Inv. 7, Part 2: The Effects of the Environment on Mitosis

First, read pages S83-S85 in the lab manual as a quick review of the cell cycle and its regulation using CDKs. Then, continue on:

Background

The health of a plant or animal depends upon both biotic and abiotic factors. Imagine the parking lot of your school. A few plants may be growing in cracks and crevices of the pavement. In these cracks there is at least a subsistence level of nutrients and water for a plant to survive. A few meters away an unpaved area with soil and little foot traffic may have more plants. The plants compete for space, water, nutrients, and light but, more than one plant is in the soil area. If you were to compare plants from the paved and soil areas you would likely see differences in the height of the stems, the number of leaves, and the number and length of the roots. This is a simple example of abiotic factors in the environment affecting plant growth.

Many biotic factors also affect plant growth. A classic example of a beneficial biotic effect is the mutualistic relationship between legumes (beans, peas, clover, and alfalfa) and the nitrogen-fixing bacterium, rhizobia. Rhizobia (singular=rhizobium), live in nodules on the roots of beans and other plants. Bean plants with rhizobia nodules are typically larger and healthier than plants that are not infected.

Not all biotic interactions benefit a plant. Parasitic interactions may harm a plant by increasing mitosis. For example, the plant pathogen Agrobacterium tumefaciens (now called Rhizobium radiobactor) causes plant cancer or galls. It does this by triggering the plant to produce certain plant hormones that promote cell division. By forcing the plant to expend more energy in that location and not in the other roots, stems, and leaves, the pathogen weakens the plant and may cause death.

The plant of choice for studying mitosis is the common onion. Onions germinate easily without soil so the chemicals provided to the plant can be easily controlled. Onion root tips also grow quickly and are only a few cells thick. A stain is used to dye condensed chromosomes—like those undergoing mitosis—a very dark color. By viewing the onion root tip using a light microscope it is easy to determine if a particular cell is in interphase or mitosis. See Figure 1 for a graphical representation of the anatomy of an onion root tip. Note that cell division occurs only in the meristem region, not in the other regions of the root tip. Recall also that 90% of the time a cell in the zone of cell division will be in interphase, since mitosis typically makes up only 10% of a full cell cycle. Onions are alive and therefore the onion slide preparation will have more than one layer of cells present in each preparation. In order to reduce the total depth of the slide preparation the onion root tip needs to be treated and then squashed between the cover slip and the microscope slide.

Part 1: Growing roots in altered environments

1. Obtain 5 green onions. Cut the roots on all onions to 0.5 cm. Cut the tops off so all onions are about 5 inches tall.
2. Label your cups: 1, ½, ¼, 1/8, water.
3. Pour 50 mL of water in the cup labeled water and place an onion in it.
4. Dissolve a 200 mg caffeine tablet in 500 mL of water. This will take lots of stirring and will not dissolve completely.
5. Pour 50 mL of your solution into the cup marked 1.
6. Discard 200 mL of the solution, so you have a remaining 250 mL. Add 250 mL of water to reduce its concentration by half. Pour 50 mL of this solution in the cup labeled ½. Repeat this step twice more to produce dilutions of ¼ and 1/8 of the original concentration.
7. Put onions in the remaining cups. Make sure your cups are labeled with your name and the concentration of the solution.
8. Place each cup in a bag and use dental floss to tie the bag around the onion. This will prevent excess water loss.
9. Set aside to wait! We will revisit the onions in a few weeks.

Thinking question: Caffeine (C_{8}H_{10}N_{4}O_{2}) has a molar mass of 194.2 g/mol. Determine the molarity of the solutions you produced.

Part 2: Measuring root growth in an altered environment
1. Remove and discard the floss and bags.
2. Measure the length of the longest root on each of the onions.
3. Pool your data with the class data.
4. Using the class data, create a graph of root length (cm) vs caffeine concentration (M). Calculate the mean, Standard Deviation, and Include ±2SEM.
5. Write a short CER (three sentences TOTAL) about this experience.

Part 3: Preparing root squashes – This is HARD to do
1. Use only the onion in the DI water and the full strength caffeine solution. Discard the other onions.
2. The goal of this procedure is to dye the chromosomes of the cells and then reduce the thickness of the root so that a light microscope can be used to discern what stage of the cell cycle each cell is in. Prepare your well plate so that you can quickly transfer your roots between solutions. This is key. You will want to have separate rows of wells for the control (water) and treated (caffeine) onions. You only need about 1mL of liquid in each well – only enough to submerge the root tips.
3. Using dissection scissors, cut three or four (one for each person in your group) roots from the control onion. Remove the entire root.
4. Trim the tapered end of each root to 0.5 cm. This root tip is the apical meristem (Fig. 1 on the front page), and contains several different zones of cells. We are interested in the “Zone of Cell Division,” which is just above the “Root Cap.”
5. Using forceps, place the 3-4 root tips in the hydrochloric acid solution for 5 minutes. The HCl begins to break down the cell walls.
6. Using forceps, remove the tips from the hydrochloric acid and place in the water for 1 minute.
7. Using forceps, remove the tips from the water rise and place in the stain for 2-3 minutes.
8. Using forceps, remove the tips from the stain and place in the second water rise for at least 1 minute. The water may need to be changed (or just put some water in an empty well). The final color of the rise water should be a clear blue. This is a key step: too much or too little stain will inhibit analysis.
9. Place a single root tip onto a microscope slide.
10. Place a cover slip onto the root tip and gently press down on the cover slip with the eraser of a pencil. Note: The cover slip may be gently spun or moved back and forth to reduce the thickness of the prep. If the cover slip breaks use forceps to remove the pieces and replace with a new cover slip. If more solution needs to be added to the prep, use a pipet to add a partial drop of
water to the edge of the cover slip. The water should move under the cover slip by capillary action.

11. Look under the microscope at your slide. Find the zone of cell division under high magnification and look carefully at the cells you observe. Can you identify a series of cells that are dividing? Look for condensed chromosomes.

12. Repeat this entire process with the onion roots in the full strength caffeine solution. What do you expect to see?

13. If you have an AWESOME slide, please call over your teacher to take a look. My goals is to obtain a collection of great images of the effects of caffeine on mitosis but projecting the microscope images on the projector and taking pictures of them. If we aren’t successful, we’ll use stock images I ordered of a related experiment.

Part 4: Counting Cells and Analyzing Data

1. Within the image, look for well-stained distinct cells. Count the cells in interphase vs. mitosis. You are NOT trying to count cells in Prophase, metaphase, etc., just interphase vs. mitosis. Count as many cells as possible in your image.

2. Each student should count one control and one treated image. Then, pool your data into class data.

My data:

<table>
<thead>
<tr>
<th>Tip</th>
<th>Number of Cells</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Interphase</td>
<td>Mitotic</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Class data:

<table>
<thead>
<tr>
<th>Tip</th>
<th>Number of Cells</th>
<th></th>
</tr>
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<tbody>
<tr>
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3. For this experiment, the number of treated cells in interphase and mitosis will be the observed values.

4. To find out what your expected values are, complete the following steps:
   a. Calculate the percentage of cells in interphase and mitosis in the control group from the class data.
   b. Multiply the percentages by the total number of cells in the treated group; this will give the expected numbers (e).

5. Complete a chi-square analysis of the data. Show all of your work, including statistical hypothesis. Don’t forget to draw a final conclusion.