Discovering plant tissues in a new dimension

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INTRODUCTION

Traditionally, students engaged in learning practical botanical microscopy in laboratory courses observe different tissues of various organs such as roots, stems, leaves, etc, using prepared slides. They usually struggle to perceive the depth of the tissues, their spatial localization and relationship with other tissues. Visualization of a whole plant organ is limited by the low clarity of the tissues, and thus requiring a clearing procedure to improve the visualization. Although there are many clearing solutions, the most commonly used is acidified chloral hydrate. However, chloral hydrate is a Federally Regulated narcotic, and requires a special permit to purchase or use, placing this technique out of reach for routine teaching. The lab exercise described herein uses a new non-toxic clearing agent, Visikol™, to clear tissues and allow students to observe the relationship structure and function of plant tissues and perception of depth in plant organs.

MATERIAL AND METHODS

Whole fresh leaves (basil and *Arabidopsis thaliana*) and fresh roots (*Arabidopsis sp*.) were submerged in Visikol until they became transparent (24 hours), then the whole organ was mounted on a microscope slide with two drops of Visikol and a cover slip was added. All the microscopic image analyses were taken on a Nikon Eclipse 80i microscope, with NIS-Elements D 3.00 SP7 imaging software (Nikon, Tokyo, Japan).

RESULTS AND DISCUSSION

Exercise Activity

Students will have a whole mount of leaf and root, and will instructed to:

1. Examine the epidermis, locate stomata, trichomes and oil glands.
2. Examine the leaf from the upper (adaxial) to the lower (abaxial) part.
3. Examine a root tip, locate different layers of root tissues.

Questions for students

A. Do you see any difference (shape, size) between the superficial layer of cells and the deepest ones? Can you relate the structure you see with the function it has?
B. How many layers of tissues you can to see form one side of he leaf to the other?
C. Why do you think the meristem in the root is located in deeper tissues and not on the surface?

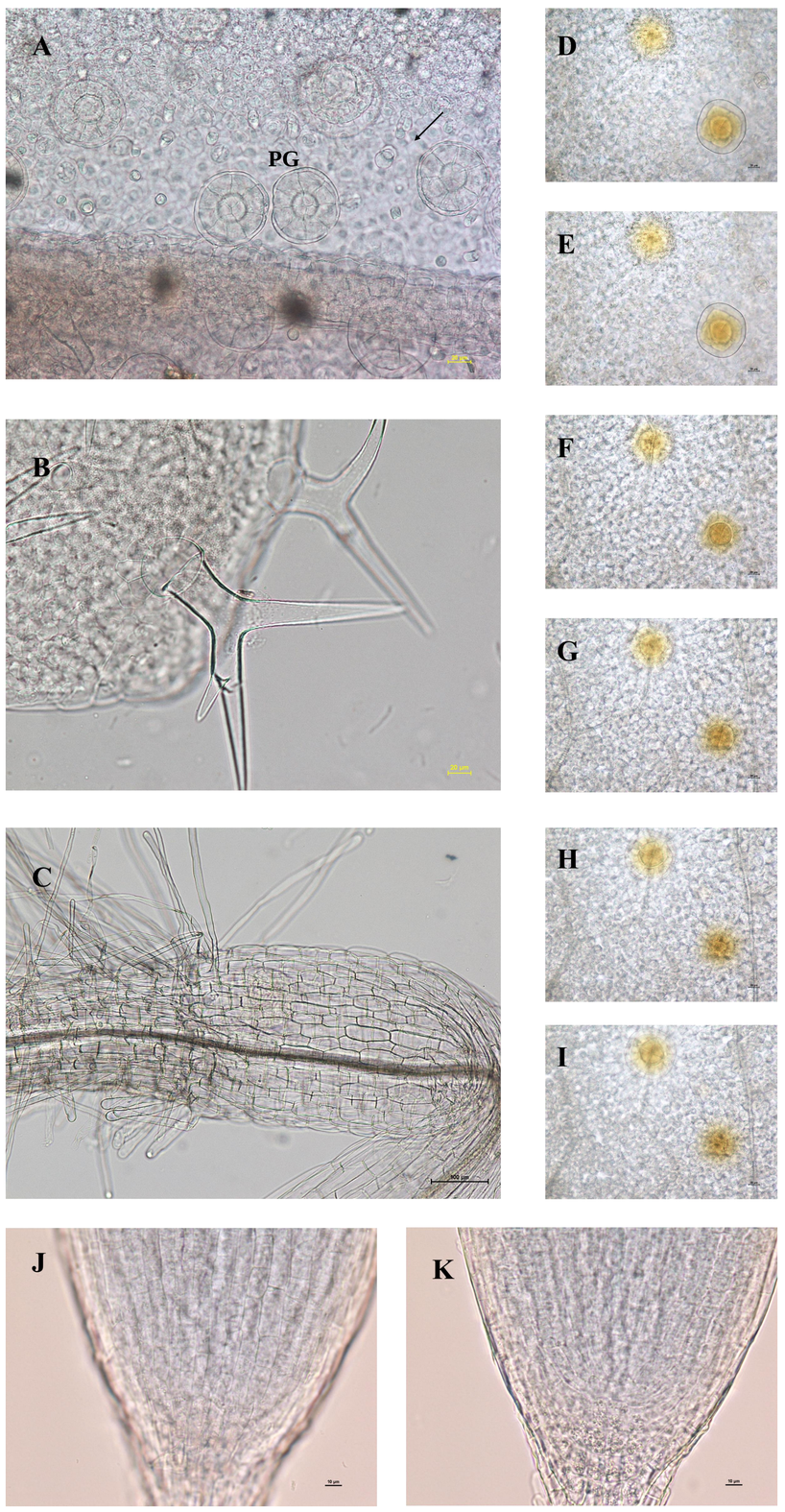

Fig 1: Light micrographs of fresh, whole mounted botanical specimens cleared with Visikol. (A) Spearmint leaf. Epidermis showing abundant peltate gland (PG) with eight-celled apical disc and stalk cell in the center and capitate glands (arrow); (B) Arabidopsis leaf, epidermis, covering branched trichomes; (C) Arabidopsis root tip, maturation zone abundant in root hairs; (D-I) Basil leaf series micrographs from upper side to lower side of the leaf showing epidermis, mesophyll cells and vascular tissues (J, K) Arabidopis root tip close up. Superficial view epidermis (J), and deeper layers of division zone showing the new source of cell for root growth (K).

The instructor can point out the importance of the epidermis as a continuous and protective layer, formed by epidermal cell, stomata and modified epidermal cells. For example, basil is an aromatic plant that synthetizes and accumulates essential oils in glands on the surface of the leaves. Two different essential oil glands, capitate and peltate oil glands could be easily distinguished (Fig.1 A). The presence, location and size of the oil glands in the epidermis can be discussed. Using the fine focus knob, different layers of leaf basil tissues were easily identified such as epidermis, with stomata, oil glands, underlying palisade cells, vascular tissues etc (Fig 1 D-I).

Another example can be illustrated using 10 days Arabidopsis plantlets. In Arabidopsis is characterized by branched covering trichomes, non- secreting epidermal cells (Fig 1 B). Cleared root tips can be used to point out the zones of root development, cell division, elongation zone and maturation zone with root hairs as extensions of epidermal cells (Fig 1C). Students can use the coarse and fine adjustment knob to investigate the meristematic tissue without cutting the tissues (Fig. 1 J-K).

Follow up questions

What is the purpose of having oils glands and trichomes on the surface in the leaf?

Do you expect to find oil glands in the epidermis of the root? Why?

This lab exercise allows students to navigate in three dimensions through the organ tissues, to understand the internal structures and relate it to the function in the plant.

CONCLUSION

We present here a lab exercise using a new clearing solution that allows students to easily observe and study whole mount plant organs in three dimensions. This hands on experience helps to motivate and engage students in biology classes, and excite them about the world of botany.

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