ICORE Development Award Proposal

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Abstract (150)
In order to introduce students to various biotechnology / molecular biology techniques, my AP Biology class will be studying the molecular characteristics of the tomato spotted wilted virus infected peanut. This will also allow students to understand and appreciate the extent of emerging pathogens and their economic burden. Typically, students perceive emerging pathogens as only affecting humans. This exercise will bring a larger perspective to them, gain an appreciation of how these pathogens evolve and allow working hands on in the topic. It will also provide a platform to launch a biotech program at school.

Rational

TSWV-infected peanut leaves will be analyzed by ELISA, PCR, PAGE-SDS and MALDI. Non-infected plants would be used as controls.
Alternatively tomatoes/red peppers, TMSV-infected or not, or grown in full media versus nitrogen or phosphate depleted media would be used as comparisons.
This will allow intermeshing pathogens of economic importance with the role of macronutrients in plant growth.

I want to do this in the classroom so that

Students

- Become aware of pathogens of economic impact
- Understand and use biotech to study emerging pathogens in a secure atmosphere
- Cover the following Sunshine State Standards for Honors Biology
- Experiment and see first hand the effect of macronutrients (biogeochemical cycles) in the life and growth of plants
- Will experiment on plant biology and photosynthesis
- Will be able to isolate DNA, RNA and proteins, and to amplify DNA and/or RNA as a mean to understand DNA replication, RNA transcription and protein translation
- Will gain evidence for the theory (law) of evolution by analyzing the sequences of selected protein digests
- Explore techniques and careers in biotechnology

Teachers

- I am expecting to create a Biotech Club at my school as a way to involve other teachers and students in biotechnology, using this discussion and experimentation forum as a platform for science fair and research projects.
- In particular, this year I will have a student assistant who will work on a project of the effects of *Euphorbia hirta* and *Euphorbia tirucalli* on the protein expression pattern of mosquito larvae. Those plants are well known for their larvicidal action. Molecular characterization has not been researched yet. Hopefully this will be a collaboration done with Dr. Cheng's and Dr. Connelly's laboratories.

School and Community

- An implementation of a biotech program with the aims of growing it into a class will give our magnet school and ability to keep pace with the needs of the market place, place our students in a position to apply for internships of higher value, improve their college placement, while keeping their AP testing, explore the option of obtaining a technical certificate in biotech skills.
- Eventually, I have requested for next school year 2011-2012, to have my AP biology class split into lectures and labs. This format would allow for the AP biology test taking and obtaining the biotech certificate. This biotech/AP program will need the support of higher learning institutions such as UF, FAU, and other local and state agencies.

Description of teaching unit or module(s), including expected outcomes

These lab experimentations will encompass several AP biology mandated labs (at least AP 5, 6 and 12) as well as several of the Sunshine State Standards units on molecular biology, evolution, plant biology, biotechnology and biogeochemical cycles.

Data collection techniques and/or student assessments

Outcomes:

Students will be able to learn the basic biotechnology skills and appreciate the way science understand the nature and understand the process of inquiry; and discovery based research. Students will:

- Grow plants and prepare growth media as a tool to understand plant biology, photosynthesis and the role of macronutrients in the soil
- Isolate DNA and learn about DNA as a diagnostic tool to discover pathogens.
- Amplify the isolated DNA using Polymerase Chain Reaction technique and study how DNA samples separate based upon different sizes using gel electrophoresis.
- Sequence the DNA using bioinformatics techniques and study their phylogenic tree.
- Run PAGE-SDS gels to separate proteins based on their size
- Digest protein samples and separate those fragments to send for sequencing
- Be able to use BLAST databases to study protein evolution

ICORE Summer Institute elements specifically included (UF connections)

- ICORE for loaner equipment PCR thermocycler, primers, peanut leaves, polyacrylamide gel electrophoresis chambers, simulation microarrays and simulation ELISAs.
- Dr. Cheng for protein fragments by MALDIs.
- Dr. Cheng and Dr. Polfer for protein fragment analysis through BLAST.
- Dr. Connelly on how to grow mosquitoes in special canisters where the *Euphorbia* extracts can be placed with the larvae
- Dr. Chuck Lawrence's microarrays for plant virus can be a good source to further explore plant pathogens.
- Dr. Dean Gabriel explained how tomato or pepper seeds could be grown rapidly onto growth media culture tubes and use as source of experiments.

Literature cited

1. Erik Kiviat. Traditional protection against biting flies. Hudsonia Ltd. Annandale, New York 12504, U.S.A. 1993

2. Ramos MV, Pereira DA, Souza DP, Araújo ES, Freitas CD, Cavalheiro MG, Matos MP, Carvalho AF. Potential of laticifer fluids for inhibiting Aedes aegypti larval development: evidence for the involvement of proteolytic activity. Mem Inst Oswaldo Cruz. 2009 Sep;104(6):805-12.
3. The effects of different levels of minerals on plant growth. SAPS Scotland Higher Biology Practical. http://www.practicalbiology.org/areas/intermediate/environment/factors-affecting-plant-growth/investigating-the-effect-of-minerals-on-plant-growth,126,EXP.html
4. John Sawyer. Measuring the nitrogen status. (2010). http://www.extension.iastate.edu/CropNews/2008/0612JohnSawyer.htm
5. University of California College Prep for AP Biology (2010). Phosphate/orthophosphate extraction and measurement. http://www.ucopenaccess.org/courses/APBioLabs/course/index.html
    a. LAB 5 AP Biology
    b. LAB 6 AP Biology
    c. LAB 12 AP Biology

Budget and budget justification

ELISA anti TSWV	
Immunostrips anti TSWV	$90.05
Agdia ACC00936, 25 bags + 25 strips	
RT-PCR of TSWV	
E-Gel Starter Kit with 6-2% Gels with SYBR Safe	$109.95
Carolina 213807	

ICORE lockers

PCR thermocycler	$ 0.00 (!!)
PAGE chambers	$ 0.00 (!!)
Simulation microarrays and ELISAs	$ 0.00 (!!)
TSWV Primers	$ 0.00 (!!)

Existing at school

VERNIER probes (for phosphates and nitrogen content of leaves)
Spectrophotometers with cuvettes
DPIP
Deoxynucleotide Mix (BIO-RAD 11760), 10 mM each of dATP, dGTP, dCTP, and dTTP. Sufficient for forty 50-µL PCR reactions.
Taq DNA Polymerase (BIO-RAD 211750). New England BioLab®s. 100 units; 5 units/µL. Supplied with 10× buffer.