Microscopic Techniques I

Plant Biology

Objectives: After completing this lab a student should be able to

1) Make simple longitudinal and transverse free-hand sections of plant material.
2) Make a wet mount slide.
3) Understand the use of the following stains: Toluidine blue, Phloroglucinol-HCL, Iodine-Potassium-Iodide, and Sudan IV.

Introduction

Plant anatomy (morphology) is a basic core subject in the study of biology, especially plant biology. In the study of plant structure, it is important to recognize that there is a fundamental difference between plant and animal development. In plants, the environment plays a greater role in regulating development. As a result, plant cells are more adapted to changes. The internal structure of the same plant can be slightly different when grown in different environments. This is also reflected in their anatomy. Although distinct cell layers and tissues can be seen, different cell and tissue types do not occur as large homogeneous masses and no sharp demarcation exists as in animal organs. To complicate matters further, an apical to basal as well as a radial gradation of “age” exists within the plant body. As a result, differing structural characteristics exist. Therefore in order to learn about plant structures, it is important to take a hands-on approach. The purpose of the following exercises is to introduce some of the simple techniques that are useful in the study of plant structures. One will soon realize that one’s own hand sections are better than prepared slides.

Dissection

Sit comfortably, with plenty of clear table space (it is NOT rude to put your elbows on the table, they steady your hand). Use a microscope on ALL possible occasions, a little magnification is helpful even for large subjects. Use a BRIGHT LIGHT to illuminate the subject. Cut as well as disassemble. Make both transverse as well as longitudinal sections (see figure below). You can stain dissections!
Free-Hand Sectioning

Most plant parts are too thick to be mounted intact and viewed with a microscope. In order to study the structural organization of the plant body, sections have to be made so that enough light can be transmitted through the specimen to resolve cell structures under the microscope. A free hand section is the simplest method of preparing specimens for microscopic viewing. This method allows one to examine the specimens in a few minutes. It is also suitable for a variety of plant materials, such as soft herbaceous stems and small woody twigs.

Procedure

- Use single-edge razor blades. Use a NEW blade ONY for preparing the final sections. Use OLD blades, a knife or clippers for initial trimming. Be sure to rinse a new blade to remove oils and grease from the surface of the blade.
- Hold large specimens between thumb and forefinger. For smaller specimens and leaves, hold the material between pieces of polystyrene (white packing Styrofoam) or carrot. You may want to try to roll leaves before cutting.
- Flood the razor with water. This will reduce the friction during cutting as sections can float onto the surface of the blade.
- Cut sections as thin as possible using a “drawing cut”. See the diagram below for how to hold your hand and the razor blade. Wedge-shaped sections are often of most use. Do not despise thick sections, however. Cut several sections at one time.
- Transfer sections to water using a brush – NOT forceps or a needle
You may wish to look at the sections fresh and immediately. They can be fixed and stored in 70% ethanol for later observation.
Mounting

The most common slide preparation is called the "wet mount" slide and utilizes a flat slide and a cover slip.

Procedure

- Place a drop of water or 50/50 glycerin/water in the middle of a clean slide.
- Using a brush, transfer one (or more) sections to the drop of water. Do not mount more sections than will fit in about a 1/3 the area of the coverslip.
- Hold a coverslip gently over the drop at an angle, with one edge touching the slide first (See Figure 1, below). Allow the liquid to spread out between the two pieces of glass without applying pressure.
- Slowly lower the coverslip so as not to introduce air bubbles.
- It takes some practice to determine just how much liquid to use. If too much is placed on the slide, the cover slip will "float", creating a water layer that is too thick. If too little liquid is used, the specimen may be crushed by the cover glass and evaporation will dry up the specimens quickly. A well prepared slide made with water will last for 15 -30 minutes before it dries up. The 50/50 glycerin/water will give you a few days.
- To extend the life of a wet mount slide you can do one of the following:
  o Seal the edges with clear nail polish. BE SURE THIS IS DRY BEFORE PUTTING THE SLIDE ON THE MICROSCOPE!!
  o Scrape petroleum jelly onto each of the four edges of the cover slip (Figure 2). Place the cover slip over the drop of water ("jelly side down" - Figure 3), and press lightly to seal it to the slide. This sealed slide may last for several days.

Bleaching

Cell contents (especially those contents that are colored) may be removed by placing sections in household bleach (Clorox; aqueous sodium hypochlorite).

Procedure:

- Transfer your section to a watch glass containing a small amount of bleach.
- Let the section bleach for 3-10 minutes. Watch this carefully as prolonged exposure to bleach will macerate (separate) the cells in your section!
- Wash the section by transferring the section back to water for a few minutes.

**Simple Stains**

Keep all stains covered in watch glasses or Petri-dishes; alcoholic stains evaporate VERY rapidly!

**General Stain (Toluidine Blue 0.1% aqueous)**

Toluidine blue stain is a polychromatic dye. This means that it reacts with different chemical components of cells differently and results in a multi-colored specimen. The colors generated can provide information on the nature of the cell and its walls. Pectin (and the middle lamella) will appear red or reddish purple; lignin will appear blue; phenolics acids will appear blue-green; sieve tubes and companion cells will appear purple; and nucleic acids will appear purplish or greenish blue. Callose and starch will remain unstained.

**Procedure:**

- Transfer your section to a watchglass with stain.
- Stain for about 1 minute.
- Rinse by returning the section to water.
- Mount in 50/50 glycerin/water.

**Phlorogluconol-HCL stain for lignin**

Lignin is a common constituent in the secondary wall of plant cells (e.g.: the walls of xylem elements and sclerenchyma tissue). Phlorogluconol-HCl will stain lignin a red-violet color.

**Procedure:**

- BE VERY CAREFUL USING THIS STAIN AS IT IS HIGHLY ACID! Keep it away from the microscopes!
- Transfer your section to a watchglass with stain.
- Stain for about 2 minutes. (You will see the section turn red).
- Mount in 50/50 glycerin/water.
- Examine these slides quickly as the color will fade.
- Wash your brush well under tap water to remove the acid
**Iodine-Potassium-Iodide stain for starch**

The iodine-potassium iodide (IKI) stain is specific for starch. The length of the starch molecule determines the color of the reaction – the shorter the molecule, the more red the color; the longer the molecule, the more blue-black the color.

*Procedure:*

- Transfer your section to a slide.
- Place a drop of IKI solution directly on the specimen.
- Wait for a few minutes.
- Apply a coverslip

**Sudan IV stain for lipids**

Sudan IV stain is soluble in apolar substances (like lipids and waxes).

*Procedure:*

- Transfer your section to a watchglass with stain.
- Stain for about 5 minutes
- Transfer the sections to 85% propylene or ethylene glycol in water. Agitate these for about 30 seconds to wash off excess stain.
- Rinse by placing the section in water.
- Mount in 50/50 glycerin/water.
Notes for making stains:

**Toluidine Blue:** dissolve 0.1 g of toluidine blue O in 100 ml of 0.1 M benzoate buffer, pH 4.4. (benzoic acid 0.25 g, sodium benzoate 0.29 g, water 200ml). This buffer is recommended for histochemical purposes. If benzoate buffer is not available, for general use, tap water can be used as the solvent for TBO.

**Phloroglucinol-HCl:** Prepare a saturated solution of phloroglucinol in 20% hydrochloric acid. The hydrochloric acid used is about 2 N. First dissolve phloroglucinol (about 2.0 g) in 80 ml of 20% ethanol solution and then add 20 ml of concentrated HCl (12 N) to it.

**IKI:** Dissolve 2 g of KI in 100 ml of water. Add 0.2 g of iodine into the KI solution.

**Sudan IV:** Dissolve 0.7 g of Sudan IV in 100 ml of propylene or ethylene glycol. Heat to 100°C and stir for several minutes. Filter hot solution through Whatman No. 2 paper. Cool. Filter again.