PROTOCOLS FOR ANATOMY/MICROMORPHOLOGY

GENERAL DISSECTION OF SPIKELETS

Remove the spikelets or portion of a spikelet you wish to examine and place it on a piece of glass or a slide or whatever surface you use for dissections. Place a few to several drops of Pohl's solution on the sample and let it sit for a few minutes. As soon as the material is softened you can proceed with your dissection. You may need to add a drop or two of Pohl's solution as you work since it does tend to evaporate quickly. Note that hairs tend to disappear in the liquid so you might wish to make observations on pubescence before adding the Pohl's solution.

HAND SECTIONS AND EPIDERMAL SCRAPES OF BAMBOO LEAVES

With a little practice, it is possible to make semi-permanent sections or epidermal scrapes of bamboo leaves that allow the observation and scoring of important micromorphological and anatomical features. It is also helpful to make sections or scrapes in this way to check for condition of the material and for useful variation before going to the more expensive and time-consuming methods of paraffin- or resin-embedding and microtome sectioning. It is assumed that the starting point will be leaf material removed from herbarium specimens but it is also possible to obtain sections or scrapes from fresh leaves.

Hand sections

1. It is important to make sure that the foliage leaf blades are selected from approximately the same position in the leaf complement and at about the same stage of maturity across the samples to be examined. Once a leaf is selected, sections are usually made from the middle third of the leaf blade. Place the leaf blade on a glass slide (cut or break the leaf blade crosswise if necessary so that some portion of the middle third is readily available for sectioning) and soak it with several drops of Pohl's solution. Let soak for several minutes until the leaf is rehydrated. Alternatively, you may soak the leaf or leaf piece in Pohl's solution in a small watchglass, but be aware that when you reach the next step, you will need to have a dropper or squeeze bottle handy to keep the material wet as the Pohl's solution evaporates rapidly.

2. Place the glass slide with the leaf piece under a stereomicroscope (dissecting microscope). Using the lowest magnification, focus on the surface that you plan to section. Secure the leaf with your forefinger and place the nail slightly behind the edge you wish to cut. Using your nail as a guide, make the cross-section using a fresh single-edged razor blade. The first cut will be to even out the surface but following cuts with the razor blade should produce sections thin enough to fall over on their sides. If the cutting edge or the cut sections start to dry out, add a few more drops of Pohl's solution. Note that the area of the razor blade used to make the cuts will dull quickly due to the presence of silica in the silica cells of the grass epidermises. Use different areas of the original razor blade until you cannot obtain a sharp cut and then discard the blade. It may be necessary to use two blades (or more) per sample until you are

practiced at the technique but eventually it is usually possible to obtain satisfactory sections with one blade.

An alternate method, if you don't feel comfortable using your fingernail, is to cover the top of the leaf with another glass slide and use it as the guide for sections. Either method will require some practice before you can produce thin sections.

3. Once you have some thin sections, prepare a glass slide by putting a self-adhesive label on it and then placing one or two drops of lactophenol/aniline blue solution on the slide. Using fine forceps, transfer the thin sections to the lactophenol/aniline blue. Usually the sections will float off into the preservative/staining solution but sometimes they stick to the forceps, so you need to make sure that they go into the solution on the slide. Pohl's solution does not mix with the lactophenol solution, so often at first you will see the lactophenol solution pull back from the section(s), but the Pohl's solution quickly evaporates and the lactophenol will completely envelop the section(s).

4. Add up to several thin sections but don't crowd them too much or you may have problems with overlapping tissues. Once you have all the sections you need in the lactophenol solution, carefully place a coverslip over the lactophenol and sections. It is very easy to introduce bubbles around the sections, so do this very gently. If there is insufficient lactophenol to fill the area under the coverslip, add a very small amount at one edge of the coverslip. If there is too much lactophenol, blot around the edges of the coverslip to remove the excess.

5. You can observe the sections immediately under the compound microscope but the full staining reaction takes about 24 hours to develop. If you have some good sections, place the slide on a tray and let it sit until the next day. If the sections are not usable, you know right away that you will need to make additional ones.

6. If you have obtained good sections and wish to preserve them for a longer period, the coverslip can be sealed in place using a couple of coatings of nail polish around the edges. This works best when all of the excess lactophenol around the edges has been wiped away. The slides can be stored on edge in a slide box but they will last the longest if stored flat.

7. If you make sections this way and then they sit for too long and the lactophenol begins to evaporate, you can usually rejuvenate them by adding a bit of lactophenol at the edge of the coverslip. If this doesn't work then you will need to make new sections.

8. If you start with fresh material, instead of dried material (either from a silica gel sample or from a herbarium specimen), you do not have to soak the sample in Pohl's solution before cutting sections. If you can work rapidly enough, it is probably best to just make sections without adding any water or Pohl's solution and immediately mount the sections in the lactophenol/aniline blue solution. If it is necessary to keep the sample wet, use Pohl's solution or distilled water, although the Pohl's solution will evaporate quickly and will not dilute the mounting solution as the water might.

Epidermal Scrapes

1. Assuming that you will be working with leaves from herbarium specimens or dried specimens, remove a section from the middle third of the leaf blade and soak it in Pohl's solution for a few minutes. Slice away the midrib. Place the desired surface downward against the slide and gently scrape away the leaf tissue from above. It will be difficult to get a large area scraped without tearing, but usually even what appears to be a small area will be large enough under the microscope for you to observe details.

2. Once you have a satisfactory scrape, cut away most of the surrounding tissue and then, using fine forceps, move the epidermal scrape to the lactophenol/aniline blue solution on a slide prepared as described in I.3. Place the coverslip gently over the scrape.

3. Follow the steps described in section I for semi-permanent preservation of the slide if desired.

CLEARING AND STAINING INTACT PLANT ORGANS

From Laboratory Exercises in Plant Anatomy, Spring 1980, by Dr. Nels Lersten, Iowa State University.

Many plant structures can be examined 3-dimensionally if the cell contents are removed and the cell walls stained. The following procedure will work for fresh, liquid-preserved, or dry material.

1. Place fresh material into 95% alcohol until chlorophyll is mostly removed. Start dry or liquid-preserved material in Step 2.

2. Sodium hydroxide (10% aqueous solution). As cell contents leach out, the NaOH may become discolored. If so, replace with fresh NaOH. Clearing time varies from a few days to several weeks. Repeat, if necessary, until material is fairly clear. If dark areas remain, place in full-strength household bleach for a minute or more (keep time to a minimum as some tissues tend to disintegrate).

3. Three changes of distilled water (5 minutes each*).

4. Place in aqueous chloral hydrate for a day or more. The tissues should become transparent except for lignified areas. The material may be stored indefinitely in this solution if necessary.

5. Three changes of distilled water (5 minutes each*).

6. Two changes of 95% ethanol (10 minutes each*).

7. Stain (0.5% chlorazol black E in 100% ethanol) for 1-2 minutes. The best time will vary considerably.

8. Two changes of absolute ethanol (5 minutes each*).

9. Xylene-absolute ethanol (10 minutes*).

10. Stain in safranin in equal parts of xylene and absolute ethanol. Time varies considerably, depending on the material. Try 10 or 15 minutes for a start.

11. Destain to desired intensity in 1:1 xylene and absolute ethanol.

12. Place in xylene to stop destaining. Because safranin particles may precipitate on the epidermis, change to fresh xylene after a few seconds. Examine, and if the stain is too dark, repeat Step 11.

13. Mount a slide using xylene-soluble mounting medium. Add one or more weights to the coverslip. Thick specimens pose some problems, chiefly from solvent evaporation, and at first more mounting medium must be added at the side of the coverslip

SCANNING ELECTRON MICROSCOPY OF BAMBOO LEAVES

This methodology was published in Dávila and Clark (1990).

Materials

In my experience this procedure works best with leaf material taken from herbarium specimens but it is certainly possible to use fresh leaves, depending on the type of scanning electron microscope available. Cut two representative samples approximately 0.5 x 1cm from the midportion of a mature foliage leaf, one for viewing the adaxial (upper) surface and one for viewing the abaxial (lower) surface. Position these side by side so that you have one with the adaxial surface facing up and the other with the abaxial surface facing up, and then cut a tiny piece off of each one on the upper right hand corner. This way, after the treatments described below, you can always be sure that you have them correctly oriented when you go to mount them on the stub.

Method

1. Removal of epicuticular wax is important in order to see surface details clearly; this also often helps to remove fungi and other debris, at least partially. Place each pair of leaf pieces into a small glass vial marked with tape to keep the identifications in place. Add enough xylene to cover the pieces generously. Place the vials in a sonicator and sonicate for 10-15 minutes. Allow the samples to air dry, always being careful to keep each pair properly identified.

2. Mount the specimens on brass discs with silver paste or double-stick tape, either both pieces of one sample on the same disc or on separate discs, but with some identifying code scratched onto each brass disc. Sputter coat the discs with Au-Pd in a sputter-coater (all of my samples to date were done in a Polaron E5100 sputter-coater, but newer models are undoubtedly available).

3. All of my samples were viewed at 15kV in a JEOL JSM-35 scanning electron microscope at the Bessey Microscopy Facility (now the Microscopy and NanoImaging Facilty) at Iowa State University. They were photographed using Polaroid Type 665 positive-negative film, but virtually all instruments available today have digital imaging as the standard.

RECIPES

Amann's Lactophenol/Aniline Blue (Sass, 1951)

phenol (melted)	20 cc
lactic acid	20 cc
glycerine	40 cc
distilled water	20 cc
1% aniline blue	5 cc

1. Remove the bottle of phenol from the freezer, place it in the fume hood, and allow it to warm up to room temperature. Phenol is very caustic and dangerous, so wear gloves and safety glasses during this procedure, and keep the whole procedure under the fume hood.

2. Loosen the lid of the phenol bottle to prevent build-up of pressure or measure out the correct amount of phenol and place in a bottle (with a capacity of at least 200 cc) with the lid on loosely.

3. Place the bottle in a hot water bath at 60-65º C until the crystals have melted.

4. Add the remaining ingredients and mix well. Note that cotton blue can be substituted for aniline blue.

5. Cool and store in a closed bottle.

Pohl's Wetting Solution or Pohlstoffe (Pohl, 1965)

dioctyl sodium sulfosuccinate (Aerosol OT)	1 cc
distilled water	74 cc
methyl alcohol	25 cc

1. Dissolve the Aerosol OT in distilled water and add the methyl alcohol. Wear gloves and work under the fume hood.

2. Store in a tightly sealed vial or jar. It is best dispensed from a dropper bottle or a small polyethylene squeeze bottle. Try to avoid shaking the solution.

Modified Pohl's Solution (Clark, unpub; I use this version)

distilled water	750 ml
1-propanol	250 ml
liquid dishwashing soap	2 ml

1. Mix the ingredients together and stir well.

2. Store in a tightly sealed vial or jar. It is best dispensed from a dropper bottle or a small polyethylene squeeze bottle. Try to avoid shaking the solution.

REFERENCES

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