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Note

Intact Sectioning of Plant Tissues with a Cryomicrotome

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In functional genomics as well as basic genomics, monitoring of gene expression in intact tissues provides various kinds of useful information. In order to characterize mutant phenotypes and conduct cellular analysis (Sylvester and Ruzin 1994) as well as monitor gene expression in tissues, thinner and uniform intact sections of tissues should be available. Although the use of a cryomicrotome which is suitable for preparing non-fixed thin sections was developed half a century ago, it had been very difficult to obtain thin-sectioned specimens of hard tissues or plant tissues. Recently, Kawamoto and Shimizu (2000) have developed a convenient system for obtaining uniform section of whole body of a rat with completely calcified bone and teeth by using a cryomicrotome. They used an adhesive film to avoid damaging frozen sections during cutting. The film is specially prepared with synthetic adhesive (Cryo-film transfer kit, FINETEC Co. Ltd. Japan) (Kawamoto 1990). Their method could also be suitable for plant samples by using the adhesive film.

We tried to apply the method to plant samples and prepared intact frozen sections of rice leaves. In order to apply the technique, the plant samples must be embedded with carboxymethyl cellulose (CMC) gel. Since plants develop a wax layer or trichome in the epidermis to protect them from rain or UV-B damage (Jansen *et al.* 1998), intact thin-sectioning of plant tissues can not be easily achieved. Parthasarathy (1994) reported that sucrose at appropriate concentrations is very effective as a penetrating cryoprotectant for plant tissues. We conducted a pretreatment with a 10% sucrose solution for sectioning rice leaves. Tween 20 as a surface-activating reagent was added to the solution enable the solution to penetrate into the intracellular spaces of the leaves. By this treatment, the leaves could be well embedded with the CMC gel and cut.

As shown in Fig. 1, we obtained intact cross-sections of rice leaves with variegated albino characteristics by combining the pretreatment and the use of an adhesive film. The structure of the leaf was preserved as satisfactorily as that of the samples embedded with paraffin wax. The sections showed clearly distinguishable cells and boundaries of green sectors in the leaf tissues of the variegated albino plant (Fig. 1-B) which were found to be highly discriminative (Fig. 1-C, D and E). Moreover, it was observed that the chloroplast developed only in the green sectors (Fig. 1-C). Frozen sections, 3-20 μm thick, were obtained from plant samples such as leaves and grains without any additional manipulations. As a result, the method was also found to be useful for thin-sectioning of plant tissues. We recommend the use of an automatically operating cryomicrotome (Leica CM3050S), though a manually operating one could be used, because cutting at a constant speed enables to obtain uniform specimens. Especially, for the sectioning of large-sized samples, cutting at a constant speed is essential.

This method can also be applied for studies in various fields, such as histology, general histochemistry, enzyme histochemistry, immunohistochemistry, in situ hybridization, autoradiography and electron microprobe X-ray microanalysis (Kawamoto and Shimizu 2000). For example, in the signal transduction pathway, it is considered that calcium ion plays an important role as a second messenger. The actual mechanism still remains unknown because intact thin-sectioned specimens of tissues to which X-ray microanalysis was applied could not be obtained. Actually, Iwano *et al.* (1999) observed drastic changes in the distributions of calcium ion in papillar cells and pollen grains in the process of cross- and self-pollination in a self-incompatible species of *Brassica* using an energy dispersive X-ray microanalysis system. However, this observation was conducted only at the surface of the stigma and it was difficult to observe the changes in elemental distribution within the stigma. In this regard, a intact sectioning method for X-ray microanalysis may enable to obtain the mapping of the elemental distribution within a tissue directly.

Thus, we describe the application of the Kawamoto and Shimizu method for the intact sectioning of plant tissues by using a cryomicrotome.

Protocol

Preparation of frozen sample block

1) Cut the plant material of an appropriate length and transfer the pieces to nylon mesh bags (Fig. 2).

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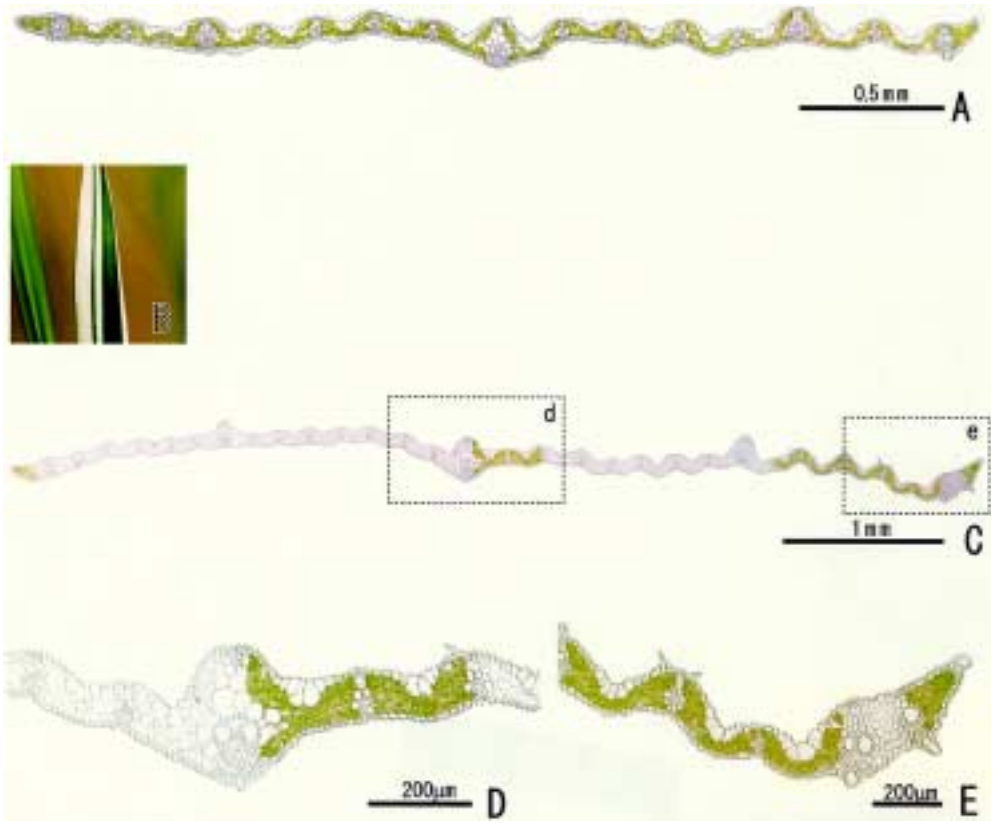

Fig. 1. Cross-section of un-fixed rice leaf (10 μm-thick). Normal (A) and variegated albino (C). B: variegated albino leaf before sectioning. D and E, Magnified view of the central portion and edge indicated by rectangles (d and e), respectively.

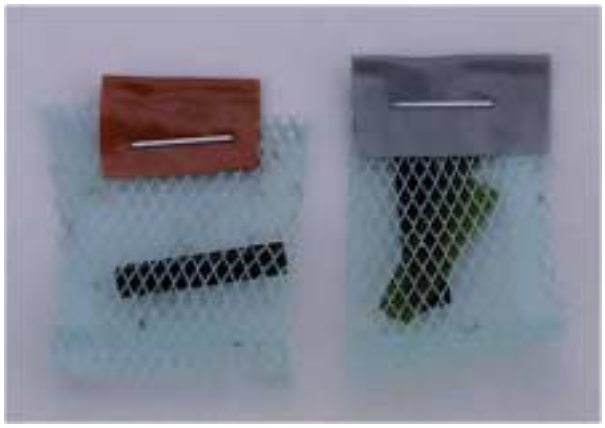

Fig. 2. Nylon mesh bag for gathering the samples and keeping them in the solution.

2) Dip the mesh bags into a 10% sucrose solution with 0.3% Tween 20 and hold them down these with stainless steel mesh (Fig. 3).
3) Put the sample in a low-pressure chamber for 10 minutes to remove air bubbles on the epidermis and inject sucrose into the intracellular spaces.
4) Immerse the pieces into a stainless steel container filled with 3-5% CMC gel and place the pieces at the bottom of the container (Fig. 4).

Fig. 3. Steel mesh in a beaker for keeping the samples in the solution in a low-pressure chamber.

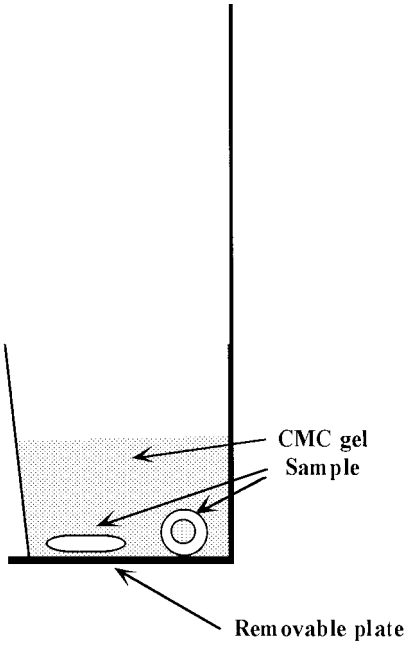

Fig. 4. Illustration of samples immersed into CMC gel in a stainless steel container.

5) Dip the container into liquid nitrogen (or hexane-dry ice) and freeze the pieces with the gel completely (take care not to splash the liquid into the container in order to avoid cracks of the gel).
6) Take the frozen CMC gel out of the container.
7) Pour the CMC gel onto the sample stage of the cryomicrotome and place the frozen CMC gel on the sample stage.
8) Place the frozen sample on the cryomicrotome and trim the sample with a disposable tungsten carbide blade (Jung TC-65, Leica Instruments).

Preparation of adhesive film and sectioning

1) Spread 100% EtOH on the acrylic board.
2) Place a polyvinylidene chloride film (10 μm thick; Asahikasei Kogyo, Japan) on the board uniformly and squeeze out excess EtOH.
3) Apply the synthetic adhesive onto the film uniformly with a specially designed brush according to a layout sheet for preparing the adhesive film (Fig. 5).
4) After approximately 5 minutes, cut the adhesive-coated film into the size of the frozen CMC block with a rotary cutter.
5) Transfer the adhesive-coated film with the acrylic board into the cryochamber.

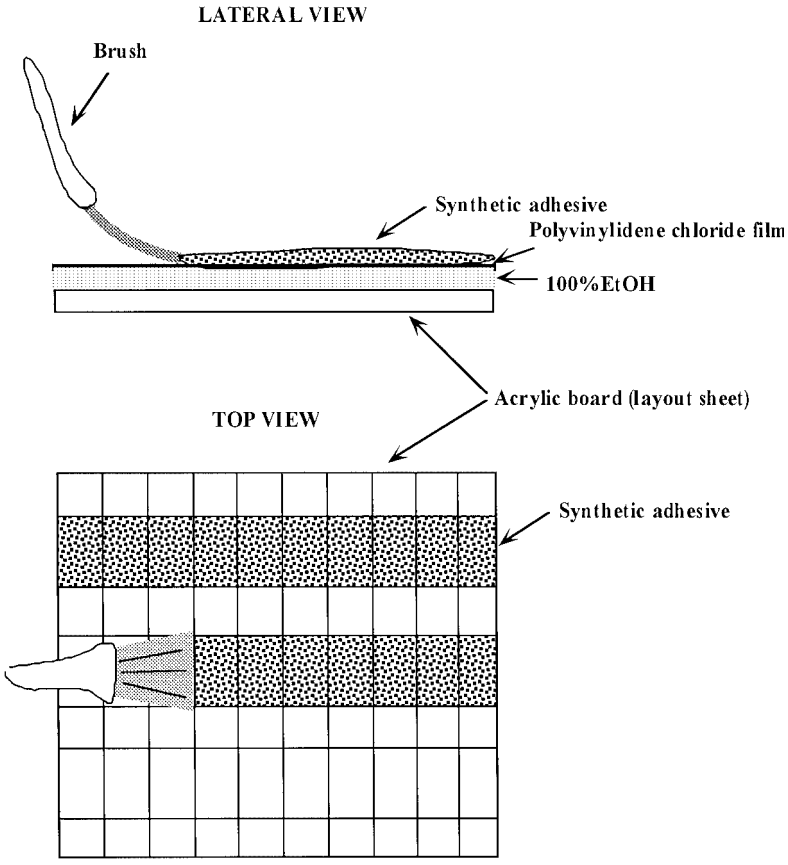

Fig. 5. Illustration of application of synthetic adhesive on a polyvinylidene chloride film on an acrylic board.

6) Hold up a piece of the adhesive-coated film with a forceps and place the adhesive side of the film on the trimmed surface.
7) Fix the film closely to the surface of the sample with a soft cotton wad or paper.
8) Cut the sample slowly at a fixed speed (press gently the boundary between the blade and the sample with a brush during cutting).
9) Hold up the film with the cut section and turn up the sectioned side of the film on a glass slide in the cryochamber.
10) Add an aliquot of a 10% sucrose solution containing 0.3% Tween 20 onto the section and turn down the sectioned side onto the glass slide.
11) Place the sectioned side of the film on the glass slide from the edge slowly so as to squeeze out air bubbles.
12) Remove the excess solution with a filter paper.
13) Record the image of the section with a digital camera.
14) Reproduce the image on a computer.

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