Idioblasts in *Dieffenbachia*

Objectives: Upon *completion of this exercise the student should be able to:*

1. Use the ocular micrometer to measure the length of an object
2. Calibrate an ocular micrometer with a stage micrometer
3. Describe the appearance of idioblasts and raphides of *Dieffenbachia*
4. Describe the impact of various osmotic agents on idioblast discharge

Pre-Lab:

1. Bring a copy of this exercise to lab.

2. Send an email to me responding to the following questions. The answers can be found in the articles by Middendorf (1983), Sakai & Nagao (1980) and the introductory chapter of Cao's (2003) thesis.
   a. Explain why *Dieffenbachia* is called commonly called dumb cane.
   b. Identify the poisonous agent(s) in dumb cane, or in other words, why is dumb cane poisonous?
   c. What is an idioblast?
   d. What is a raphide?
   e. How many kinds of idioblasts occur in dumb cane? Describe them.
   f. Identify three hypothesized functions of idioblasts in *Dieffenbachia*.
   g. What is the hypothesized mechanism for raphide discharge from *Dieffenbachia* idioblasts.

Introduction: As we learned from our pre-lab investigations, raphides are ejected from idioblasts with a broken tip. It is hypothesized that the crystals are ejected when polysaccharides inside the idioblast swell as they absorb water. We will test this hypothesis and attempt to develop a mechanism to explain raphide discharge from the idioblasts of *Dieffenbachia*.

General Protocol: We will use Middendorf's (1983) technique to prepare idioblasts.

1. Grind a piece of leaf in a small amount of water with a mortar and pestle.
2. Add tap water to bring the volume to about 10 mL
3. Strain the liquid through a small piece of screen into a conic centrifuge tube. This will remove the larger leaf debris
4. After a short wait, remove a drop of the liquid from the bottom of the tube
5. Place a drop on a microscope slide, add a coverslip and examine.

Exercise 1. *General Observation of Idioblasts*

Question: What do the idioblasts of *Dieffenbachia* look like? How large (length, width) are the idioblasts?

Hypothesis: The idioblasts will appear similar to published reports and will be approximately 165 x 40 μm (Sakai & Nagao, 1980).

Protocol: Use an ocular micrometer to measure the length and width of 5 idioblasts. Calibrate the ocular micrometer with a stage micrometer. Obtain data from at least one other individual and calculate the mean size of the idioblasts. Make a sketch or prepare a digital image of a typical idioblast with raphides.

Results: Create a data table (Table 1) to collect your results. You will need columns for both omu's and μm for length and width. Calculate the mean length and width (in μm). Include a sketch or image of an idioblast (Fig. 1).

Analysis: Do our data support our hypothesis? Why or why not? What conclusions can you draw from these data?

Exercise 2. ***Idioblast Discharge in Water***

Questions: Are the idioblasts shooting raphides? If so, what percent? Are raphides ejected from intact idioblasts or only those with broken tips?

Hypotheses: Idioblasts with broken tips will eject raphides. Idioblasts with intact tips will not release raphides.

Protocol: Collect data to determine the state of discharge of the idioblast sample. Examine idioblasts and score them on the following basis: 0 = tips intact, no discharge; 1 = tips broken, no discharge; 2 = less than 25% raphides discharged; 3 = 25 – 75% raphides discharged; 4 = more than 75% discharged; and 5 = 100% discharged, idioblast empty.

Results: Create a data table (Table 2) to collect your results. You should have data from a minimum of 50 idioblasts. Plot a histogram (Fig 2) showing the frequency distribution of idioblast discharge in water. Calculate the mean state of discharge.

Conclusions: What can you conclude about our questions based on your data?

Exercise 3. ***Rate of Idioblast Discharge***

Question: How rapidly are raphides discharged from idioblasts? Are raphides discharged one or a time or more than one?

Protocol: Collect data to measure the rate of raphide discharge. A stop watch is available. It might be easiest to work with a partner; one person can be the timer while the other counts the number of raphides discharged during the time period. As you observe discharge, note if the raphides are discharged singly or in multiples.

Results: Create a data table (Table 3) to collect your results. You should have data from a minimum of 5 idioblasts. Plot (Fig. 3) number of raphides discharged vs. time (sec). Calculate the rate of raphide discharge (=slope). Summarize in a table (Table 4) the percent of raphides discharged singly or in other numbers.

Exercise 4. ***Mechanism of Idioblast Discharge***

We have hypothesized that water uptake by polysaccharides in the idioblasts is the driving force to eject raphides. If this hypothesis is true, then idioblast discharge should be dependent on the osmotic environment in which the idioblasts occur. In this exercise we will isolate the idioblasts in different osmotic environments and then observe the impact on idioblast discharge.

Question: Does the osmotic environment affect the discharge of idioblasts?

Hypothesis: Idioblast discharge will be affected by the osmotic environment. More idioblasts will discharge in water than in solutions with high osmotic concentrations of sucrose. Idioblast discharge will occur at a greater rate in water than in solutions with a high osmotic concentration of sucrose.

Protocol: You will be assigned a solution of sucrose (0.1 – 0.8 molal) to determine the effect on crystal discharge. Prepare idioblasts using the general protocol except substitute your assigned sucrose solution for water. Collect data as in Exercise 3 and 4 (state of discharge, rate of discharge) to test our hypotheses.

Results: Create data tables (Tables 5, 6) to summarize your results. Share the results (mean state of discharge, rate of discharge, % multiple raphides discharged) with other members of the class.

Post-Lab:

Create a summary data table (Table 7) and graph(s) (Fig 4, etc) that includes the class results from all osmotic concentrations (0 – 0.8 molal) for rate of discharge, average discharge state and frequency of multiple discharge. Then write a standard lab report documenting the results of your investigations. Address the question: By what mechanism are the raphides from *Diffenbachia* discharged?

References:

- Cao, H (2003) The distribution of calcium oxalate crystals in genus Dieffenbachia Schott. And the relationship between environmental factors and crystal quantity and quality. MS Thesis, University of Florida.
- Franceschi, VR, Nakata, PA (2005) Calcium oxalate in plants: Formation and function. *Annu. Rev. Plant Biol*. **56**: 41 – 71.
- Middendorf, E (1983) The remarkable shooting idioblasts. *Aroideana* 6: 9 – 11.
- Sakai WS, Nagao, MA (1980) Raphide structure in *Dieffenbachia maculata*. *J. Amer. Soc. Hort. Sci.* **105**: 124 – 126.