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A simple technique for cross-sectioning Gymnosperm needle leaves using microtome

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Abstract: If a staining-permanent slide is not needed, this simple and faster microtome technique is very useful for cross-sectioning Gymnosperm needle leaves. The technique employs 2× polyvinyl alcohol-lactic acid-glycerol (PVLG) without the steps of fixation, dehydration, clearing, embedding, deparaffinisation, and hydration. Conveniently, this technique can also be applied to thick leaves and other organs of Angiosperms. The procedure is very simple and is suggested for use in plant anatomy research.

Key words: Cross-section, Gymnosperm, microtome, needle leaves, PVLG

Introduction

With the invention of the microscope, there were many efforts to observe the structure of plants using anatomical cross-sections. The microtome was invented by George Adams Jr in the 18th century (Smith, 1915), and now the machine, which was improved by many researchers over the years, is important in various fields of research. Andrew Prichard improved the vibration problem in microtomes by affixing the device to the table, by separating the operator part from the amputation part (Smith, 1915). As a result, the microtome was able to cut more clearly. In the 19th century, Jan Evangelista Purkyně became the first person to create a thin slice using a microtome for microscopic examination (Henry, 1953; Šteiner et al., 1988). Researchers were able to make a very thin layer continuously for microscopic observation and develop dyeing methods.

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Currently, nuclei or micro-organisms at the cell level can be observed and a unique pathological infected area can be checked using the botanical microtechnique. This technique enables understanding of the ultrastructure of a plant by using an electron microscope (Moor et al., 1961; Eshratifar et al., 2011). However, the obtaining of a good permanent slide can be a costly and time-consuming process, because it consists of many steps, including fixation, evaporation, and dyeing. Therefore, I have developed a method that more simply allows for the examination of the structure of a needle leaf under a microscope, and I have had good results from applying this method to fresh needle leaves in several gymnosperms.

Materials and methods

Traditional botanical microtomy is used to cut a hard material like wood. Therefore, the botanical

microtome, which has a heavy blade, cannot make as thin of a cut as a regular microtome (Carlquist, 1992; Esteban & Palacios, 2009). In the traditional histological technique, materials are hardened by replacing the water in the materials with paraffin so that they can be cut. The steps required in using paraffin to make a permanent slide are time-consuming and require much effort (Spence, 2001; Güvenç & Duman, 2010). As a result, cryosectioning, which does not use paraffin, arose as an alternative for turning water-rich tissue into hard material by freezing the tissue with tissue-freezing medium and cutting the frozen tissue with a microtome within a freezing chamber (Barthel & Raymond, 1990). However, cryosectioning requires a staining step to remove water from tissue, and so it does not reduce the necessary time or money. Therefore, through many trials, I devised a new procedure to remove water from tissue using another fixative.

The technique

1. Cut fresh needle leaves to a suitable size (0.3-0.4 mm) to put on a mounting block in a cryomicrotome chamber (Leica CM1850 Cryostat Cryocut Microtome, Leica Instruments, Germany).
2. After the sample is put on the mounting block, a tissue-freezing medium (Leica Instruments) is used to embed the sample. This step should be carried out quickly and accurately.
3. After embedding, cut the sample at a constant speed at a temperature below –20 °C. In my experiment, the needle leaves were cut to a thickness of 60 μm.
4. After placing thin layers of the sample on a glass slide, drop the new fixative (2× polyvinyl alcohol-lactic acid-glycerol (PVLG): 100 mL of distilled water, 200 mL of lactic acid, 20 mL of glycerol, and 33.2 g of polyvinyl alcohol) onto the thin layers and lightly put on the cover glass.
5. This slide can be observed with a microscope, and it is possible to take a picture of it with a digital camera. The slide can be preserved for at least 1 week using a colourless nail polish.

Results and discussion

Cross-section examples of needle leaves using this technique are shown in Figure. All cross-sections of needle leaves are shown at low magnification (×63; Leitz, Wetzlar, Germany). In this technique, some steps, including fixation, staining, and dehydration, are skipped entirely. Normally, in order to make a permanent slide, at least 3 or 4 days are required using the conventional techniques, while the new fixative technique only requires 30 min to complete. This means that more time could be saved and the anatomical structure of the material could be observed right away (Figure).

The PVLG fixative is used for making slides of arbuscular mycorrhizal fungi spores (Koske & Tessier, 1983). Thus, this fixative is not an original invention, but was rather improved upon and newly applied to microtome procedures. The colourless quality of the original fixative is a very good characteristic, but the thin viscosity was not enough, in application, to make a slide. Thus, 2× PVLG was invented. PVLG is thicker in consistency and also protects the slide from oxidation.

When 2× PVLG is used, the PVLG cannot remove the water from the tissue. It only pushes the water out of the tissue. Colourless polish is better than Canadian balsam, because Canadian balsam forms a yellowish clot when reacting to water. Using the 2× PVLG fixative in making cross-section slides will help researchers create transparent slides and allow them to apply digital tools to the slides.

One example of applying digital tools to slides is the measurement of morphological characters for taxonomic research. This requires images to be captured by digital cameras and analysis software. Much relevant software has been developed. This study made use of ImageJ freeware, which is used in many fields, including molecular biology and cell pathology (Abramoff et al., 2004).

Using the microtomy application suggested in this paper, many samples can be handled with high throughput. This system can also be applied to Angiosperm plants with thick leaves. In most cases, anatomical research is approached more effectively and efficiently through the application of the 2× PVLG technique.

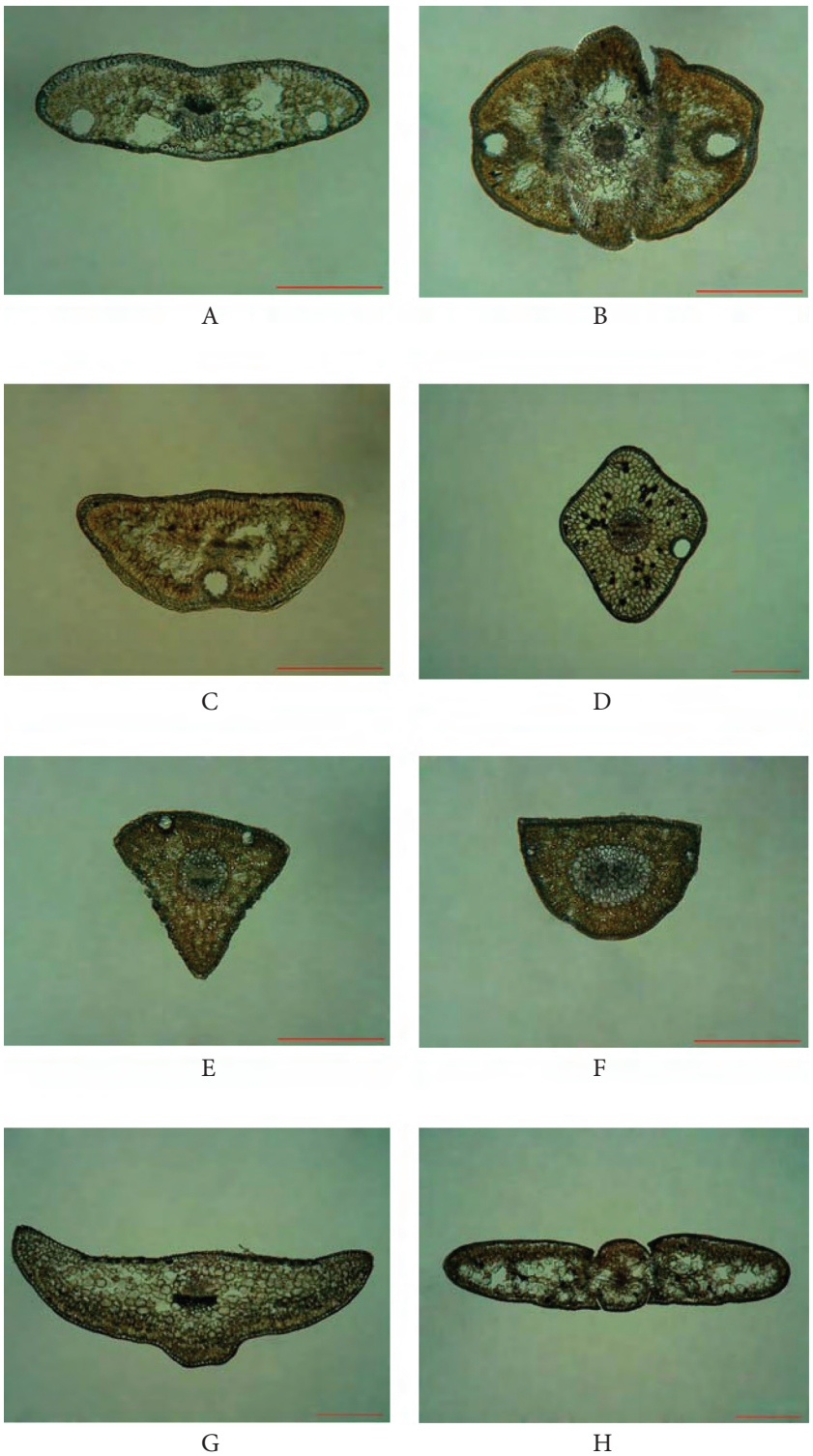

Figure. Cross-section view of Gymnosperm needle leaves: A) *Abies holophylla*, B) *Juniperus chinensis* scale leaf, C) *J. chinensis* needle leaf, D) *Picea abies*, E) *Pinus parviflora*, F) *Pinus densiflora*, G) *Taxus cuspidata*, H) *Thuja occidentalis*. Scale bar: 500 μm.

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