The Effects of Lectin on Cell Mitosis

Scientists reported that a fungal pathogen may affect the growth of soybeans (*Glycine max*). The soybean growth was decreased during three years of high 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 may be secreted by the fungus, was found in soil surrounding the soybean roots. Lectins accelerate mitosis in some root apical meristems; however, in many instances, rapid cell division weakens plant tissues.
We are using onions instead of soybeans since onion root tips are more easily grown and studied.

PreLab Questions

These questions will help your students learn to design an experiment based upon an observation. Students can answer these questions for homework or as a group activity.

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

Notes about the lectin: Phytohemagglutinin (PHA-M) is a lectin; a lectin is a protein that binds to specific carbohydrates. PHA-M induces mitosis (acts as a mitogen) in cultured T-lymphocytes by binding to the T-cell receptor for antigen (part of the Ti-C3 complex). This causes an intracellular signal involving Ca2+ release from the endoplasmic reticulum, ultimately causing cell replication.

Preparing Chromosome Squashes

1. Place the onion root tip in 6 M HCl for 4 minutes.
2. Transfer the tip to Carnoy's fixative for 4 minutes.
3. Place the onion tip on a slide, and cut off the distal 2 mm portion of the tip; discard the remainder of the tip.
4. Cover the root tip piece with carbol-fuschin stain for 2 minutes.
5. Blot off excess stain and cover tip with 1–2 drops of H_2O.
6. Place the cover slip over tip and cover the cover slip with a scientific cleaning wipe.
7. Firmly press down on the cover slip with your thumb or with the eraser end of a pencil. Do not twist the slide.

Counting Cells and Analyzing Data

1. Observe the cells at high magnification (400–500 X).
2. Look for well-stained, distinct cells.
3. Within the field of view, count the cells in each phase. Repeat the counts in two other root tips. Identification of these stages is prerequisite knowledge.

4. Collect the class data for each group, and calculate the mean and standard deviation for each group.
5. Compare the number of cells from each group in interphase and in mitosis.
6. Use a chi-square distribution test to statistically analyze the data.

Table 1: Onion Root Tip Cell Phase Data; Control

	Number of Cells		
Tip	Interphase	Mitotic	Total
1			
2			
3			
Total			

Table 2: Onion Root Tip Cell Phase Data; Lectin-treated

	Number of Cells		
Tip	Interphase	Mitotic	Total
1			
2			
3			
Total			

1. Enter the number of treated cells in interphase and mitosis as observed (o).
2. Calculate the percentage of cells in interphase and mitosis in the control group from **Table 4**.
3. Multiply the percentages by the total number of cells in the treated group; this will give the expected numbers (e).
4. Calculate the chi-square ($\chi 2$) value for the test.
5. Compare this value to the critical value in **Table 6**.

Table 3: Class Data

Group	Number		
	Interphase	Mitotic	Total
Control			
Treated			

Table 4: Percentage of Cells in each phase (class data)

Group	Number	
	Interphase	Mitotic
Control		
Treated		

Table 5: Calculation of Chi-Square $\chi^2 = \Sigma (o-e)^2/e =$

	# observed (o)	# expected (e)	(o-e)	$(o-e)^2$	$(o-e)^2/e$
Interphase cells					
Mitosis cells					
				$X^2=$	

Table 6: Critical values of Chi Square Distribution

Probability	Degrees of Freedom (df)				
	1	2	3	4	5
0.05	3.84	5.99	7.82	9.49	11.1
0.01	6.64	9.21	11.3	13.2	15.1
0.001	10.8	13.8	16.3	18.5	20.5

1. The degrees of freedom (df) equals the number of groups minus one. In this case, there are two groups, interphase and mitosis; therefore, df = 2-1, or 1.

2. The p value is 0.05, and the critical value is 3.84. If the calculated chi-square value is greater than or equal to this critical value, then the null hypothesis is rejected.

- If the calculated chi-square value is less than this critical value, the null hypothesis is not rejected. In terms of this part of the investigation, what does it mean if your null hypothesis is rejected?

Post Lab Review

These questions can be answered in a report, as a group activity, or as homework.

- 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 determine how fast the rate of mitosis is occurring in root tips?

Designing and Conducting Independent Investigations

Students can design and conduct an investigation to determine what substances in the environment might increase or decrease the rate of mitosis. Consider, for example, abiotic soil factors such as salinity, temperature, and pH, or biotic factors, including roundworms, which might alter root growth.