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RESEARCH ARTICLE

Improvement of plant cryosection

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Abstract **Cryosection in plants is usually challenging because of the larger amounts of water contained in plant cell than animal cell. The formation of ice crystals within the plant cells easily destroys subcellular detail during freeze processing. And cell walls make freezing plant tissues hardly sliced and preserve the cell structure in subtle ways. In this study, the technique was improved on infusion, embedding and flatting sections with the winter wheat Variety Aikang58 as raw material.**

Keywords cryosection, wheat, histochemistry

Introduction

Frozen section technique is a valuable tool to rapidly prepare slides from tissue for microscopic interpretation (Asan, 2003; Gal, 2005). Although frozen section technique is used in a myriad of clinical and research setting (Jensen et al., 2010; Stierhof and El, 2010; Baltaci et al., 2011; Kang et al., 2011; Turhan et al., 2011; Vitha and Osteryoung, 2011; Ye et al., 2011), its application is not quite common in botanical microtechnique. Many problems, in which the present day workers are interested, have remained open. It is a particularly promising method of specimen preparation for the application of cytochemical and immunocytochemical procedures, since it is likely to allow greater retention and preservation of antigenic or enzymatic activities of macromolecules than most other methods (Stierhof and El, 2010).

Materials and methods

Materials and chemicals

The winter wheat variety, Aikang58 was provided by the Wheat Improvement Center of Henna Institute of Science and Technology. All the chemicals were of analytical grade.

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Fixation

After rapidly rinsing the tissues at room temperature in bi-distilled water, the wheat tissues were dissected into the length of 1 cm. Then, the tissue pieces were fixed with 70 % ethyl alcohol or FAA (formalin-aceto-alcohol, FAA) for 2 h under vacuum to accelerate the removal of air from the intercellar spaces of mesophyll and the penetration of the fixative into the pieces. FAA should be made fresh as needed. Fixative volume should be 5–10 times of tissue volume. The fixation was always carried out in an ice bath.

Infusion

The tissue segments were infiltrated in 5%–15% glycerol (v/v) for 30 min by maintaining a partial vacuum, and then incubated 3 h at room temperature. The hardness of the frozen tissue segments were inversely related to the glycerol concentration within a certain range (Table 1).

Sectioning

The Leica CM1850 cryostat (Leica, Inc., Germany) and its accessories were used in this study. After infusion, the tissue segments were placed on a specimen holder and covered with the same concentration of glycerol to infusion, to keep it in a form convenient for sectioning (Table 1).

Once the tissue block has frozen into place, move the block into place and cut the sections to the desired thickness (7–20

Table 1 Glycerol concentration selection chart

Tissue	Glycerol concentration (%)*
Leaf	8–10
Stem	8–13
Root	11–15
Spikelet	5–10

* Above concentration values are based on long-term experience but are only approximate values.

microns). Slow the speed of sectioning (approx. 5 mm/s) to ease the manipulation of the sections as they were coming off the block face. To achieve ideal sections, it was critical that the knife (Feather A35 microtome blade, Feather, Inc., Japan) edge should be as sharp as possible. Trim the block face to a knife sharp with the long axis oriented vertically. This orientation could make removal of the sections from the knife edge easier, and minimize handling damage of the tissue. Use a small camel hair brush to guide the section off the block face and transfer it to egg white subbed slide. The above procedure was finished at −22°C. The egg white subbed on glass slide was able to prevent tissue crush during section melting procedure, and get more perfect tissue morphology.

Staining and microscopy

For staining, a 0.1% safranin O solution was left in contact with the sections for 1–15 s before removing most of the staining solution. An Eclipse 80i Microscope (Nikon, Inc., Japan) was used for observation and examination.

Result and discussion

Morphological structure of the winter wheat seedling various organs could be observed clearly in frozen sections, only leaves were presented here by Fig. 1. The main steps to improve cryosection protocol were summarized in Fig. 2. It took less times, lower costs and simple instruments than conventional section method, but the same clear results were obtainable without much experience. The most basic factor affecting the quality of section was the plasticity of the block and the sections.

In principle, sections of frozen tissues should retain structures, which are free from chemical and structural artifacts produced during the dehydration and embedding procedures of conventional paraffin section. However, structural delineation in frozen section has not generally been up to the standards of conventional paraffin section. One important challenge is the control of the sectioning consistency of frozen tissues (Tokuyasu, 1973). To improve the sectioning quality, Iglesias embedded tissue pieces in a methyl cellulose solution after preembedding in a gelatin solution. Moreover, Bernhard used a gelatin solution at 37°C for embedding. And Farrant embedded tissues in a bovine serum albumin solution at near 0°C (Ripper et al., 2008;

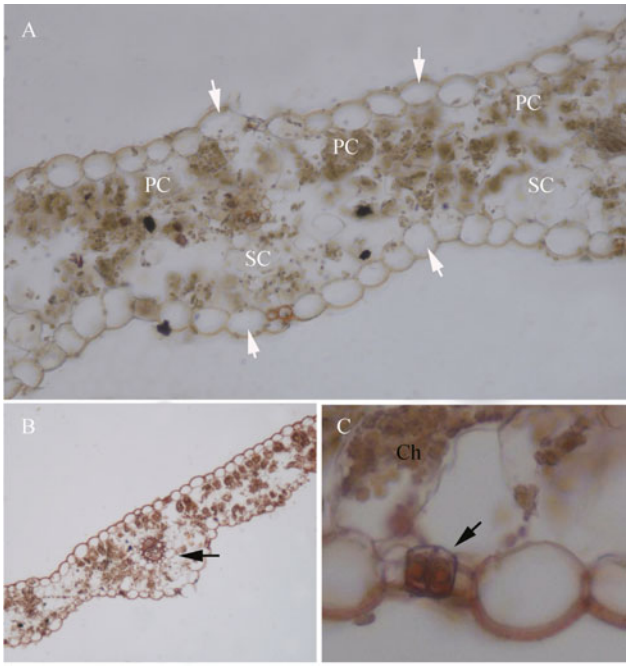

Figure 1 Parts of leaf pieces in *Triticum aestivum* Linn. (transverse section). A: Smoothly sectioned wheat leaf is show (no staining). Palisade cell (PC), spongy cell (SC) and epidermal cell (white arrows) are clearly seen. × 400; B: leaf vein is indicated by a black arrowhead. × 65; C: chloroplasts (Ch) and a pair of guard cell (black arrowhead) are observed in lower epidermis of wheat leaf. × 1200.

Stierhof and El, 2010; Vitha and Osteryoung, 2011). However, all these macromolecules do not penetrate into cells and the above embedding methods could not improve the intrinsic sectioning quality of cellular interiors or cytoplasmic organelles.

A more positive manner to control the plasticity of the specimen and reduce ice crystal damage is to infuse chemically inert, such as glycerol or sucrose, into fixed tissues pieces before freezing them. Tokuyasu infused small spinach leaf with 1.6 M sucrose before sectioning. Before embedding, the apical buds of *Sinapis alba* L. were infiltrated in 0.1 M sucrose for 2 h followed by 1.3 M sucrose in PBS for one night at 4°C by Jacqmard et al. (2002). Ice-cold 10 % (w/v) PVP/2.07 M sucrose in phosphate-buffered saline (PBS) was used to infuse pea chloroplasts by Morin and Soll (1997). Highly lignified fruits of 4 weed species (*Rumex dentatus* Linn., *Achyranthes bidentata* Blume, *Medicago polymorpha* Linn. *and Torilis scabra*) were pretreated with 15 % glycerol for infusion, the internal structure adaptive to floating could be clearly observed. Liu et al. (2006) selected 12%–15% glycerol for infusion with *Schisandra chinensis* (Turez.) as raw materials. Ball found that specimen infused with proper concentration of glycerol could be smoothly sectioned while frozen.

Another difficulty in obtaining high quality sections is how to prevent smooth spreading of sections. Some researchers

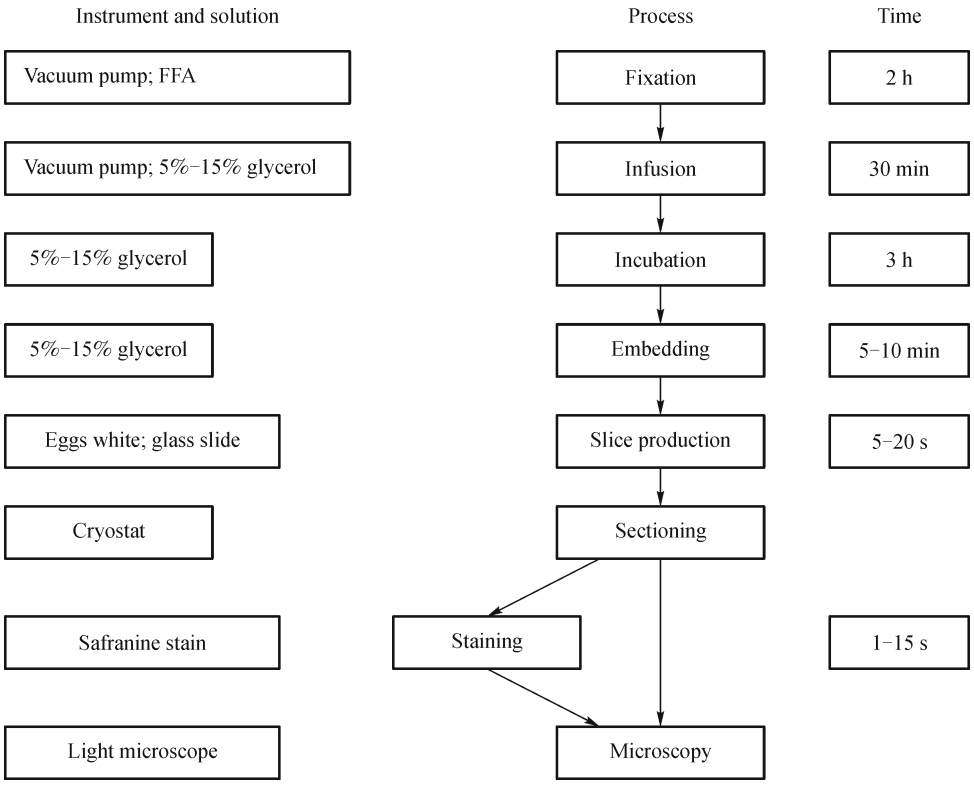

Figure 2 Main steps in the protocol for the plant tissues cryosection improved.

used a 50% DMSO solution to float sections, which were used for cytochemical purpose (Bernier et al., 1972). Bernhard recovered the sections by using 50% glycerol solution (Ripper et al., 2008). Now, the section is directly picked up on a glass slide by an eyelash probe or a brush in large number of frozen section protocol. In this study, we transfer frozen sections to a pre-cooled glass slide with a daub of egg white, which cannot only solve the problem but be beneficial for the following stain procedures.

There were three technological innovations in our method: (1) Infusing samples with lower concentration glycerol (5%–15%). By choosing the proper glycerol concentration, a wide variety of plant tissues, e.g. leaves, stems, roots, even blooms could be smoothly sectioned. The 5%–15% glycerol was roughly equivalently to 0.7–2 M in molar concentration. Another commonly used substance for infiltration is sucrose. The range of sucrose concentration carried out for infiltration was 20%–150% in previous reports (Morin and Soll, 1997; Jacqmard et al., 2002), which is 0.5–2.3 M in molar concentration. Therefore, the sectioning consistency seemed to be strongly dependent upon the amount of chemically infiltration constituent. In some researcher's opinion, the fixed tissue pieces stored in 0.1 M phosphate buffer for 0.5–1 day generally showed better results than those sectioned immediately after the completion of fixation. In fact, the significant difference between the two manners was not found in our study; (2) Embedding with the same concentration glycerol as infusion, which could prevent the non-uniformity of samples and Tissue Freezing Medium caused by OCT (SAKURA Tissue-Tek® O.C.T. Compound, Order Number 4583, USA), and obtain the serial sections with high quality; (3) Egg whites were used as the agent of adhibiting tissue. The egg white subbed on glass slide was able to prevent tissue crush during section melting procedure, so that got more perfect tissue morphology. Although the further improvement of fixation, freezing, and staining procedures could be acquired, the technique of the cryosection presented in this study could be considered as an alternative to conventional paraffin sectioning for the optical microscopic structural studies of plant tissues.

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