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Publication No. 11180

Altering the Rate of Mitosis

Introduction

All new cells come from previously existing cells. New cells are formed by the process of cell division, which involves the replication of the cell's internal structures and the division of the cytoplasm (*cytokinesis*). What factors affect the rate of mitosis?

Concepts

- Mitosis
- Abiotic and biotic factors
- Plant hormones

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=rhyzobium), 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.

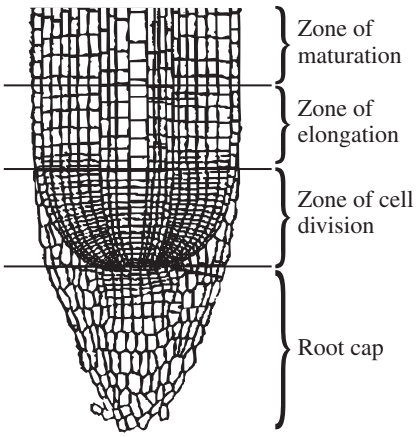

Figure 1. Apical meristem

Materials

Ethyl alcohol, 95%, 20 mL
Gibberellic acid, 2 mg
Hydrochloric acid solution, HCl, 1 M, 2 mL
Indole-3-acetic acid, 2 mg
Toluidine blue stain, 1%, 2 mL
Water, deionized (DI)
Onions, green or onion sets, 50
Compound microscope with 40X objective
Cover slips, 6
Cups, plastic, 8
Dissection scissors
Forceps
Glass slides, 6
Paper towels
Pencil with eraser
Permanent marker or wax pencil
Pipets, disposable, 2
Sand, white
Spot plate

Safety Precautions

Toluidine blue stain is moderately toxic by ingestion and is a permanent stain on many objects. Hydrochloric acid solution is toxic by ingestion or inhalation and severely corrosive to skin and eyes. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Avoid contact of all chemicals with eyes and skin and wash hands thoroughly with soap and water before leaving the laboratory. Please follow all laboratory safety guidelines.

Pre-Lab Preparation

1. Prepare the indole-3-acetic acid or gibberellic acid solution (2 mg/L)
   a. In a 1-L volumetric flask, dissolve 2 mg of either indole-3-acetic acid or gibberellic acid in 10 mL of ethyl alcohol.
   b. Fill the volumetric flask to the 1-L mark with tap water and mix well.
   c. The solution is stable at room temperature for several weeks.
2. Prepare the (control) water solution.
   a. Add 10 mL of ethyl alcohol to a 1-L volumetric flask.
   b. Fill the volumetric flask to the 1-L mark with tap water and mix well.
   c. The solution is stable indefinitely if stored at room temperature.
3. Germinate root tips.
   a. Fill a plastic cup about ⅓ full with fine white sand.
   b. Pour the sand into a tared weighing dish on a balance. Use the same mass of sand in each cup.
   c. Fill 8 plastic cups with the same massed amount of fine white sand.
   d. Label 4 of the cups “control” and the remainder “treatment.”
   e. Remove any long roots or dried roots from the bottom of each onion.
   f. Insert onions into the cups. Place several small onions per cup.
   g. Add enough control solution to the “control” cups to completely wet the sand. Depending upon the size of the cup used the amount varies from 20 to 40 mL.
   h. Add the same amount of the treatment solution to the “treatment” cups to completely wet the sand.
   i. Loosely place plastic wrap on top of each cup to prevent excess evaporation.
   j. The onions do not need to be placed in direct light but the room should be 65–75 °F. Check for root growth every day. Roots are typically ready after 36–48 hours.

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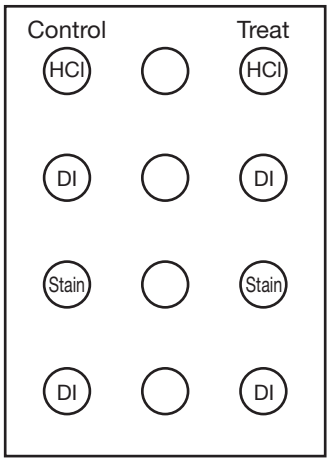
Procedure



1. Fill the spot plate as follows: top row with 1 mL of the hydrochloric acid solution, second and fourth rows with deionized water, and the third row with 1 mL of the stain.
2. Cut three roots from actively growing onions using dissection scissors. Remove the entire root.
3. Trim the tapered end of each root to 0.5 cm. Only the tapered end of the root tip will be used. The remainder may be discarded in the trash.
4. Place each tip into the hydrochloric acid solution for 5 minutes. The HCl begins to break down the cell walls.
5. Remove the tips from the hydrochloric acid and place in the DI water for 1 minute.
6. Remove the tips from the water rinse and place in the stain for 2–3 minutes.
7. Remove the tips from the stain and place in the second DI water rinse for at least 1 minute. The water may need to be changed. The final color of the rinse water should be a clear blue. This is a key step. Too much or too little stain will inhibit analysis. Achieving the correct depth of color may take some practice.
8. Place a single root tip onto a microscope slide.
9. 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.
10. Locate the meristem area of the root tip and identify mitosis or interphase for a minimum of 300 cells or completely count two fields of view, whichever is greater. *Note:* Count all cells in the meristem region. Take care not to count the same cell more than once. Also, do not only count cells in mitosis. This would skew the results.
11. Repeat with the remaining root tips.
12. Pool data with that of the other lab groups.
13. Complete a chi-square statistical analysis of the data (optional).

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures, and review all federal, state and local regulations that may apply, before proceeding. Excess hydrochloric acid may be neutralized with base and then flushed down the drain with plenty of excess water according to Flinn Suggested Disposal Method #24b. Excess Toluidine blue solution, control solution and IAA or gibberellic acid solution may be flushed down the drain with an excess of water according to Flinn Suggested Disposal Method #26b.

Connecting to the National Standards

This laboratory activity relates to the following National Science Education Standards (1996):

Unifying Concepts and Processes: Grades K–12

Systems, order, and organization
Evidence, models, and explanation
Constancy, change, and measurement
Evolution and equilibrium
Form and function

Content Standards: Grades 9–12

Content Standard A: Science as Inquiry
Content Standard C: Life Science, the cell, biological evolution, matter, energy, and organization in living systems,

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Tips

- A minimum of 2,000 cells should be counted for each treatment.
- Carbol fuchsin stain may also be used to stain the chromosomes. Please read the MSDS before proceeding.
- Although only IAA and gibberellic acid are mentioned in the procedure, other plant hormones will also affect plant growth. Each has a concentration range that promotes plant growth and affects mitosis. Concentrations above this range will inhibit plant growth, while lower concentrations will not show a statistically significant effect. These plant hormones are:

  Abscisic acid (Dormin) (Catalog No. A0298) 0.1–10 mg/L
  Gibberellic acid (Catalog No. G0042) 0.01–5.0 mg/L
  Indole-3-acetic acid (Catalog No. I0001) 1–3 mg/L
  Indole-3-butyric acid (Catalog No. I0003) 0.1 –10 mg/L
  1-Naphthalene acetic acid (Catalog No. N0066) 0.1–10 mg/L

- Red kidney bean lectin (*Phytohemagglutinin*) is used in AP Biology Investigation 7 by the College Board. Lectin is a lyophilized powder that must be kept refrigerated. The recommended concentration is 10 mg in 200 mL of deionized water. Once diluted, it must be stored in the refrigerator. It is only active for a few days. Lectin significantly increases mitosis in the roots. Please read the MSDS before using lectin. Lectin is available from Flinn Scientific, Catalog No. L0114.
- Caffeine inhibits mitosis. Onions treated with 1% aqueous caffeine failed to produce any roots after several weeks. A 0.1% aqueous caffeine solution did produce short roots. However, when stained the cell cycle phase is difficult to determine and may not produce quantifiable results. Caffeine is very toxic with an oral (rat) LD_{50} of 192 mg/kg. Please read the MSDS before using caffeine. Caffeine is available from Flinn Scientific, Catalog No. C0344.
- The auxin 2,4-dichlorophenoxyacetic acid (2,4-D) was tested at a concentration of 1 mg/L using the above procedure. Numerous root tips germinated but they remained very short and made it difficult to quantify the results. 2,4-D is available from Flinn Scientific, Catalog No. D0056.
- Other factors that may affect the mitotic rate are salinity, temperature, mineral limiting factors, pH, roundworms, soil bacteria or fungus, other plant hormones, amount of light, acetaminophen, aspirin, ibuprofen, vitamins and minerals, heavy metals, antibiotics, and certain plant competitors that excrete inhibiting chemicals.
- Store bought onions may be treated with rooting inhibitors. Onion sets are available from Flinn Scientific (Catalog No. FB1468).
- This write-up is based on the kit Environmental Effects on Mitosis—An Advanced Inquiry Lab (Catalog No. FB2031).

Materials for *Altering the Rate of Mitosis* are available from Flinn Scientific, Inc.

Catalog No.	Description
AP6399	Spot Plate, Polystyrene, Pkg. of 12
FB1468	Onion Sets, Pkg. of 100
G0042	Gibberellic Acid, 0.5 g
H0013	Hydrochloric Acid Solution, 1 M, 500 mL
I0001	Indole-3-acetic Acid, 1 g
L0114	Lectin, 10 mg
S0004	Sand, Fine White, 2 kg
T0048	Toluidine Blue O Solution, 100 mL

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.

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