Toluidine Blue: A Simple, Effective Stain for Plant Tissues

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For years educators have stressed the importance of the presentation of biological concepts with balance between botany and zoology. Though animal biology often draws greater emphasis in biology courses, the central role of plants in keeping the Earth alive is a cornerstone of environmental science and ecology. Since the role of plants is so vital, the study of biology should include many of the sub-disciplines of botany such as physiology, histology, development, and pathology.

The rediscovery and adaptation of simple laboratory techniques that yield high quality results are always welcomed by educators. Publications such as *Discovering Plants: A Nature and Science Book of Experiments* (Klein and Klein 1968) offer a variety of these experiences, and when utilized in the classroom they can carry botany far beyond general studies of anatomy and taxonomy. In this article we call attention to a method capable of yielding such experiences—the preparation of botanical sections stained with toluidine blue 0.

Animal histologists have long recognized the usefulness of this staining agent, which has the remarkable property of giving different colors to different tissue components (polychromasy). O'Brien, Feder, and McCully (1964) describe simple methods of utilizing toluidine blue in botanical staining. These methods, although still relatively little known, markedly facilitate the study of plant tissues for students at every academic level. Though complete histological explanations for the polychromastic effects of toluidine blue are still lacking, the results are highly reliable and comparable to those of many complicated, multi-stage techniques. The method for making fresh, temporary mounts begins with the preparation of a solution of 0.05% (50 mg/100ml) toluidine blue in distilled water or 0.01 M phosphate buffer (pH 6.8). In some areas with "soft water," good results may be obtained using tap water. The solution is quite stable and may be kept for a year or more without loss of staining properties.

Hand-cut sections are recommended since they can be made without fixing or embedding the plant material. Sections are cut with a single-edged razor blade, or a double-edged blade with one edge protected with adhesive tape. Several approaches are possible. For example, the material to be cut may be placed on a flat, non-skid surface (such as a blotter pad or a block of wood), and the cut made from above, downward and slightly away from the body, thus producing an oblique slicing motion, causing less cell damage than a cut pushed directly downward (fig. 1a). Students must be cautioned against moving the sharp edge of the blade toward their fingers. Indeed, since students will be handling sharp and potentially dangerous instruments, a demonstration of the techniques involved in hand sectioning, and a clear warning as to the hazards involved, must be given. Additional information on the hand sectioning of delicate materials is given in Morholt *et al.* (1966).

For the preparation of the plant material, the following procedure is recommended (figs. 1a-f):

a) hand cut sections (10-50μ) with a razor blade;

TABLE 1. Differentiations Observed in Cell Types and Tissue Structures Using Toluidine Blue

Tissue Element or Structure	*Color Developed by Toluidine Blue*
Xylem	Green or Blue-Green
Phloem	Red
Sclerenchyma	Blue-green, sometimes Green
Collenchyma	Red-Purple
Parenchyma	Red-Purple
Callose, Starch	Unstained

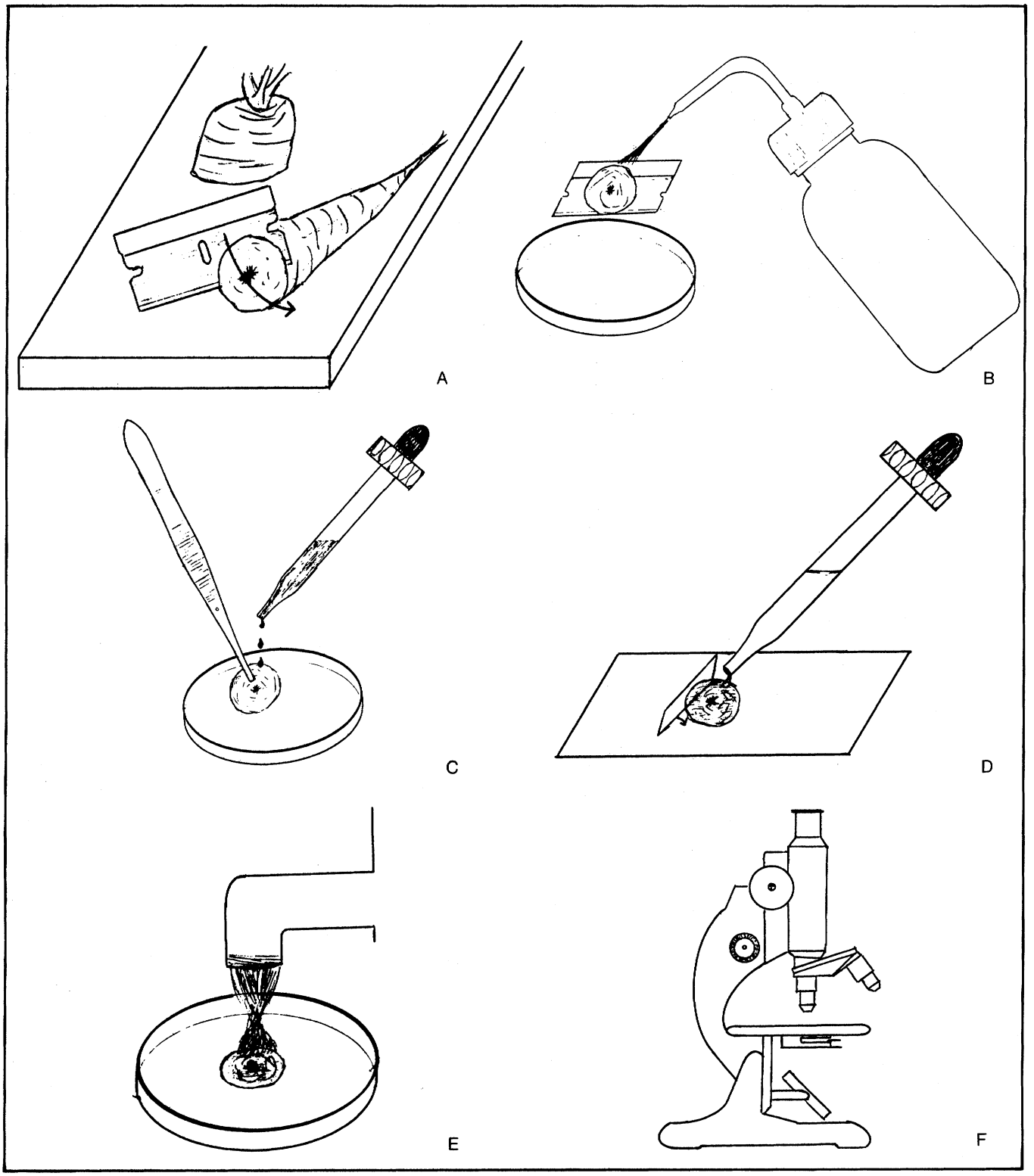

FIGURE 1. A) Place the plant material on a non-skid surface such as a block of wood or a blotter pad and make an initial cut to prepare the surface. Grasp the plant material firmly and slice as thin a section as possible. This section should be quite translucent. B) Flush the section from the razor blade into a small petri dish, using a wash bottle filled with tap water. Soak in the tap water for 2-3 minutes. C) Transfer the section to a second petri dish and add 2-3 drops of toluidine blue to the section. Allow the dye to soak the tissue for one minute. D) Transfer the section to the last petri dish and rinse under gently running water for two minutes. E) Mount on a clean glass slide with a drop of tap water and cover with a coverslip. F) Examine under a microscope at powers up to about 250X. Some sections of outstanding quality will give good results at higher powers without additional preparation.

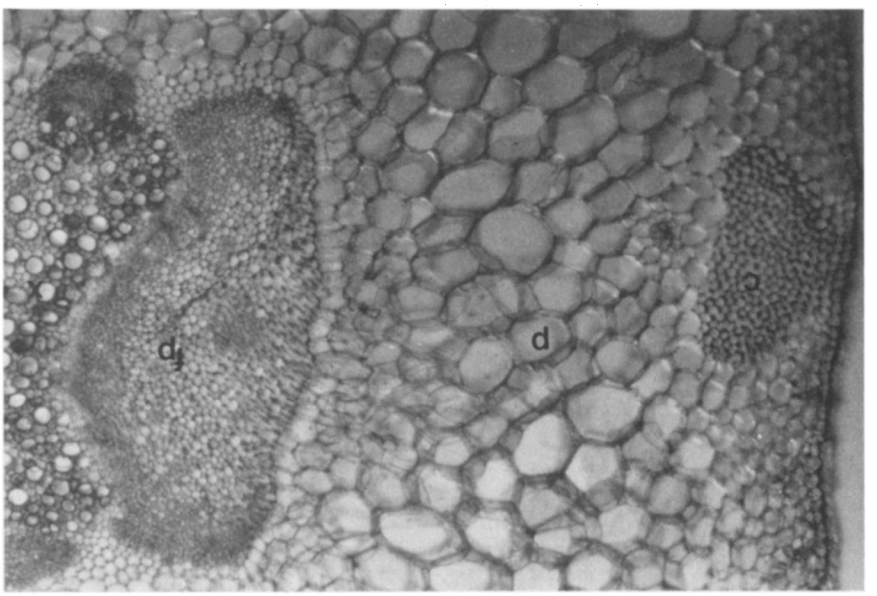

FIGURE 2. Hand-section of celery *(Apium)* stained with toluidine blue at magnification of 200X showing delineation of cell walls. Also shown are areas of collenchyma (c), parenchyma (p), fiber with associated phloem (fp), and xylem (x).

b) transfer sections to tap water and soak for 2-3 minutes;

c) transfer sections to the staining solution and immerse for one minute;

d) transfer stained sections to tap water and rinse for two minutes;

e) mount with a drop of tap water under a cover slip; and

f) examine at magnifications up to about 250X.

For examination under higher power, or to make permanent mounts, see O'Brien, Feder, and McCully (1964).

Toluidine blue gives impressive results in differentiating many cell types and tissue structures (table 1). It provides excellent discrimination among structures with a high density of different cell types, such as root tip or bud. For example, the characteristic green to bluish-green coloration of lignified structures provides striking delineation of cell walls, a location of high lignin content (fig. 2).

In the classroom toluidine blue can give the students results ten minutes after they set up their microscopes, leaving time for the comparison of many types of plants. Virtually any fresh plant material is suitable for study with toluidine blue, for example, geranium leaf, *Anacharis* (*Elodea*) stem, carrot root tip, asparagus bud, pea epicotyl, etc. Common vegetables and fruits provide excellent results and can arouse interesting discussion on the function of the different cell types and their value in nutrition.

Toluidine blue is readily available from scientific supply companies; a gram or two of the dye would supply the needs of classes over the course of many years. The wealth of information that can be tapped by the use of a razor blade and a few drops of toluidine blue should make this procedure very attractive to teachers and students alike.

References

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