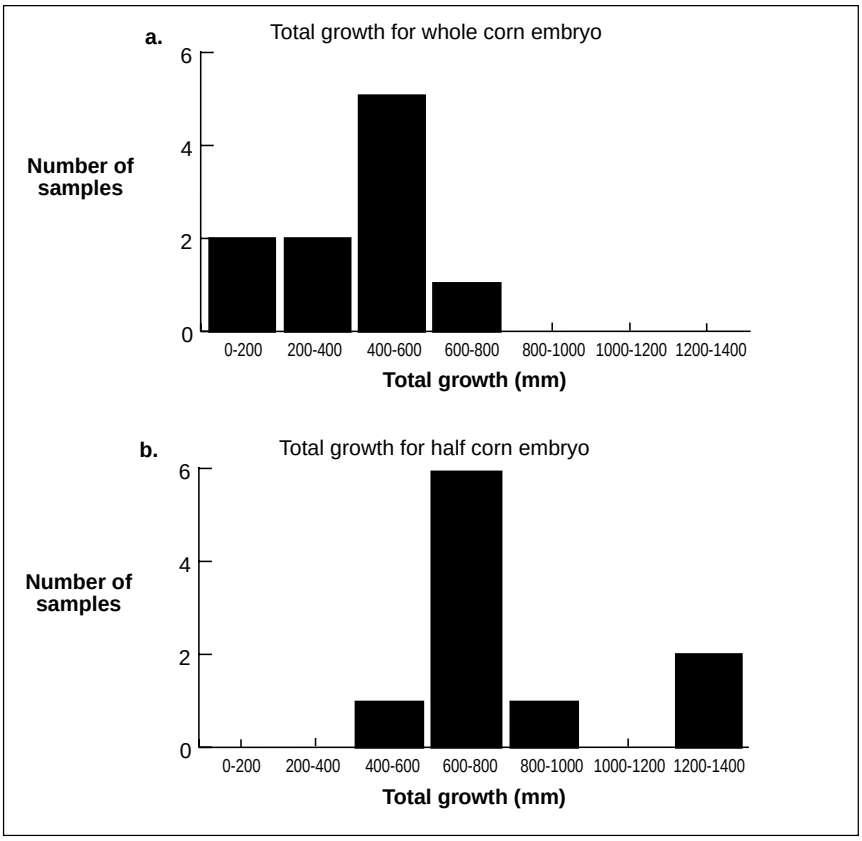
TEACHER'S NOTES

Interpretation

Statistical analysis reveals that the growth of half and complete embryos differs. We accept the hypothesis. Inspection of these data reveals that complete embryos grew faster than half embryos.

Answer to Test Question

C



Graph D. Total growth for whole (a) and partial (b) corn embryos grown for 13 days.

TEST QUESTION

One group of students recorded the following data for an exercise in which they wanted to compare growth of embryos.

Table 11. Growth of partial and complete corn embryos over 3 days.

Time (days)	Partial embryos		Complete embryos	
	Average shoot length (mm)	Average root length (mm)	Average shoot length (mm)	Average root length (mm)
1	2	1	2	3
2	3	2	3	6
3	4	6	4	12

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

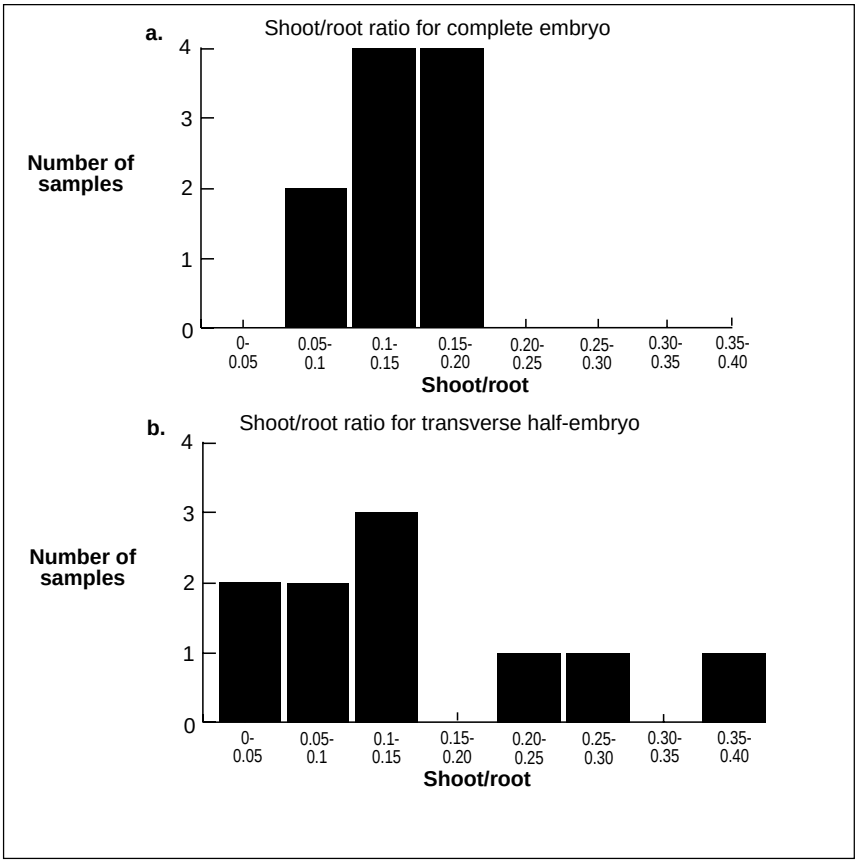
Sample Data

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

Replicate	Transverse half-embryos			Complete embryos		
	Root length (mm)	Shoot length (mm)	Shoot/root	Root length (mm)	Shoot length (mm)	Shoot/root
1	610	89	0.15	660	91	0.14
2	730	76	0.10	1210	105	0.09
3	950	93	0.10	520	79	1.15
4	410	0	0	560	107	0.19
5	680	104	0.15	520	97	0.19
6	690	0	0	630	81	0.13
7	170	50	0.29	860	102	0.12
8	380	75	0.20	520	97	0.19
9	550	70	0.13	1240	96	0.08
10	210	75	0.36	590	93	0.16

Interpretation

Although our half embryos grew more slowly than our complete embryos, their growth was not different than that produced by complete embryos. Reject the hypothesis and accept the notion that values as different as those observed are likely to occur by chance alone.



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

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?

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.

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.

TEACHING TIPS

- Students can collect their own grass seeds for this exercise or you can obtain seeds from a health food store or feed store.
- Accessory floral structures adhere to some grass seeds and should be removed before the seeds are surface sterilized. The extra tissues make it more difficult to sterilize the seeds and more difficult to remove the embryo. Soak the seeds for an hour in 30°C water before removing the floral parts.
- If students practice removing embryos under magnification with a dissecting microscope, they will be able to judge the right amount of seed to remove the embryos without magnification when they need to excise them aseptically.
- The basic structure of grass flowers is a floret. It consists of the flower enclosed in a lower bract (the lemma) that encloses an upper bract (the palea) and the flower. Several florets are combined in a spikelet and usually several spikelets in a more complex inflorescence.
- Figure 8 is provided to help you guide your students in removing the accessory tissues, not to create a vocabulary lesson in grass flower structure. The parts to be removed are what Garrison Keillor refers to as "oat hulls and wheat chaff."

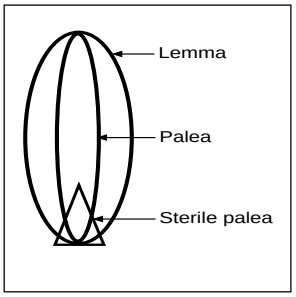


Figure 8. An unmilled grass seed will look like this diagram. The embryo is on the far side of the seed, opposite the surface showing in this diagram.

- All of the grasses used here have a groove opposite the side where the embryo is located.

TEACHER'S NOTES

Sample Experimental Procedure

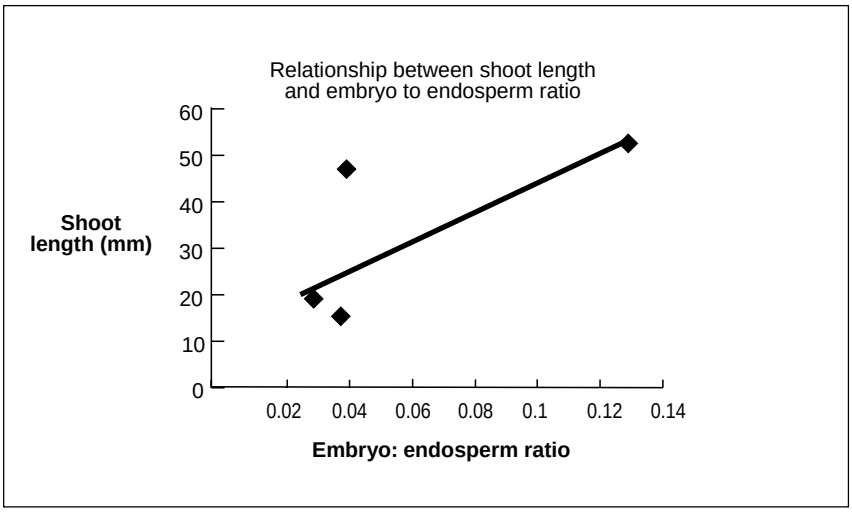
1. Soak seeds for about 1 hour in 30°C tap water to soften the accessory floral parts before removing them. Discard any seeds that have begun to germinate.
2. Sterilize the surface of seeds as in the Core Experiment.
3. Excise the embryos by cutting them away from the endosperm.
4. Transfer 5 embryos 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.

Grass genus	Replicate	Shoot length (mm)	Root length (mm)	Endosperm/ embryo
Zea (corn)	1	71	210	0.128
	2	59	92	
	3	54	113	
	4	43	290	
	5	57	165	
Agropyron (wheatgrass)	1	27	32	0.033
	2	25	20	
	3	17	38	
	4	20	46	
	5	11	21	
Triticum (wheat)	1	23	25	0.038
	2	12	17	
	3	15	36	
	4	23	33	
	5	12	20	
Hordeum (barley)	1	53	27	0.039
	2	51	36	
	3	50	56	
	4	28	60	
	5	40	30	



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 H_2O
- 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 (R^2= 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.

TEACHER'S NOTES

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

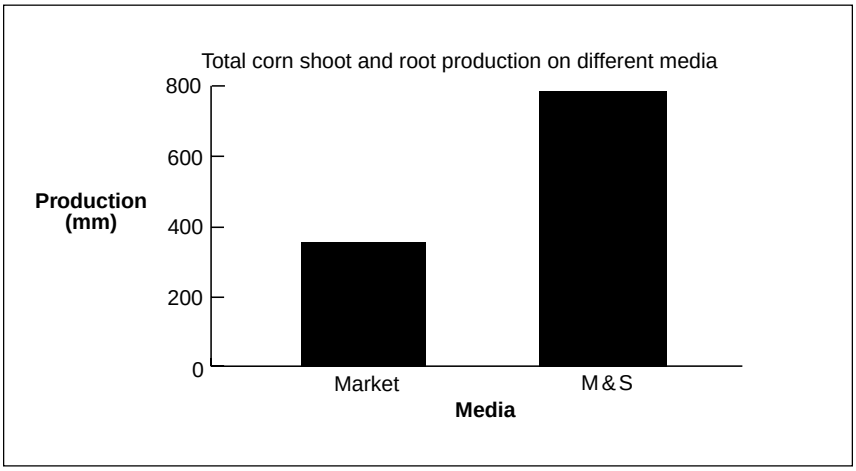
1. Surface sterilize seeds following directions of the Core Experiment.
2. Using the procedures of the Core Experiment, extract embryos and place 5 embryos horizontally on M&S Basal Medium and 5 embryos on Market Tissue Culture Medium.
3. Seal the petri dishes with Parafilm™ or PetriSeal™ and place the 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.

Replicate	Medium type	
	Market	M&S
1	155	599
2	280	617
3	288	667
4	450	741
5	490	1315
Mean	333	788



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.

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.

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.

Replicate	Market Medium			Endosperm present		
	Root length(mm)	Shoot length	Root/shoot length(mm)	Root length(mm)	Shoot length	Root/shoot length(mm)
1	240	40		64	28	
2	130	25		25	24	
3	250	38		9	8	
4	400	50		8	10	
5	430	60		33	17	

Answer to Test Question

Table 16. A comparison of the root to shoot ratio produced by corn grown with different nutrient sources.

Replicate	Market Medium			Endosperm present		
	Root length(mm)	Shoot length	Root/shoot length(mm)	Root length(mm)	Shoot length	Root/shoot length(mm)
1	240	40	6.0	64	28	2.3
2	130	25	5.2	25	24	1.0
3	250	38	6.6	9	8	1.1
4	400	50	8.0	8	10	0.8
5	430	60	7.2	33	17	1.9

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.

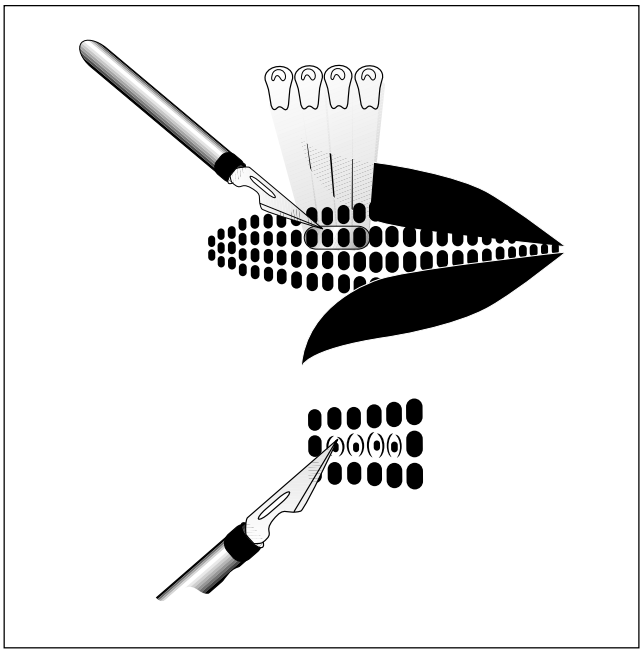


Figure 1. Excising the embryo from a corn kernel.

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?

You must have your teacher approve this protocol before you begin this experiment.

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?