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Making the Most of Onion Root Tip Mitosis

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Prepared slides of onion (*Allium cepa* L.) root tips are commonly used to study the process of mitosis. In many instances the student may simply be required to recognize and identify the various mitotic stages. These same slides may be used to fuller advantage both to provide a greater insight into the process of mitosis and to demonstrate the roles of cell division and cell elongation in the growth of the root.

The objectives of this article are to present two laboratory exercises using onion root tip slides which I have found to be successful in my introductory college botany course. The first, demonstrating the duration of successive mitotic stages, is incorporated into the laboratory on mitosis. The second, part of the root lab, graphically demonstrates the roles of cell division and cell elongation in the growth of the root. The only equipment needed are slides of onion root tips and compound microscopes.

Duration of Mitotic Stages

This exercise is conducted after students have learned to recognize the various stages of mitosis as viewed under a microscope.

Each student must obtain an onion root tip slide and locate the region, slightly behind the root cap, where the greatest mitotic activity occurs. This area should then be centered, under high power, in the field of view. Once a field is chosen, the slide should not be moved and only the cells within that field will be examined. Each student should then proceed to fill in the section of table 1 labeled "STUDENT, Number of Cells/Stage." The individual student data are then cumulated to provide "CLASS" data.

One basic assumption of this exercise is that in an active meristematic region, the frequency of occurrence of a given mitotic stage is proportional to its duration. That is, the longer the duration of a stage, the more cells in that stage will be found. A second assumption is that the duration of the cell cycle in onion root tips, from the beginning of interphase to the end of telophase, is about 24 hours (Mazia 1961). The duration of each mitotic stage may now be estimated using the following equation:

$$\text{time/mitotic stage} = \frac{\text{number of cells/stage}}{\text{total number of cells}} \times \frac{24\,\text{hr.}}{\text{mitotic}} \times \frac{60\,\text{min.}}{1\,\text{hr.}}$$

Students should now complete table 1 by estimating the duration of each stage based on their own data and the cumulative class data. The data presented in table 1 are representative for a class of twenty students. The estimates obtained from this procedure are relatively accurate. The durations of prophase, metaphase, anaphase, and telophase in onion root tips are 71, 6.5, 2.4, and 3.8 minutes respectively (Cohn 1969). The lower-than-expected figure for prophase nuclei is probably due to the students' inability to differentiate between interphase and early prophase, but this is a minor deficiency. In addition, the exercise demonstrates the use of large population sampling to obtain more accurate results. A number of students may completely lack one or even two stages in their individual results, but these gaps will be balanced by their classmates' data.

TABLE 1. Duration of mitotic stages

	STUDENT		CLASS	
	Number of Cells/ Stage	*Duration (min.)*	*Number of Cells/ Stage*	*Duration (min.)*
1. Total number of nuclei	500	–	12,890	–
2. Number in prophase	26	74.88	376	42.00
3. Number in metaphase	4	11.52	61	6.81
4. Number in anaphase	2	5.76	31	3.46
5. Number in telophase	3	8.64	30	3.36
6. Total mitotic figures	35	–	498	–
7. Number in interphase (#1–#6)	465	1,339.20	12,392	1,384.37

Root Elongation

Most introductory botany texts describe the root tip as being divided into three more-or-less distinct regions: the region of cell division; the region of elongation; and the region of maturation. The distinction between these regions is not clear. Jensen and Kavaljian (1958) have shown that the frequency of cell division varies at different levels and in different tissues of the root tip. Likewise, even an actively meristematic cell will elongate during the interphase between successive divisions. The following exercise is one which I use to demonstrate the relative roles of cell division and cell elongation at various levels in the root tip. It also serves to emphasize the gradual, rather than sudden, shift in importance of these processes as one proceeds away from the tip.

In order to complete this exercise it is necessary that the field diameter, under high power, be calculated for each student microscope. This should be done at the beginning of the lab period if it has not been done previously. Students should then obtain onion root tip slides and position them on their microscopes so that the bottom of their field of view is on the root cap-root apex junction. (*Note*: Median or near-median sections provide the best results in this exercise.) This field of view represents zone I. It should be arbitrarily divided into three equal-sized subzones (tables 2 and 3). Zone IA borders on the root cap, and IB occupies the center of the field, followed by zone IC. The student should estimate both the number of nuclei and the total number of mitotic figures for each subzone and enter their results in table 2. Before moving the slide, the students should also count and average the number of cells in three separate files within each subzone, i.e., a median file of cells and one to each side. These data should be recorded in the first four columns of table 3.

The students should now move the slide so that the far edge of zone IC becomes the bottom of the field for zone II. This and subsequent zones need not be subdivided. After collecting the data for zone II, the slide should again be moved the diameter of one field to delineate zone III and subsequently zone IV. In each zone, the number of nuclei, number of mitotic figures, and average number of cells per file should be determined for the entire field of view.

Once the raw data are collected, the students may calculate the average cell length within each zone (subzone) by dividing the diameter of the zone (subzone) by the average

TABLE 2. Mitotic index at various levels in the root tip

	STUDENT			CLASS			
Level	*Number of Nuclei*	*Number of Mitotic Figures*	*Mitotic Index*	*Number of Nuclei*	*Number of Mitotic Figures*	*Mitotic Index*	%
IA	180	2	1.1	4,750	38	0.8	10
IB	170	19	11.2	3,840	307	8.0	100
IC	170	7	4.1	3,110	115	3.7	46.25
II	500	10	2.0	10,100	242	2.4	30
III	150	0	0	2,700	23	0.9	11.25
IV	75	0	0	1,550	0	0	0

TABLE 3. Average cell length at various levels in the root tip (diameter of field at 430X = 420 γm)

	STUDENT					CLASS	
Level	*Number of Cells/ Field Diameter*				*Average Cell Length (γm)*	*Average Cell Length (γm)*	%
	file 1	*file2*	*file 3*	*Ave.*			
IA	9	9	8	8.67	14.82	15.52	10.95
IB	10	9	10	9.67	14.82	14.56	10.27
IC	10	9	9	9.33	15.36	14.65	10.34
II	20	18	24	20.67	20.80	21.73	15.33
III	5	6	7	6.0	71.67	70.11	49.46
IV	4	4	3	3.67	117.17	141.75	100.00

number of cells within a file. Average cell length should be recorded in table 3 and the individual data for this as well as the number of mitotic figures and number of nuclei are then cumulated to provide "CLASS" data.

The mitotic index (M.I.)—the percentage of cells undergoing mitosis—may now be estimated for each region using the formula:

$$\text{M.I.} = \frac{\text{number of mitotic figures}}{\text{number of nuclei}} \times 100$$

The class mitotic indices (table 2) and average cell lengths (table 3) for each region may now be used to complete the graph in figure 1. However, I feel it is easier to compare the relative importance of these phenomena in each region if the data are standardized, i.e., expressed as a percentage of the maximum observed. Thus, the highest mitotic index and longest average cell length are expressed as 100 percent and all other values are expressed as a percentage of the maximum. Comparison of the two factors is thus facilitated by having both ordinate axes drawn to the same scale.

The data presented in tables 2 and 3 and figure 1 are representative of a class of twenty students. It is immediately apparent from figure 1 that the transition from a region of predominantly cell division to a region of predominantly cell elongation is a gradual one with the latter becoming increasingly important at greater distances from the tip. A second feature of importance is the sudden increase in M.I. between regions IA and IB. The low M.I. in region IA is due to the quiescent center, located in the center of the root apex just beneath the root cap. Surrounding this center are the most actively meristematic cells of the root tip, as evidenced by the dramatic increase in M.I. in region IB.

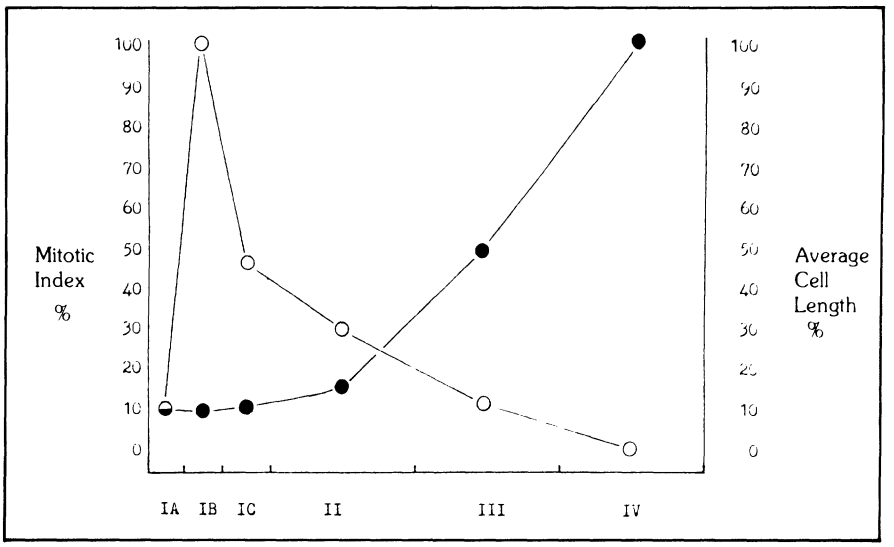

FIGURE 1. Comparison of mitotic index and average cell length at various levels in the root tip. Mitotic index and average cell length expressed as percentage of maximum observed.

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