BIO LAB: Effect of Environment on Mitosis (Week 11A)

Description:

You will set up and analyze an experiment using onion bulbs. The lab experiment is based on the premise that lectins increase the rate of mitosis in roots. Lectins are proteins that bind to specific carbohydrate groups. Scientists reported that a fungal pathogen may affect the growth of soybeans (*Glycine max*). The soybeans growth was decreased during three years of heavy rainfall. The soybean roots were poorly developed. Close relatives of *R. anaerobis* are plant pathogens and grow in the soil. A lectin-like protein, which might have been secreted by the fungus, was found in the soil surrounding the soybean roots. Lectins accelerate the mitosis in some root apical meristem; however, in many instances, rapid cell division weakens plant tissues. You will be using onions instead of soybeans because onion root tips are more easily grown and studied. It will take you the whole week to complete this lab part of it you will do independently on your own.

Notes about lectin:

Phytohemagglutinin (PHA-M) is a lectin. Lectins are proteins that bind to specific carbohydrates. PHA-M induces mitosis, in other words acts as a mitogen in cultured T-lymphocytes by binding to the T-cell receptor for antigen (part of the T-C complex). This causes an intracellular signal involving Ca^{2+} release from the endoplasmic reticulum, ultimately causing cell replication.

Prelab questions:

Form groups of two or three and answer these questions:

- What is your experimental hypothesis? What is your null hypothesis? How are these different?
- How would you design an experiment with onion bulbs to test whether lectins increase the number of cells in mitosis?
- What would you measure? How would you measure it?
- What would be an appropriate control for you experiment?

Materials:

Each of you will be provided with the following materials to perform the experiment:

- Onion sets, scallions
- Jars with lids, 8-10 cm in diameter
- Sand
- Ethanol
- Glacial acetic acid (17.4 M)
- Hydrochloric acid (12 M)
- Carbol-fuschin (Zeihl-Neelson) stain
- Lectin (phytohemagglutinin, PHA-M, from *Phaseolus vulgaris*)
- A razor blade
- Forceps
- Dissection scissors
- Slides and cover slips
- Scientific cleaning wipes
- A coplin jar
- Petri dish
- Disposable gloves
- A compound microscope

Procedure:

1. Dissolve 10 mg of lectin in 200 ml of water. Exposure to lectin may cause irritation so do wear your gloves!

2. Mix 125 ml of the glacial acetic acid with 375 ml 95% ethanol to prepare your Carnoy's fixative.

3. Fill two jars with approximately 1.5 cm of fine sand. Label one "control" and the other "lectin".

4. Wet the sand in the control with water.

5. Wet the sand in the lectin jar with 50-75 ml of lectin.

6. Cut off the green leaves and the dry roots of the onion bulb with the razor blade.

7. Insert the bulbs in the sand until they reach the bottom of the jars.

8. Store the jars in the dark for two days.

9. Wearing gloves, take other the bulbs and rinse the sand with water.

10. Cut off the roots from each bulb using the dissection scissors. Be careful not to mix the lectin and the control bulb!

11. Place the cut roots in your Carnoy's fixative for 18 hours.

12. Decant off the fixative and rinse the tips with 25 ml of 70% ethanol.

13. Place the tips in 70% ethanol and store them covered at 4°C (in the fridge, NOT the freezer).

14. Place the tips in 1 M HCl for 4 minutes.

15. Transfer the tips into the Carnoy's fixative for 4 minutes.

16. Remove the slide from the Coplin jar containing 70% ethanol, dry with scientific wipe and label properly.

17. Place the onion tip on the slide and cut off the distal 2 mm portion of it; discard of the remainder.

18. Cover the root tip with the carbon-fuschin stain for 2 minutes.

19. Blot off the excess stain and cover the tip with 1-2 drops of water.

20. Place the cover slip over the slide and clean with a scientific wipe.

21. Observe the cells at high magnification (400-500 X).

22. Look for well-stained, distinct cells.

23. Within the field of view count the cells in each phase; repeat with two more root tips.

24. Collect the class data for each group and calculate the mean and standard deviation.

25. Compare the number of cells in interphase and in mitosis; use a chi-square distribution test to statistically analyze the data.

Postlab questions:

Form groups of two or three and answer these questions:

- What was the importance of collecting the class data?
- Was there a significant difference between the groups?
- Did the fungal pathogen lectin increase the number of cells in mitosis?
- What other experiments should you perform to verify your findings?
- Does an increased number of cells in mitosis mean that these cells are dividing faster than the cells in the roots with a lower number of cells in mitosis?
- What other way could you use to determine how fast the rate of mitosis is occurring in root tips?

Assignment:

I expect to have your full lab report by the end of next week, including your lab notebook where you took down your notes while conducting the experiment. Please review the requirements for the lab report given to you in the first lab assignment of the year.