Idioblasts in *Dieffenbachia: Effect of SDS on Raphide Ejection*

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. Be prepared to discuss in class 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. Water pressure is believed to be the driving force for ejecting crystals from the idioblasts. Pressures presumably develop in the idioblasts because of osmotic entry of water from the surroundings. If this is true, then if the idioblast membrane is damaged, we expect that this will discharge the pressure and ejection should stop. We will test this hypothesis by treating idioblasts with an ionic detergent (sodium dodecyl sulfate) 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. A drop of neutral red (0.04%) can help visualize the idioblasts.

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 digital 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. ***Idioblast Discharge in response to SDS***

We have hypothesized that water pressure is the driving force for raphide ejection. If this hypothesis is true, then destroying the idioblast membrane should prevent discharge. In this exercise we will isolate the idioblasts in different concentrations of SDS and observe the impact on idioblast discharge.

Question: Does SDS affect the discharge of idioblasts?

Hypothesis: Idioblast discharge will be affected by the SDS 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 one concentration of SDS (0.001, 0.01, 0.1, 1, 10%) to determine the effect on crystal discharge. Prepare idioblasts using the general protocol except substitute your assigned SDS solution for water. Collect data as in Exercise 2 (state of discharge) to test our hypotheses.

Results: Create a data tables (Table 3) to summarize your results. Share the results (mean state of discharge) with other members of the class.

Post-Lab:

Write a standard lab report documenting the results of your investigations that includes the data from our experimental work.

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.