Comparison of Indigenous and Non-indigenous Palm Species to Study Resistance to Lethal Yellowing

Melvin Poole, Jr.
Hawthorne Middle/High School

Abstract:

The goal is to designed a ready teaching unit that will give students practice with integrate biotechnological tools to solve societal problems. Students will do a genetic comparison of native and non-native Florida palm trees for the purpose of trying identify possible genes that may confer resistance to Lethal Yellowing. Lethal Yellowing (LY) is a disease of palm trees that presents with the yellowing and drooping of palm fronds followed by the death of the tree as the spear leaf collapses and bud dies. LY is caused by a phytoplasma, a fastidious cell wall-less bacterium, and transmitted by the planthopper *Myndus crudus*, a common planthopper in southern Florida.[1] Fortunately LY only affects palms that are not native to Florida.[2] A rudimentary understanding of LY resistance in native palms can be approached by doing a genetic comparison of native palms with non-native palms via electrophoresis.

Description of teaching unit and Expectations:

This inquiry will be used as a part of a unit exploring the impact of biotechnology on the individual, society and the environment.[3] I expect students to learn to use the technique of electrophoresis as it applies to agricultural problems.

Here's how I envision the context of the lesson I'm proposing:

1. Lesson on Plant anatomy and Physiology[4]
2. Introduction to the economic significance of the palm tree and palm tree products.
3. Introduction of the Problem: outbreak of lethal Yellowing threatens to come to Florida (do I study for test now or the morning of?)
4. Introduction to the tools and techniques of Biotechnology[5] (I'm thinking I may need to teach about the tools and techniques prior to this lesson . . . but will there be time?)
5. Brainstorm: How can we use the tools of biotechnology to insure the palm tree industry remains sustainable?[6]
6. Lesson on what is known about the palm tree geome
7. Strategize our plan of attach towards getting a solution.
8. DNA extraction
9. PCR
10. Electrophoresis
11. Southern blot: may need to do a simulation at this stage depending on whether we get bands or not.
12. DNA BLAST unique sequences to determine possible proteins and functions?
13. Not sure where to go from here; some sort of closure to our inquiry (remember Melvin, the goal is to get at the molecular bases for native palm's resistance! Have student weigh strategies[7] with each

[1] Document P-222 of the Plant Pathology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, 2005.
[2] Emerging Pathogens materials compiled by Charles Lawrence, MPH, Ph.D
[3] SC.9.12. L.16.10 – Biotechnology and Society
[4] SC.9.12.L.14.7 – Plant anatomy and Physiology
[5] Extraction, PCR, Electrophoresis, southern blotting, western blotting, ELISA
[6] SC.912.N.4.1 – Science and Society

other, perhaps? I'm going to have to spend more time thinking about where I want them to go with all this . . .)

Data Collection techniques/Student Assessments:

A pre and post test will be used to test how well students transfer skills in DNA extraction, PCR, electrophoresis, and et cetera to application of societal problems.

ICORE Summer Institute Elements:

Procedural assistance/Equipment for the following techniques:

-DNA extraction

-PCR

-Electrophoresis (e-gels)

-Southern Blotting or DNA Blaster (Can't remember if this gives a protein from a DNA sequence)

Literature Cited:

Please see footnotes

Budget:

Reagents and equipment for DNA extraction

PCR tubes and Thermocline (CPET-UF)

E-gels (Perhaps a longer gel may need to be run; maybe Dr Chen can inform and help on this)

Southern blotting?

[7] SC.912.N4.2 – Weighing options for solving problems

Palm Pathogen: A Genetic Pursuit

KEY QUESTION(S):

1. What are the specific skills involved in conducting a **scientific inquiry** of a particular phenomenon?
2. Specifically, how would particular skills of scientific inquiry be used to solve **scientifically investigatible societal problems**?
3. In what ways is scientific knowledge and processes useful for some areas of study and but limited for use in other areas?
4. What aspects of **molecular genetics** have been applied and used to create the **reagents** and processes used in **biotechnology**?
5. What are the **techniques of biotechnology**? How do these techniques integrate with each other to help give insight to or solve investigatible societal problems?

***SCIENCE SUBJECT**: Biology

***GRADE AND ABILITY LEVEL**: 9th and 10th Grade Honors

SCIENCE CONCEPTS: Applied Science Process Skills, Biotechnology Laboratory techniques, Genetic and Molecular Biology

OVERALL TIME ESTIMATE: Three Days (This lesson involves a series of activities).

LEARNING STYLES: Visual (molecular models and diagramed processes in molecular biology), auditory (lecture/discussion), and or kinesthetic (laboratory), tactile (laboratory).

KEY VOCABULARY:

1. **scientific inquiry**
2. **scientifically investigatible societal problems**
3. **molecular genetics** (DNA, RNA, Polymerase, leading strand, lagging Strand)
4. **reagents** (buffer)
5. **biotechnology**
6. Amplification
7. Polymerase Chain Reaction
8. Protoplasm primers (and associated reagents)
9. Electrophoresis
10. Agarose Gel
11. Digested and Amplified protoplasm DNA (to run on gel to make comparison)
12. **techniques of biotechnology (DNA extraction, Polymerase Chain Reaction, Electrophoresis, Southern Blotting, western blotting, ELISA)**

LESSON SUMMARY: Student will use the processes of scientific inquiry and apply their knowledge of molecular genetics and acquired skills in biotechnology procedures to positively identify the protoplasm responsible for lethal yellowing thru the processes of DNA extraction, PCR amplification, digestion, and electrophoresis. Students will be challenged to integrate and generate meaningful understanding of how biotechnology is used in problem based context thru laboratory and classroom discussion.

***STUDENT LEARNING OBJECTIVES WITH STANDARDS:**

Learning Objective	**Florida Science NGSSS**	
Given a biotech problem, student should be able to 1. identify or predict appropriate biotechnology techniques to use, 2. give the sequence by which those techniques are to be used, and 3. tell the purpose for those techniques.	L16.12 – DNA Cloning SC.912.L.16.10 - Evaluate the impact of biotechnology on the individual, society and the environment, including medical and ethical issues.	-DNA extraction -Polymerase Chain Reaction -Electrophoresis
Identify natural processes in molecular biology that are borrowed and modified for use in biotechnology.	L.14.2 – Plant and animal cell components L14.3 – Prokaryotic & Eukaryotic Cells L16.3 – DNA Replication 16.5 – Cell Cycle & Cancer 16.8 – Protein Synthesis	-Hypotonic solutions -DNA Replication -Rates of movement for different fragments

Number your objectives for easy reference. Focus on what students will KNOW, FEEL, or be able to DO at the conclusion of the lesson, not what they are doing during the lesson. Use specific, measurable, observable verbs. Avoid general terms like "know," "understand," or "appreciate." Include at least ONE objective for EACH subject area addressed. Remember that each objective will require at least one specific assessment. Correlate lesson objectives to state or national standards.

***MATERIALS:**

DNA Extraction of phytoplasmas from Palm Fronds:

Student Work Station	Source	Number required per group	Number required for class
Lysis Buffer: Trizma (1.21 g) Na_2EDTA (0.4 g) CTAB (2.0 g) Buffer Additive NaCl (8.12 g)	Aldrich	2	12
Leaf Samples		2	12
Micro pipette 20-200ul	UF	2	12
Sharpie	Classroom		
Microcentrifuge tubes	Fisher		
Sterile blue micropestles	Fisher		
0.2 PCR Tubes	Fisher		

P 20 Pipette tips	Fisher		
P200 Pipette tips	Fisher		

Amplification of Phytoplasma DNA

Student Work Station	Source	Number required per group	Number required for class
Thermal cycler	UF	1	
Phytoplasma DNA			
70% ethanol			
Analytical Scale	UF	3	
P1000 pipette tips		1 box	
Kimwipes	Classroom		
1.5 ml microcentrifuge tubes		Four per group for each aliquot	
Sterile Distilled Water	Invitrogen	1 tube	

Electrophoresis of Amplify DNA with Commercial Phytoplasma DNA

Student Work Station	Source	Number required per group	Number required for class

BACKGROUND INFORMATION: Assume the teacher knows very little about your lesson topic. Provide accurate, up-to-date information from reliable sources. Define all key vocabulary. Usually a minimum of 3 paragraphs and maximum of 2 pages double-spaced.

ADVANCE PREPARATION: Explain what the teacher needs to do to get ready for the lesson, e.g., choose and mark a study site; prepare a solution; prepare a list of local organisms; obtain news clippings regarding an issue; etc.

***PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:** This is the "heart" of your lesson plan. Assume you are writing this lesson for a substitute teacher to follow. (They do not have any idea what to do unless you clearly explain it to them).

Divide your procedure into numbered steps with time estimates (in minutes) for each step. Be specific. Don't just say "Introduce concept of food webs." Instead, explain HOW the concept will be introduced. Are you giving a brief lecture? Doing a demonstration? Asking a series of whole-class questions? Showing a film clip?

Middle school lessons are generally 40-90 minutes (regular or block schedule) and high school lessons are usually 50-100 minutes (regular or block schedule). Elementary lessons can be as brief as 30 minutes.

When including discussion questions in a lesson plan, list specific questions to ask and provide sample acceptable answers in parentheses. Don't just say: "Conduct a discussion of the impact of global warming." Instead, list exactly what questions the teacher should ask.

***ASSESSMENT SUGGESTIONS**: Describe specific assessments for EACH objective:
For objective 1…

For objective 2… etc.
Don't just say "Collect student worksheet " or "Give an oral quiz." Need to clearly indicate the exact question or task that will be used to assess each objective.

EXTENSIONS:

ACTIVITIES: Are there other activities you know of from other resources that relate to this lesson?

LITERATURE: Are there trade books, novels, journal articles, or other print materials that focus on the same topic(s) as this lesson?

***RESOURCES/REFERENCES:** List all print and/or web-based references/resources used for either lesson ideas of content background information. Provide complete citations for all references.

Opener: I know what you're thinking . . . : Palm Trees? Really, Mr. Poole? Of all the things to study . . .? But in actuality, turning our scientific inquiry skills on the pursuit of palm trees is anything but trivial (notice the trivial pursuit allusion)

OK Better Opener: Something more with a more let's find an interesting scientific question to this question with some prodding from Mr. Poole.

Standard to consider: Essential Questions