BIL 151 - Mechanisms of Mitosis

PART III. Collecting Data

You and your teammates have chosen to examine the outcome of treating a rapidly dividing tissue (onion root tip) with a substance that either promotes (Indole-3_butyric acid) or inhibits (trifluralin) mitosis. Your background research should have given you some insight into these chemicals’ mechanism of action at the cellular and molecular level.

Today you will actually collect data by performing chromosome squashes on a sample of treated and untreated onion root tips, and then counting the number of cells in mitosis (and in each phase that you can identify) as well as the total number of cells in a given field of view.

I. Laboratory Equipment and Techniques

You will have the same materials and equipment as in the session you used for practicing chromosome squashes. For your convenience, we have reproduced the squash method here for you to follow, step-by-step.

A. Materials

Refer to the previous chapter for the list of supplies, if necessary.

B. Chromosome Squash and Toluidine Blue Staining Procedure

Onion root tips have been incubated for 24 hours in either plain water or in water containing indole-3-butyric acid or trifluralin. You and your teammates should do chromosome squashes on ten individual root tips from treatment onions and ten from control onions. DO NOT USE MORE THAN ONE ROOT TIP FROM THE SAME ONION, AS THIS WOULD BE A FALSE REPLICATE.

REMEMBER: BECAUSE SOME OF THE STAINS WILL PERMANENTLY MAR CLOTHING, BE SURE TO EITHER WEAR CLOTHES YOU DON’T MIND GETTING STAINED, OR BRING AN APRON OR OTHER COVERALL TO PROTECT YOUR CLOTHING.

Make sure that all of your materials (beakers, plants, microscope slides) are properly labeled before you start this procedure. (When you examine root tips that have been treated with a compound next week, all microscope slides should be labeled appropriately as treatment or control and with the treatment start-time.) Your team may have 10-20 slides out at any time, so it is critically important to label everything properly!

Before you begin, make sure all the microscope slides you plan to use are extremely clean. If you need to clean a microscope slide or coverslip, place 1-3 drops of 95% ethanol on the slide and wipe well with a Kimwipe. Do this on both sides of the slide, and repeat, as necessary, until the slide is very shiny and clean.

Because some of the reagents you will be using are a bit caustic, WEAR THE NITRILE GLOVES PROVIDED AND YOUR OWN SAFETY GLASSES WHILE YOU PERFORM THE CHROMOSOME SQUASH. Do not waste gloves! One pair per student, or bring your own gloves if you plan to use more than one pair.

1. Snap an entire healthy root from an onion. The root tip is the most delicate part of the root and it desiccates very easily. You will need healthy, living cells, so keep your onion root wet at all times! Do not leave onion roots out of the water or lying on the lab bench.

2. Place the root on your labeled slide. Using the dissecting scope, identify the root tip. In plants, mitotic division occurs in the meristem cells, stem cells that can differentiate into any other type of cell. The apical (i.e., located at the apex, or tip) meristem is about one millimeter from the apparent tip of the root (the root cap) (Figure 2). If you cut off too much of the root, you will see long, rectangular cells in your squash. These cells are no
longer undergoing mitosis, so you should not use or count them. Carefully cut off just the meristem region of the root tip with a sharp razor blade, and use that for your squash.

3. Place just the root tip containing the apical meristem into a 1.5 ml microcentrifuge tube (Figure 3) with your fine-tipped forceps. (Handle fine-tipped forceps with care. The tips are fragile, and will bend or break if you drop the forceps or handle them roughly.)

4. Fill the centrifuge tube halfway with 1M HCl from the dropper bottle onto your root tip in the tube. This step will soften the connection between the cells. ***Use caution as HCl is a strong acid.

5. Close the tube and place in a hot 60°C waterbath for exactly 8 minutes (leaving the tip in the hot acid too long results in a soggy mass of cells that will disintegrate when you rinse).

6. **Remove the tube carefully from the hot bath.** To remove the 1M HCl, fill the tube with deionized (DI) water and remove it with a plastic squeeze pipet. Place all removed waste water into your 400mL beaker labeled “WASTE SOLUTIONS”. Rinse a total of three times.

7. Add 2 drops of 0.5% toluidine blue to stain the root tip in the tube. Incubate at room temperature for 5 minutes, gently flicking the tube with the flat of your fingernail about once a minute to distribute the stain. Make sure the root tip stays in the stain.

8. Rinse off the excess toluidine blue as you did for the HCl: fill the tube with DI water and then remove with the plastic squeeze pipet. Repeat a total of three times, always placing the waste solution into the “WASTE SOLUTIONS” beaker. You should be able to see your blue root tip clearly by the time you have removed the last bit of rinse water.

9. Remove almost all of the last rinse, then use the dissecting probe (Figure 3) to gently push the root tip onto a clean, labeled slide.
10. Add one drop of DI water to the stained root tip, and then place a coverslip on top of it. You should now have "sandwich" of slide/root tip in water/cover slip.

11. Place a sheet of bibulous paper (from the booklet supplied on your tray) over the coverslip and GENTLY PRESS STRAIGHT DOWN onto the coverslip with root tip underneath. Be careful not to break the coverslip, or you'll have to start over. **DO NOT PLACE YOUR SLIDE INSIDE THE BIBULOUS PAPER BOOKLET! Please keep the pages clean and uncontaminated for your future slide preps.**

12. Remove the bibulous paper and place the slide on the compound microscope to observe your stained root tip. **ALWAYS BEGIN YOUR MICROSCOPE OBSERVATIONS ON LOW POWER.** Find your root tip cells in the viewing field on low power, and then focus until the view is clear. Then swivel the objective to the next higher objective, and focus again. Do this until you are properly focused with the 40X objective, which you will need to use to see nuclear material clearly.

13. Examine your squash. You should be able to see cells in various stages of mitosis.

**D. Data Collection**

The cells you should count will be round or square and flattened into a single cell layer. (Do not count long, rectangular cells, as these are no longer undergoing mitosis.) Choose an area that is properly squashed (cells should be in a single layer and clearly visible) and count all of the cells you can see (around 50-200 cells). From among those, count how many cells—each—are in (1) **interphase**, (2) **prophase**, (3) **metaphase**, (4) **anaphase**, and (5) **telophase**. Record these numbers and repeat for 3-5 fields of view to obtain a good sample from your onion (about 250-600 cells per root tip).

**one sample = all the cells counted in one root from one onion**

**AVOID PSEUDOREPLICATION!** Do not take multiple roots from the same onion and do not count multiple fields of view as separate experimental samples. All samples from a single individual onion are part of the same treatment, and it would be invalid to count them as separate samples. **All the cells counted from a particular, individual onion plant should be considered one sample.**

1. Count the number of cells you can identify in each stage of mitosis.

2. **When you are completely finished with your slide preparations, place them in the Broken Glass Disposal Container at the front of the lab room. Teams leaving slides or other materials at their station will be docked 5 points.**

3. On **each** of your two data collection days, your team should perform cell counts on
   - 10 control root tips
   - 10 treatment root tips
   for a **total of twenty samples in each group** at the end of two weeks.

**E. Preliminary Data Analysis: Mitotic Indices**

For each sample, record the number of actively dividing cells and divide by the total number of cells you counted. This will give you a **Mitotic Index (M)** for that sample.

\[
M = \frac{n_m}{N}
\]

\[n_m = \text{total number of mitotic cells in the sample}\]
\[N = \text{total number of cells counted in the sample}\]

For each of your treatment and control samples, calculate and record a Mitotic Index, and record these values in the table provided. Provide an appropriate legend for the table.
You can calculate indices for each phase of mitosis by counting the number of cells in a particular phase of mitosis and dividing it by the total number of mitotic cells. This will allow you to determine whether treated onion root tips have been affected in a particular stage of mitosis. For example, if you wish to calculate the proportion of mitotic cells in metaphase:

\[ M_p = \frac{n_p}{n_m} \]

- \( n_p \) = # of cells in prophase in the sample
- \( n_m \) = total number of mitotic cells in the sample

You can calculate indices for any stage of mitosis in this way. Indices for each stage of mitosis will allow you to determine whether any particular stage is more or less prevalent in treatment vs. controls. This, in turn, might give you insight as to the mechanism of action of the treatment chemical. (Tables for recording mitotic indices are provided below)

If you observe any polyploid cells (i.e., those with multiple chromosome sets)? What might be the significance of this finding? Do a quick search on “plant polyploidy” for some ideas. (HINT: some commercially grown plants are intentionally cultivated as polyploids. How might this be done? And why?)

<table>
<thead>
<tr>
<th>Treatment Sample #</th>
<th>Mitotic Index (M)</th>
<th>Control Sample #</th>
<th>Mitotic Index (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment Sample #</th>
<th>Prophase Index (( M_p ))</th>
<th>Control Sample #</th>
<th>Prophase Index (( M_p ))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Save these data, making sure each team member has a copy. When you are completely finished collecting data, your team will statistically analyze them.