



Kansas Corn: Plant Tissue Culture of the African Violet

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Grade Level: High School

Overview

The African Violet is a great species of plant to introduce tissue culture to your students. The goal of this lab is to propagate as many genetically identical plants as possible from a small tissue sample. It is essential to maintain a strict, sterile environment. One fungal spore or bacterium in contact with the growth medium will quickly reproduce and outcompete your explant for nutrient resources. Students should be able to see a round mass of undifferentiated cells, called a callus, form in about 2 to 4 weeks. This will be followed by the growing of shoots another 2 to 4 weeks later. At this point, you will be able to cut away these plantlets and transplant them into fresh medium that will promote the development of roots. Root formation is usually seen at about 6 to 8 weeks. By the end of this lab, students will have the knowledge and technical ability to effectively and rapidly grow their favorite plant species. This is a small but necessary skill that can be used to help feed the world and protect its plant species.

Kansas College and Career Ready Standards

- **HS-LS1-2.** Develop and use a model to illustrate the hierarchical organization of interacting systems that provide specific functions within multicellular organisms.
- **HS-LS1-5.** Use a model to illustrate how photosynthesis transforms light energy into stored chemical energy.
- **HS-LS1-6.** Construct and revise an explanation based on evidence for how carbon, hydrogen, and oxygen from sugar molecules may combine with other elements to form amino acids and/or other large carbon-based molecules.
- **HS-LS2-7.** Design, evaluate, and refine a solution for reducing the impacts of human activities on the environment and biodiversity.
- **HS-ESS3-4.** Evaluate or refine a technological solution that reduces impacts of human activities on natural systems.
- **HS-ETS1-1.** Analyze a major global challenge to specify qualitative and quantitative criteria and constraints for solutions that account for societal needs and wants.
- **HS-ETS1-2.** Design a solution to a complex real-world problem by breaking it down into smaller, more manageable problems that can be solved through engineering.
- **HS-ETS1-3.** Evaluate a solution to a complex real-world problem based on prioritized criteria and trade-offs that account for a range of constraints, including cost, safety, reliability, and aesthetics, as well as possible social, cultural, and environmental impacts.

Kansas Corn: Plant Tissue Culture of the African Violet

Grade Level: High School

Learning Objectives

- How to create and maintain a sterile growing environment with sterile equipment.
- How to prepare a growth medium specific to the species of plant wanting to propagate.
- How to produce clones of a specific plant species.
- Growth of plant tissue in a controlled environment into a fully developed plant that can be transferred to soil.

Materials

- Plant Tissue Culture PowerPoint (available at www.kscorn.com)
- Murashige African Violet/Gloxinia Pretransplant Media - from phytototechlab.com
- 1 L sterile distilled water
- 15 g sucrose (table sugar) per 500 mL
- 5 g agar per 500 mL (use according to instructions for specific agar brand)
- 1 L container for growth media
- 1 M NaOH or HCl to adjust pH to 5.7
- pH paper or pH meter that measures in tenths
- Petri dishes, test tubes or small containers with caps or lids to house explants
- Saran wrap
- Autoclave, pressure cooker or oven
- Aluminum foil for sterilization
- Sterile chamber options:
 - Cardboard box lined with plastic sheeting or
 - Glass aquarium or
 - Plastic storage bin or
 - PVC scaffolding lined with plastic sheeting or
 - Oven cooking bag
- Adhesive tape for plastic lining and covering
- 8-in. forceps or tweezers
- Glass stirring rod
- Scalpel, razor blade or cork borer
- Gloves
- Hot plate or microwave
- Petri dishes or glass plates for cutting samples
- Beakers or mason jars with screened lids for washing plant tissue
- Soft toothbrush or paintbrush
- African Violet plant
- 10% bleach solution

Kansas Corn: Plant Tissue Culture of the African Violet

Grade Level: High School

- 70-90% isopropyl alcohol in spray bottle
- 2 gal. of sterile distilled water
- Dawn detergent, unscented
- Well-lit area with fluorescent or grow lights – no direct sunlight
- Outlet timer
- 25-40 2-4-in. diameter nursery pots – find the best pot for your specific African Violet variety
- African Violet potting mix

Safety Considerations

- Make sure to work in a well ventilated area – do not breathe in fumes from cleaning solutions.
- Bleach solution will be used – be careful with work area and clothes, especially to avoid skin and eye contact.
- If samples become infected, rinse with 10% bleach solution before disposal.

Procedures for Instruction

Length of Time for Preparation

- 1 hour for setup of sterile area
- 1 hour for solution preparation

Length of Time for Classroom Teaching

- 50-minute class period for plant tissue culture lecture: theory and procedure
- 50-minute class period for culture of explant
- Periodic observation of tissue culture over the next 4 to 6 weeks
- 50-minute class period for each subsequent micropropagation
- 50-minute class period for transplantation of plantlet to pot

Preparation Procedure

Creation of Sterile Chamber

- Cardboard box lined with plastic sheeting and plastic sheet covering opening.
- Sterlite storage tub with plastic sheet covering opening.
- PVC scaffolding with plastic sheet lining and plastic sheet covering opening.
- Oven cooking bag. Sterile lab procedure carried out inside bag.

Kansas Corn: Plant Tissue Culture of the African Violet

Grade Level: High School

Preparation of Sterile Chamber and Equipment

- Initially, sterilize the inside of sterile chamber with a 10% bleach solution.
- Use a spray bottle for ease of use. Allow time to dry.
- Constantly sanitize chamber, equipment and hands with 70-90% isopropyl alcohol throughout lab procedure.
- Equipment – forceps, tweezers, scalpels, razor blades, paper towels and gloves – can be sterilized by wrapping utensils in aluminum foil and placing in an autoclave or pressure cooker at 121°C at 15 psi for 20 minutes or an oven at 350°F for 2 hours.
- You can also sterilize equipment and containers using a 10% bleach solution. Equipment should soak for at least 15 minutes. Rinse with sterile water.

Preparation of Growth Media

This will produce 500 mL of growth medium that will make approximately 25 explant tubes at 15-20 mL each or 35 petri dish plates at 12-15 mL pours.

- Add 400 mL of distilled water to your 1-L media container.
- Set container on top of a hot plate and set to continually stir with a magnetic stir bar.
- Heat should be set to low.
- Add 2.33 g of Murashige African Violet/Gloxinia Multiplication Media to the media container.
 - (For a later Pretransplant stage: 2.20 grams of Murashige African Violet/Gloxinia Pretransplant Media)
- Add 15 g of sucrose to media container.
- Add 5 g of agar to the media container. Use the amount according to instructions for the specific brand of agar.
- Add enough distilled water to bring the total volume of media to 500 mL.
- Adjust pH of media to 5.7 by adding 1 M NaOH or HCl, drop by drop.
- Slowly bring the media to a boil for 10 minutes. Be careful. The solution will quickly boil over.
- Turn off the heat and let it cool to the touch.
- Pour media into sterilized test tubes or petri dishes. You want about 15–20 mL for each container.
- Cover test tubes with test tube caps and petri dishes with its top plate immediately and let cool.
- Tilt the test tubes and petri dishes at a slight angle so water drains down and doesn't drown the tissue samples.
- Store in a sanitized area.
- If individual containers are not sterile, pour media and place containers in an autoclave or pressure cooker at 121°C at 15 psi for 20 minutes. If using an oven, set at 350°F for 2 hours. Wrap in aluminum foil. If using plastic equipment, only use plastic approved for autoclave use.

Kansas Corn: Plant Tissue Culture of the African Violet

Grade Level: High School

Background Information

By 2050, the global human population will surpass 9 billion, which will present new challenges to humanity and the world. One of these challenges will be the ability to feed this increasing number of people on less available land for farming. One solution will be to utilize techniques that quickly grow specific plants year-round in a small space that will be ready for planting in areas when the growing season is ready to begin. This would also work great for species of plants that are slow to develop or in areas that have shorter growing seasons. Another solution is to grow many, identical plants that can be available for scientific testing as well as for the genetic engineering of specific plants with specific traits. These traits can protect the plant from pests and disease and allow them to better tolerate drought and flooding conditions.

A tool that scientists have been using for a while now is called plant tissue culture. It goes by many other names, such as in-vitro culture, cell culture and micro-propagation. Tissue culture can produce many plants from just pieces or even a small sample of cells from another plant called the mother plant. The pieces can come from the petals, leaves, stems or roots of the plant. These tiny tissue samples are called explants and are then placed in a growth medium under a sterile, controlled environment. The explant will be able to rapidly produce new shoots called plantlets. These plantlets can then be divided up and each placed in a new growth medium for further development. This can lead to the rapid growth of certain species – going from one small tissue sample to hundreds, or even thousands, of identical plantlets. These plantlets will grow into sturdy, healthy plants which are then transferred into soil and are grown outside as they would in normal conditions.

View the Plant Tissue Culture of an African Violet PowerPoint at www.kscorn.com for additional background information and topics covering plant physiology.

Classroom Discussion

- Why would we want to grow plants in a lab rather than in the ground?
- What conditions and nutrients are needed to grow a plant?
- What do we mean when we say that something is a clone?
- What uses are there for growing hundreds or thousands of identical plants?
- What does it mean to have a sterile environment?
- Why would we want a sterile environment when cloning plants?

Kansas Corn: Plant Tissue Culture of the African Violet

Grade Level: High School

Procedure for Lab

Sterilizing Plant Samples

Best to be done in an area near a sink.

1. Take plant sample(s) – petals, leaves, buds, ovaries, seeds, anthers and/or nodal segments – and place in a 250-mL beaker or similar sized glass container. A mason jar with a screened lid would be ideal.
2. Fill the beaker with tap water. Swirl your plant samples around and pour out the water, keeping the plant samples in the beaker. Repeat this at least two more times to rinse off any dirt and debris.
3. Fill the beaker with water again and then add about 1 mL of Dawn detergent to rinse off any oils.
4. Use a glass rod to carefully swirl the plant samples around the soapy water. Continue for at least 10 minutes.
5. Toward the end of the 10 minutes, use a soft toothbrush or paintbrush to gently scrub the plant samples.
6. Rinse the plant samples thoroughly with tap water until no detergent remains.
7. Spray plant samples with a 70% alcohol solution. Wait 30 seconds, then rinse thoroughly.
8. Fill the 250-mL beaker or with a 10% bleach solution – enough to immerse your plant samples – and add a few drops of detergent to kill any remaining bacterial or fungal pathogens.
9. Leave the samples in the bleach solution for 15 minutes, gently swirling or stirring every few minutes. This sterilizes both the plant samples and the inside of the container.
10. Rinse the plant samples thoroughly with sterilized water and cover with a sterilized lid.
11. Place the container with the sterilized plant material beside the sterile chamber. Do not place the container inside the sterilized chamber as the outside of the container will contaminate your chamber.

Transfer of Explants in a Sterile Chamber

1. Sterilized utensils should be soaking in a 70-90% isopropyl alcohol solution inside the chamber.
2. A glass plate or petri dish for cutting and the containers for the prepared explants should be in the chamber.
3. Take off any jewelry on your hands and wrists.
4. Use the 70-90% isopropyl alcohol solution in a spray bottle to mist your hands or gloves. Rub them together to adequately disinfect.
5. Use sterilized 8-in. forceps or tweezers to remove your plant samples from the washing container and transfer to the glass plate or petri dish.
6. Use a razor blade, scalpel or cork borer to cut out a small sample of plant tissue about a ½ cm in diameter. Be sure to include any veins from the plant leaf to ensure an adequate number of growth cells.
7. This tissue sample is now called the explant.
8. Take a growth media container and mist the outside of it with the 70-90% isopropyl alcohol.
9. Take the forceps and place the explant on top and in the middle of the growth media in your sterilized test tube or petri dish containers.

Kansas Corn: Plant Tissue Culture of the African Violet

Grade Level: High School

10. Cover the container with its cap or lid and set aside. Place Saran wrap over the top to protect from microorganisms.
11. Place your utensils in the 70-90% isopropyl solution between transfers.
12. Repeat this procedure for each explant you want to culture.

Growing of Plantlets

1. Set the containers with the explants in a well-lit area, but not in direct sunlight.
2. Provide 12-16 hours of light with fluorescent or grow lights at 8-10 in. overhead.
3. Use an outlet timer for consistent lighting.
4. Keep explants at a consistently warm temperature between 72°F and 82°F. Do not exceed 85°F.
5. Check explants daily and inspect for signs of infection. Bleach and dispose of any infected samples.
6. After 2-4 weeks, a mass of cells will start forming. This is called a callus.
7. Around 4-6 weeks, you should start to notice the formation of shoots called plantlets.
8. Once the shoots of the plantlets grow about an inch in length, you may separate them from each other and place them in a larger container with new Murashige African Violet/Gloxinia Pretransplant media by repeating the Transfer of Explants procedure.

Potting of Plantlets

1. When the plantlets show signs of well-developed roots, they are ready to be transferred to a soil pot.
2. Remove each plantlet from its container.
3. Carefully rinse off any media with distilled water.
4. Place the plant in a nursery pot filled with African Violet potting mix.
5. Place the pots in a tray and cover with a clear plastic sheet as they are not used to the drier air of the lab.
6. Continue to provide 12-16 hours of light each day, though still no direct sunlight.
7. After 1-2 weeks, remove the plastic cover in order to acclimate the plants to the outside conditions of the lab.

Teachers Tips

- These plants are now identical clones of each other. You will be able to use them in classroom experiments involving other variables as their genetic makeup is now considered a constant.
- Test the different types of sterile chambers seen above in Preparation of a Sterile Chamber under Lab Setup.
- Test growth media with different ratios of hormones specific to the plant type to see which will grow faster or have healthier plants.
- Test different plant species to see which will perform better with this culture technique.

Kansas Corn: Plant Tissue Culture of the African Violet

Grade Level: High School

- Always spray hands or equipment with 70-90% isopropyl alcohol before handling.
- Students must work fast, but controlled as the longer samples are exposed to air the higher likelihood of contamination.
- Full attention must be given to heating the media to a boil. It will boil over fast. Have oven or heat gloves and tongs next to the hot plate in order to immediately pull away media container from the heat source.
- Disinfect contaminated samples with a 10% bleach solution before disposal.

Lab Analysis

- Keep a digital photo journal of plant growth with stages of development and plant characteristics labelled.
- Document how many of the samples were infected. What percentage survived?
- Measure plantlet length over time.
- Try different sections of the same plant – leaf, stem, root, or flower – to see which explant part grows fastest.
- Count the number of shoots each explant grows. Find the average.

Reflection and Conclusion

Have students research and answer the following questions:

- What is the main goal of using plant tissue culture?
- What are other names for plant tissue culture?
- What are the benefits of plant tissue culture instead of using seeds?
- What other plants would make good candidates for plant tissue culture?
- What is a sterile environment, and why is it important in tissue culture?
- What are some ways to sterilize equipment? A laboratory environment? A plant tissue sample?
- What was your success rate of samples growing without infection? Hint: $(\text{surviving explants} / \text{total explant samples}) \times 100\%$.
- What are some factors or procedures that caused your tissue samples to become infected?
- A small sample of tissue is taken from a mother plant in order to clone many new plants. What is this small tissue sample called?
- What do we call an explant that develops into a mass of undifferentiated cells?
- When do we call the sample a plantlet? A plant?
- Research and report: How could tissue culture specifically be used to help Kansas corn farmers?

Kansas Corn: Plant Tissue Culture of the African Violet

Grade Level: High School

Science and Agriculture Careers

- Agronomist
- Analytical chemist
- Animal geneticist
- Arborist
- Bioinformatics scientist
- Biological technician
- Biosecurity monitor
- Biostatistician
- Conservationist
- Crop advisor
- Crops systems specialist
- Ecologist
- Environmental engineer
- Florist
- Greenhouse manager
- Horticulturist
- Hydroponics producer
- Microbiologist
- Molecular biologist
- Plant biologist
- Plant breeder
- Plant geneticist
- Plant pathologist
- Research associate
- Research scientist
- Soil scientist
- Viticulturist
- Weed

Sources

- Ohio Corn Education - <https://ohiocorneducation.org>
- Tissue Culture Propagation: Class 101 - <https://www.youtube.com/watch?v=qDOGrEhUe8A>
- Tissue Culture with Bill Graham - <https://www.youtube.com/watch?v=B3yi5U9Cg64&index=22&t=0s&list=PLcyQYW7ZguqjQc23hAhddcyRLUXy55LZg>
- Plant Tissue Culture - <https://www.youtube.com/watch?v=6y13hYGPi8Q>

Any educator electing to perform demonstrations is expected to follow *NSTA Minimum Safety Practices and Regulations for Demonstrations, Experiments, and Workshops*, which are available at <http://static.nsta.org/pdfs/MinimumSafetyPracticesAndRegulations.pdf>, as well as all school policies and rules and all state and federal laws, regulations, codes and professional standards. Educators are under a duty of care to make laboratories and demonstrations in and out of the classroom as safe as possible. If in doubt, do not perform the demonstrations.