Laboratory 1 - Vascular Plant Anatomy

One of the major distinctions between the study of morphology and that of anatomy is that cells themselves do not organize the plant. Rather, the organization of the plant determines that of its cells. Therefore, cell form and expression does not determine plant structure. Instead, plant structural demands determine how their component parts need to be organized and then cells are formed accordingly. This underlies the classical distinction between the two closely allied areas of morphology and anatomy, yet remain as distinct as the theories of light—whether it is a particle or a wave. Both approaches have demonstrable utility. In order to conduct morphology, we must know how plants are anatomically organized to understand how the plant is formed.

I. Cell Types: Organization and Relationships

There are only five (5) major cell types in vascular plants: parenchyma, collenchyma, sclerenchyma (fibers and sclereids), xylem (tracheids and vessel members) and phloem (sieve cells and sieve tube members). These are in turn organized into tissues, such as epidermis, cortex, pith; in some cases (e.g., xylem and phloem) the name of the cell and tissue is essentially the same. Some cells are specialized within their immediate cellular environment (ie., vascular parenchyma, etc). Cell organization is summarized in Gifford and Foster, pp. 36-42). Sachs' method (Gifford and Foster, p. 34-36) is used here to identify the general plant organization into dermal, ground and vascular systems.

A. Ground Tissues

The ground tissues are principally parenchyma. Parenchyma cells are particularly important in the plant: they make up most of the living plant and most of the developmental potential of the plant resides in these cells. These cells have only thin, primary cell walls and are located in many regions of the plant. Obtain a thin cross section of a "stalk" of celery. Did you notice what organ it represents in the plant? Now, prepare and observe the wet mount using a compound microscope. The large cells with nuclei and relatively thin cell walls are parenchyma. How can you tell that they are alive? Keep this slide for the next section on collenchyma.

B. Support Tissues

Collenchyma and sclerenchyma form the two major support tissues. Collenchyma is living cell type with thick, pearly cell walls. It is located near the periphery of the plant and remains living during function, depending on turgor pressure to remain strongly supportive. Re-examine the celery section and look for these distinctive structures. How do they look from outside of the plant? Where are they located in the petiole and in relation to the vasculature? What happens to the plant when collenchyma cells lose their turgor? Do the cells differ in length or width when the tissue loses turgor? (Retain the celery for further observation of vasculature in Section C.)

Sclerenchyma is typically dead at maturity, with thick, irreversible lignified cell walls. These are often centrally located in vascular tissue or mixed in other areas of the plant. Obtain a cross section of Hoya stem. Where are the sclerenchyma cells typically located? What is their general shape? What clues can you obtain from their staining color and what does it indicate? Obtain and observe a slide of Linum. Where are these cells located in the stem? Guess their shape in three-dimensions. Cells that are less than 10 times longer than wide are known as sclereids and are typically separate or
in small groups, particularly in ground tissues. The longer cells are known as fibers and are commonly grouped together near or within the vasculature. What type of cells are found in Hoya? What type in Linum? Would the dried tissues of this plant be flexible or resistant?

C. Vascular Tissues

Xylem and phloem form two cell types found in vasculature. Xylem forms a dead pipeline with lignified wall thickenings. Cells that will become xylem "line up" during their formation, develop necessary structural cell wall supports for water conduction, form inter-cellular pores, and then undergo pre-programmed cell lysis to become water conductive cells. There are two types of cells: tracheids and vessel tube members. Phloem cells are food-conductive cells that remain alive during function; however, at maturity they lose their nucleus and rely on surrounding cells for protein synthesis and metabolic needs. These also "line up" during their formation, develop necessary cell wall structures, and develop inter-phloem cell pores. Identify phloem and xylem cells within the vasculature of the celery petiole. How can you tell the difference? Which is on the inside of the petiole (adaxial)? Outside (abaxial)?

The order of primary xylem maturation may be determined in cross sections or in longitudinal section. The first to mature is protoxylem, which is smallest in cross sectional diameter. Next to mature is metaxylem, which may be significantly larger. Identify these in celery. Did you see the structure of any of the secondary xylem wall thickenings?

Phloem is not easy to see under the best of conditions. Try to identify some based on cell wall characteristics, cell size and relationships with companion cells. If you do not identify any, find someone who did and look at their preparation, so that you may convince yourself that this tissue exists.

II. Anatomical Relationships

A. Organization of the Stem

The angiosperm stem is a highly variable organ that we will have an opportunity to examine at a later date. At this time, only the most general features are to be stressed: epidermis, cortex, vascular bundles and pith. Not all stems have all of these features. The lower vascular plants will have essentially no secondary growth, for example. Obtain a prepared slide of a young stem of Pelargonium in cross section. Identify all of these tissues and the basic cell types. What is the function of each? Is there any secondary growth?

B. Organization of the Root

The root consists of the following tissues: epidermis, cortex and vasculature. Secondary growth is rare in the lower vascular plants. In monocots, there may also be a pith in the root, but this tissue is rarely seen in vascular plants in general. Examine a root of Ranunculus. Did you find all the tissues? This is a dicot, so if you saw a pith, you should check again.

III. Technical Data Acquisition

Several basic techniques are used to gather the data used by morphologists in their scientific interpretations. Four of the most common techniques include the following, each of which is available on a demonstration basis:
A. Maceration

Cells are separated from one another to determine the size and shape of individual cells. Typically, the cells are chemically fixed to retain their shape and size, and then are dissociated by acids, bases or enzymes, depending on how resistant the material is. One of the most common is a xylem cell preparation. Observe the native form of the dried cells and observe some under a microscope at the side table.

B. Sectioning

Sectioning of material may be accomplished using freehand sections with a razor blade or with a microtome. The material may be fixed or frozen, and is typically embedded in a support matrix to facilitate sectioning. The use of chemical solvents replaces water (and other soluble elements) of the biological material with either paraffin wax or plastic resin. Using wax, sections as thin as 1 micrometer (1/1000 millimeter) can be made by the most skillful technicians. Plastic embedding is necessary to make thinner sections (as thin as 30 nanometers [30/1,000,000 mm]), like those required for transmission electron microscopy. This is followed by staining to give contrast to the object to be studied. A wide variety of stains are used and several journals are devoted to development of new staining techniques. Sectioning is advantageous in determining cellular arrangements, where higher resolution is required; however, three-dimensional reconstruction may be ultimately required to "put it back together." Interpretation of three-dimensional data from sections is an acquired ability.

C. Clearing

Clearing involves the extraction of all of the opaque materials of the plant in order to visualize the object of interest. Clearings render the organ transparent, although a stain like safranin or others is usually added to contrast the vasculature. Leaf skeletonization is done by simply continuing the process and beating the leaf until only the xylem remains. Internal tissues, such as those within the ovule, are usually observed by using special optics (phase contrast or interference contrast microscopy) to see unstained soft tissues. Clearings retain all of the three dimensional information, in situ, but do not always provide the degree of resolution needed to answer all of the questions that could conceivably be posed about an organ.

D. Surficial techniques

Occasionally, observing the surface of the plant alone is more important morphologically than seeing the internal organization. The emergence of primordial organs, in particular, may be facilitated by examining just the surface layers. The easiest method includes the use of a simple dissecting microscope. The usefulness of this may be extended by using off-axis illumination or stains to increase visibility of specific structures. Crystal violet, for example, may render external cell walls more visible, if applied briefly to the surface.

For greater resolution and depth of focus, the scanning electron microscope is used to visualize surfaces. Different techniques may be used for tissue preparation. Chemical fixation is usually followed by chemical dehydration and critical point drying—a process whereby the phase change between liquid and gas is done with specific temperature and pressure requirements so that a phase boundary or drying front does not pass through the object. A relatively new development in this area is cryoSEM observation, where a quickly specimen is frozen and kept at liquid nitrogen temperatures throughout observation, thus eliminating many of the drying artifacts of other techniques.