Discovering Plant Tissues in a New Dimension

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Background

In botanical laboratory courses students observe different tissues using thin sections of various organs. Visualization of whole plant organs is limited by the low clarity of the tissues, and thus requiring a clearing procedure to improve the visualization. This lab exercise uses a new clearing agent (Visikol®) and allows students to easily observe and study whole mount plant organs in three dimensions. This exercise helps to understand and reason about the relationship between structure and function of plant tissues. This hands-on experience motivates and engages students in biology classes, and excites them to learn more about the world of botany.

Materials

• Visikol for Plant Biology (Visikol Inc., New Brunswick, New Jersey, USA, www.visikol.com)
• Whole fresh leaves. It is suggested leaves of an aromatic (basil, mint, etc.) and no aromatic plant (Arabidopsis thaliana) for comparison of different structures that can be found in the epidermis.
• Fresh young Arabidopsis sp. plantlets. If enough seedling are available it is suggested different ages (1-10 days-old).
• Leaves (aromatic plant) and 10- days old Arabidopsis plantlets cleared 12-24 hours in Visikol™.
• Stainless steel forceps
• Microscope glass slides and cover slips
• Gloves
• Microscope
• Camera with imaging software (if available)

Introduction

Traditionally, students engaged in learning practical botanical microscopy in laboratory courses observe different tissues using thin sections of various organs such as roots, stems, leaves, etc. These thin sections of tissue provide a great deal of information, yet students experience difficulty in imagining the same issues in a 3-dimensional orientation. 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 (Lersten, 1967; Herr 1993). 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. Recently a new simple clearing method for clearing plant tissues was described (Villani et al., 2013). Here, we have designed a lab exercise that uses a new clearing agent, Visikol, to clear plant organ and tissues that allows students to observe and reason about the relationship between structure and function of plant tissues. This simple lab exercise introduces students to basic leaf anatomy and primary root structures and allows them to navigate in three dimensions through different organs, to understand the internal structure of tissues

and relate it to the function in the plant. In addition, students will observe that root tissues arise from apical meristems in three dimensions and follow differentiation of different type of cells, tissues and tissues systems. This lab exercise is presented as is supplementary to the traditional botanical microscopy labs that learning is based on studying plant tissues in 2- dimensional space using thin section of fresh or preserved organs. This simple hands-on experience helps to motivate and engage students in biology classes, and excite them about the world of botany.

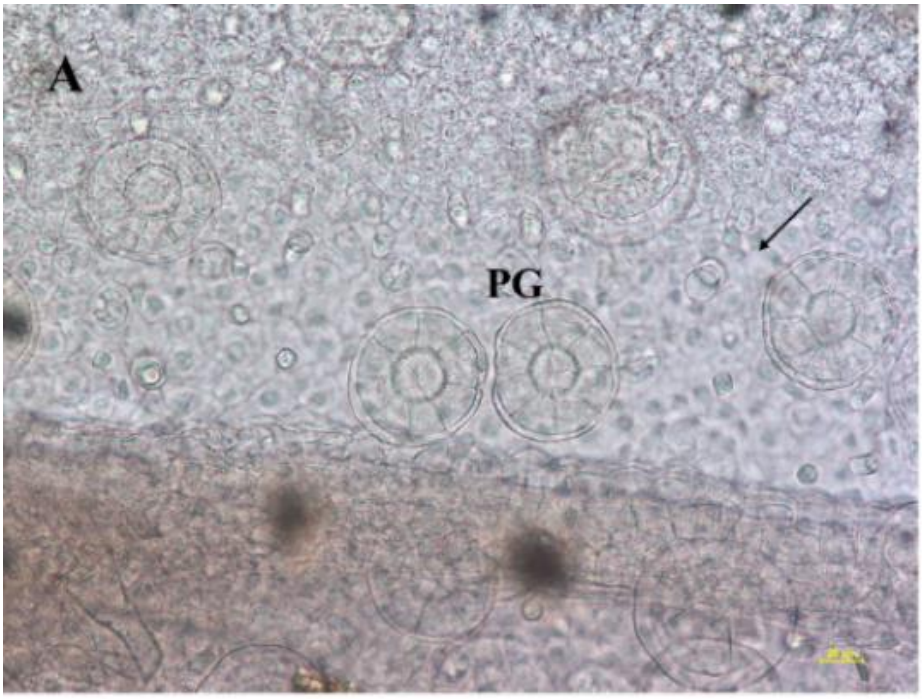
Notes for the Instructor

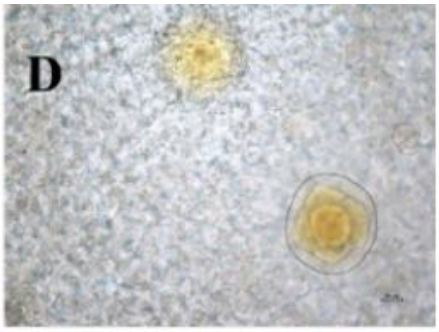
We have selected two lab exercises, one that introduces to leaf structure, and the second one that introduces root structure. Keeping in mind that leaves are the site of photosynthesis, transpiration, it is crucial that students consider the surface of light harvesting and gas exchange, permeability of the epidermis to gases (presence of stomata), intercellular spaces, and the distribution of the vascular tissues. Whole fresh leaves (oregano, basil or mint are suggested because of the essential oil glands and trichomes) were submerged in Visikol until they became transparent, then the whole leaf was mounted on a microscope slide with two drops of Visikol and a cover slip was added. Using the fine focus knob, different layers of leaf tissues were easily identified such as epidermis, with stomata, oil glands, trichomes, underlying palisade cells, vascular tissues etc. 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. Hairs or trichomes can be glandular (oil glands) or non-glandular (covering) hairs. There is a great diversity of forms and these hairs may be used for Koroch, Villani and Simon 4 Tested Studies for Laboratory Teaching identification. The function of hairs is for protection, in some species is related to water conservation in the leaf, others have anti-herbivore function. For example, aromatic plants synthetizes and accumulates essential oils in glands on the surface of the leaves.

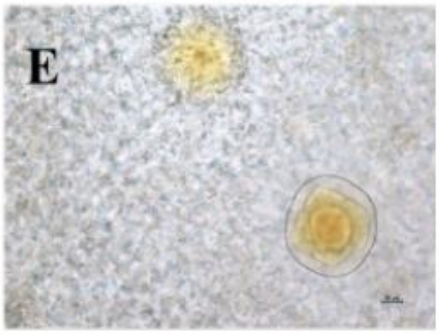
Two different essential oil glands, capitate and peltate oil glands could be easily distinguished (Fig. 1A). Essential oils are synthetized to protect the plant against different organisms and thus exhibit many biological activities such as antimicrobial, antifungal and antiviral activities) (Koroch et al., 2007). 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. 1D-I). These observations may lead to further discussions of the importance of having a complete three dimension image of the localization of each specialized cell in the leaves and thus understanding the morphology of the leaf. Another example can be illustrated using 10- days old Arabidopsis plantlets. Here, with Arabidopsis students will observe branched covering trichomes, that are non- secreting epidermal cells (Fig 1B). 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). These exercises can be easily performed with any plant material students can bring to class.

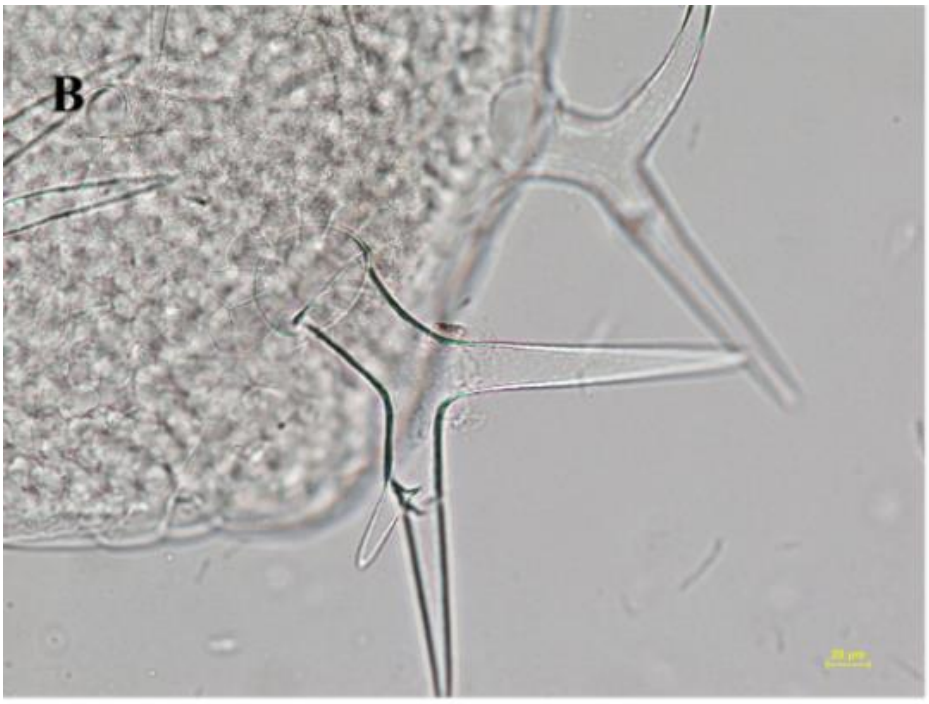
In advanced classes, students can be engaged in conducting comparative studies of structures and organ formation using either a wider variety of plants (for which students can directly sow and then observe over time) for which this lab practicium can be performed or use a series of plantletes of different ages of Arabidopsis (e.g 0 to 10-day- old). Acknowledgments We are grateful to the Science Dept., Borough of Manhattan Community College, to the New Use Agriculture and Natural Plant Products program, Dept. Plant Biology and Pathology, School of Environmental and Biological Sciences, Rutgers University and to Jim White, Rutgers Department of Plant Biology for the use of his microscope. We also thank Phytosys LLC for their donation of Visikol used in this work. This new clearing and mounting agent, Visikol was invented by the authors and is patent pending, filed by Rutgers University. Rutgers has granted an exclusive license to Phytosys LLC (co-owned by the authors) to commercially market Visikol.

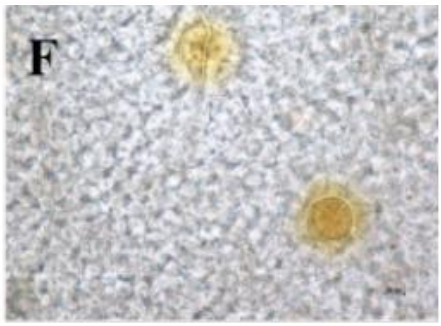
Sample Images

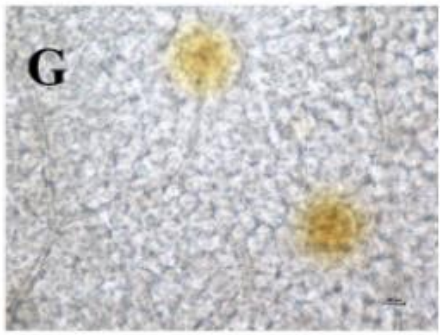












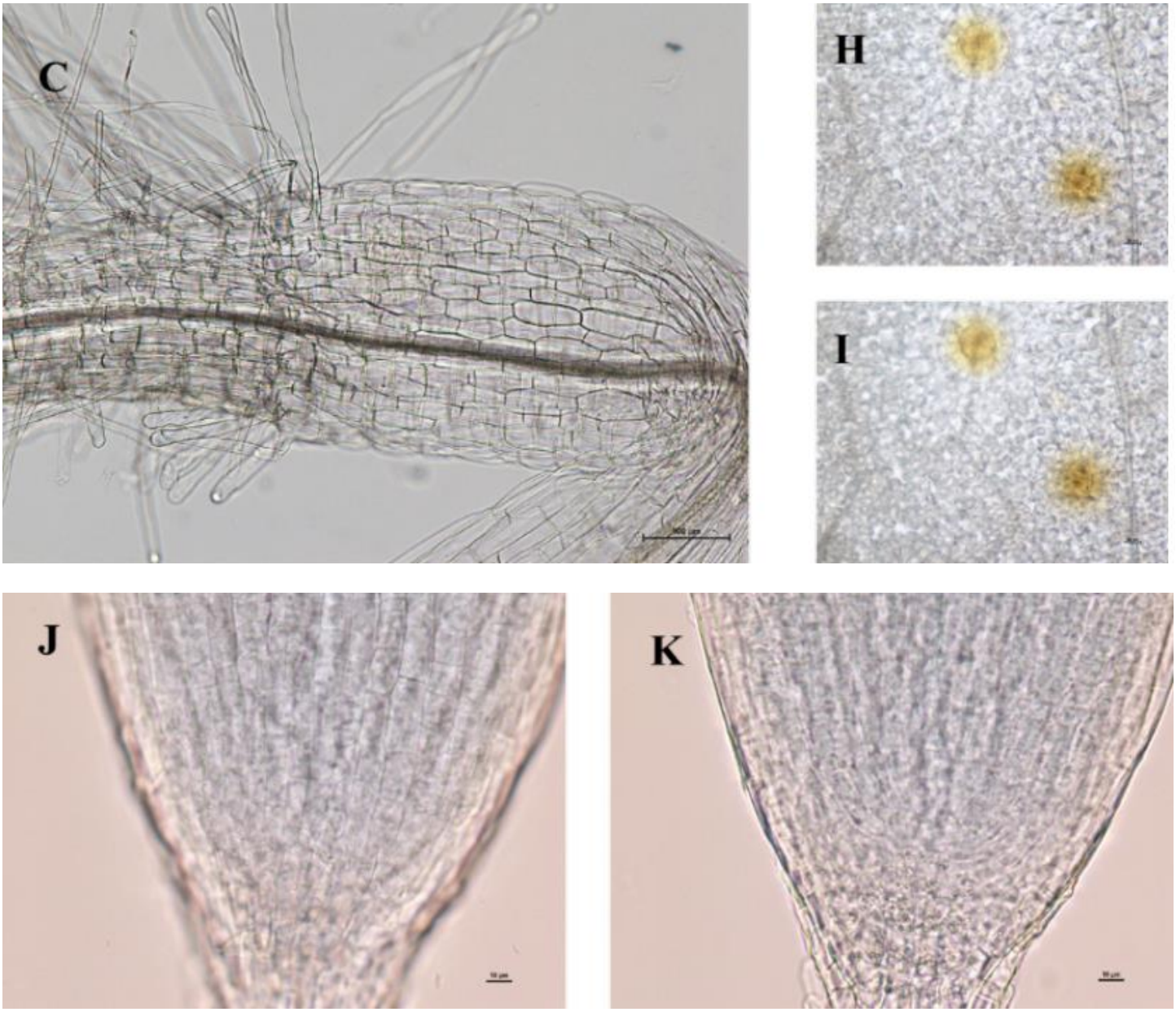


Figure Legend. Samples of light micrographs of fresh, whole mounted botanical specimens. (A) Spearmint leaf. Epidermis showing abundant peltate gland (PG) with eight-celled apical disc and stalk cell in the center and capitate glands (arrow); (B) Arabidopsis leaf, epidermis, covering branched trichomes; (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) Arabidopsis root tip close up. Superficial view epidermis (J), and deeper layers of division zone showing the new source of cell for root growth (K).

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