A Simple Guide to the Use of Compresstome in Plant Research

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Abstract

Microscopic examinations of plant tissue are essential to address biological questions, and protocols to enhance the quality and integrity of the specimens have been developed. These include pretreatments such as fixation and dehydration, which are essential for preserving the structural integrity of the tissue but are often not compatible with the detection of more sensitive and labile molecules or antigens, requiring fresh tissue. Free-hand sectioning and, more recently, the use of vibratomes have facilitated analyses of fresh tissue, although both techniques pose some limitations. The inconsistency in the section thickness and the difficulties in dealing with minute specimens limit the use of free-hand sectioning, while chatter marks and tissue displacement are common drawbacks of vibratomes. These limitations can be overcome by using compresstomes, inexpensive and versatile instruments able to produce good quality sections from a variety of materials and for a wide range of applications. Despite the many advantages of the compresstome over the vibratome in animal research, the use of this instrument in plant research is very limited. This chapter examines the benefits of the compresstome and, most importantly, provides a step-by-step procedure that inexperienced users can utilize.

Keywords

Compresstome · Fresh tissue sectioning · GFP · Nitric oxide · Vibratome

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D. T. Nhut et al. (eds.), *Plant Tissue Culture: New Techniques and Application in Horticultural Species of Tropical Region*,
https://doi.org/10.1007/978-981-16-6498-4_4

4.1 Introduction

Methods and techniques for studying animal and plant tissue often rely on pretreatments, such as dehydration, fixation, and embedding preceding histochemical and/or immunohistochemical analyses. Despite the considerable improvements in the selection of chemicals and protocols minimizing artifacts, these procedures can result in the loss of cellular constituents. They might not be suitable for detecting molecules or antigens sensitive to harsh changes in chemical or physical environments. Therefore, the utilization of fresh and untreated tissue is often required to answer biological questions using histological and immunological analyses targeting specific cell, tissue, and organ types.

There is plenty of literature dealing with studies using free-hand sectioning, a technique that, if performed correctly, produces sections comparable to those obtained with precision instruments, such as microtomes (Yeung 2015). Applied also to specimens requiring hardening through ethanol treatments, free-hand sectioning with razor blades (Fig. 4.1a) can be significantly enhanced by using methods to support the specimen during sectioning better. This can be achieved using fresh carrot tissue, which can be split longitudinally to accommodate the proper orientation of the specimen. The use of hand microtomes (Fig. 4.1b) can further facilitate the procedure as the carrot cylinder supporting the specimen can be bound to the well of the microtome, previously lubricated with low melting point wax or grease. Minute adjustments allow the support block and specimen to advance, producing sections as thin as a few microns.

While inexpensive and accessible to all users, proper free-hand sectioning with or without the assistance of a hand microtome often cannot be applied successfully to minute specimens difficult to immobilize, support, and orient during sectioning. The technique also requires a significant amount of practice and optimization, which can hamper the processing of specimens not readily available or requiring labor-intensive dissections. This is often the case of tissue culture explants, such as seed embryos, which are prone to damage and difficult to extract from the embedded maternal tissue. Furthermore, even when properly mastered, hand sectioning has some major drawbacks, including tissue shattering and shearing, uneven sectioning, and/or imprecise cutting angles. These limitations can be a deterrent to many, especially those new to plant anatomy.

Vibratomes can be a successful alternative to hand sectioning; routinely used in animal and plant research (Steele-Perkins et al. 2005; Rocha et al. 2014; Hunziker et al. 2019), they can generate good quality thin sections without the need to freeze or fix.

Common vibratomes use disposable razor blades, which vibrate while moving horizontally over the tissue imparting a cutting pattern resembling a sine wave. The tissue, which can be fresh or partially fixed, is trimmed and glued onto the vibratome's support stub. Desirable results with the vibratome have been obtained for small specimens, including plant embryos (Lentini et al. 2020), and even for tissue preparation to be used in gene expression analyses (Olsen and Krause 2019). This latter study showed that the quality of vibratome-produced sections was

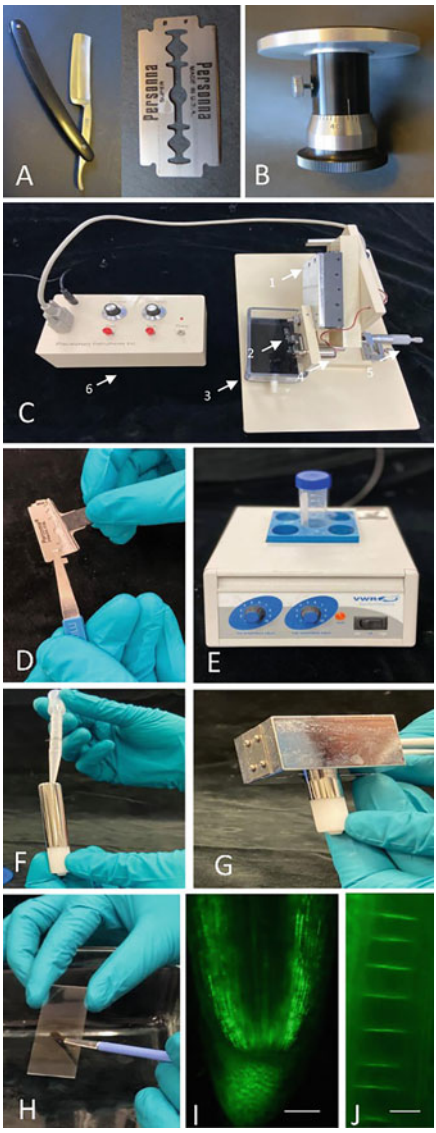

Fig. 4.1 Razor blades are commonly used for free-hand sectioning. (**a**) Hand microtome; (**b**) Compresstome composed of a vibration head (1), blade holder (2), reservoir tank (3), specimen tube (4), manual dial (5), and operation box (6); (**c**) Razor blades are placed on the holder using glue; (**d**) Before embedding the sample, the agarose solution should be kept warm using a dry bath; (**e**) Tissue is embedded by filling the space created between the plunger and the sleeve with the agarose solution, ensuring that the specimen is fully submerged; (**f**) To rapidly cool down the agarose, a cold chilling block is placed over the specimen tube and held for about 1 min; (**g**) Sections are placed on a microscope slide for further treatments using a small brush; (**h**) Visualization of nitric oxide (NO); (**i**) PIN1: GFP signal; (**j**) In corn roots. Scale bar = 300 nm (i) and 10 nm (j)

suitable for laser microdissection and RNA extraction, thus replacing the laborious fixing and embedding procedures for tissue preparation.

The use of the vibratome is convenient; it does not require special blades. Most importantly, it is effective on fresh tissues without pretreatment, such as fixation and dehydration that might cause artifacts typical of cryo- or paraffin-embedded sections. Vibratome sections preserve good structural and ultrastructural characteristics, and adjustments to the vibrating razor blades in terms of speed and vibration amplitude permit the user to optimize sectioning on diverse types of specimens. Furthermore,

sectioning can be performed at low temperatures by filling the reservoir tank with ice and a suitable buffer designed for a specific application. These features, not compatible with free-hand and hand microtome sectioning, are crucial when dealing with liable antigens sensitive to suboptimal temperature and chemical environments.

Despite these advantages, however, vibratomes' use has several major drawbacks that need to be considered and addressed when embarking on a new project. Vibratome-generated sections are often characterized by chatter marks (also referred to as vibratome lines), which can be more or less conspicuous depending on the nature of the specimens. Furthermore, compared to microtomes, vibratome sectioning can be tedious and very slow as the movement of the vibrating blade is almost undetectable; this is a problem for projects requiring large numbers of sectioned material. Finally, a very common drawback when using the vibratome is that its blade often slides on soft tissues while "catches" on hard or fibrous tissues displacing components and compromising the integrity of the specimens. This has been demonstrated in several studies, including during the analyses of lymph node and spleen tissue (Abdelaal et al. 2015). These limitations can be partially overcome by using a compresstome, an automated instrument designed for rapid sectioning with frequent applications in animal tissues (White et al. 1997; Skinner et al. 2000; Yang et al. 2010) but far less in plant tissues (Mira et al. 2020). Like the vibratome, the compresstome is versatile for generating free-floating sections (from a few microns to several hundred microns) from fresh or fixed material but, unlike the vibratome, does not have the same drawbacks. This was clearly documented in a study comparing the compresstome VF-300 (Precisionary Instruments Inc., San Jose, California) and the vibratome 3000 (Technical Products International, St. Louis, MO) for sectioning lymphoid and genital tissues of primates for immunofluorescence applications (Abdelaal et al. 2015). The authors observed that while the time required for sample preparation was very similar for two instruments, both allowing the ability to control temperature during sections, the compresstome offered several advantages. The cutting speed of the compresstome was significantly faster than that of the vibratome, a difference ascribed to the larger size of the block supporting the specimens. These characteristics also resulted in larger sections obtained with the same instrument. Unlike vibratome sections, compresstome sections were devoid of chatter marks and, most importantly, retained better structural integrity as the compresstome blade did not cause displacement of tissue. No differences in the quality of the sections for microscopy analyses were noted between the two instruments (Abdelaal et al. 2015).

Based on the advantages and wide range of applications of the compresstome described above, it is surprising to see a very limited utilization in plant research. The purpose of this chapter is to illustrate the step-by-step procedure for the utilization of a compresstome VF-300 (Precisionary Instruments Inc., San Jose, California) and provide case studies for localization of nitric oxide (NO) and GFP signal in corn roots.

4.2 Overview of the Compresstome VF-300

The instrument (Fig. 4.1c) is composed of the following components: a vibration head connected to a blade holder, a reservoir tank, a specimen tube comprising an external metal sleeve with an internal plunger, and a manual dial. The blade holder can accommodate diverse types of razor blades, ranging from ceramic blades, tungsten carbide blades to double edge razor blades that can be glued directly onto the holder. The reservoir tank holds the buffer solution for the free-floating sections. The selection of the buffer and the ability to control its temperature enhance the preservation of the antigen or molecule of interest and the structural integrity of the tissue. With an external metal sleeve securing the tube to the reservoir tank, the specimen tube houses an internal plunger to which the specimen is glued. Advancement of the plunger during sectioning is regulated by either a manual dial equipped with a scaled crank or, in newer models, by a controlled box with an automated advancement controlled system. An operation box with a stop and start function regulates the degree of oscillation of the blade and its advancement speed during sectioning.

4.2.1 Tissue Preparation

The compresstome can be used to section fresh or fixed tissue. For fresh tissue preparation, it is highly recommended to cut the specimen in iced cold Petri plates with either a scalpel or a double edge razor blade. For fixed specimens, the most suitable fixation buffer should be selected depending on the application. It is recommended to wash the tissue with a buffer after fixation. Fresh or fixed tissue should be trimmed to the size of the base of the plunger of the specimen tube, making sure that the height of the tissue does not exceed its width. This precaution will prevent the specimen from wobbling during sectioning. Once trimmed, the specimen can be sectioned directly without any further treatment, or embedded in low melting point agarose, a process yielding uniform sections and highly recommended when dealing with minute specimens that cannot be easily oriented. From our experience, we found embedding in agarose be essential when sectioning Arabidopsis embryos used as explants for somatic embryogenesis and to generate cross-sections of roots. The procedure for agarose embedding is presented in Sect. 4.2.3.

4.2.2 Mounting of the Blade

Depending on the nature of the specimens, different blades can be used, although double edge razor blades are the most commonly used, being relatively inexpensive and disposable. After removing the blade holder from the vibration head using an Allen key, squeeze a small amount of glue (in our hands, Krazy glue or Gorilla glue gives the best results) on a petri dish. With a narrow spatula, scoop some glue and gently apply it to the blade holder, making sure to cover the whole surface housing

the blade. Avoid using more glue than necessary as this will interfere with the positioning of the blade and sectioning, especially if the glue comes in contact with the sharp side of the blade edge. To cut the blade, hold one half of the blade with a pair of pliers, and with another pair of pliers, gently twist the other half. The twisting motion will sever the two halves, each of which can be used as a cutting blade. Gently place the cutting blade on the holder and apply pressure with the forceps to ensure the whole surface of the blade adheres uniformly to the glue (Fig. 4.1d). Avoid touching the sharp side of the blade as this might cause scratches on the sections, and most importantly, do not allow the glue to stick on the side of the blade to prevent tissue damage during sectioning. Leave the blade to dry for at least 5 min before mounting the blade holder back onto the vibration head of the compresstome.

It is imperative to clean the blade holder thoroughly before mounting a new blade, as this might interfere with the adhesion of the blade to the mount. Do so by detaching the old blade from the mount using a pair of forceps and then immerse the blade older in acetone to remove residual hardened glue. Wipe the mount with a clean paper towel and repeat the wash in acetone if needed. The blade holder's surface needs to be completely smooth and devoid of any debris before mounting a new blade. Acetone-resistant containers should be used for the washes.

4.2.3 Embedding the Specimens

When possible and depending on the sensitivity of the antigen or molecule of interest, it is recommended to embed the tissue in a low melting point agarose. While some instruction manuals suggest using agarose tablets for consistency in results, embedding can be performed using any powdered low melting point agarose. Generally, a 2–3% agarose solution is recommended, although, from our experience, the agarose gel's consistency should be empirically determined. The best results are obtained when the consistency of the gel is close to the consistency of the specimen, as this will prevent the tissue from detaching or lifting during sectioning.

When using tablets, the instruction manual will provide details on the number of tablets and the volume of water to be used for the desired percentage of agarose. Furthermore, when possible, replacing water with a buffer can further stabilize the antigen or molecule of interest. Using a 50 mL Falcon tube, dissolve the tablets in water (or a buffer solution) by swirling the tube, and then microwave for a few seconds several times until the tablets are fully dissolved. This procedure also applies when using powered agarose. The agarose solution can then be placed in a hot water bath, or a dry bath (Fig. 4.1e) set anywhere between 32 and 35 °C, depending on the melting point of the agarose used, for several minutes. It is crucial to stabilize the agarose solution's temperature before starting the embedding process, making sure it does not congeal as this might have negative effects on the embedding process. It is therefore recommended to check the temperature of the water bath frequently with a hand thermometer.

To help solidify the agarose solution uniformly, compresstomes are equipped with a metallic chilling block that needs to be precooled in the fridge (or ice) for at least 30 min, or, alternatively, −20 °C for about 15 min. While the block is chilling, the tissue can be glued to the specimen tube. After retracting the metallic sleeve of the specimen tube, a drop of glue can be placed on the base of the white plunger; with a pair of forceps, gently press the specimen against the glue in the desired orientation. The glue will be cured in about 1–2 min, although more time might be required. It is very important not to use too much glue. Ensure the glue does not leak down along the plunger's surface and sides, as this might glue the metallic sleeve to the plunger. Tissue paper moistened in acetone can be used to remove excess glue. Glue can also interfere with the visualization of antigens or molecules of interest; a problem often encountered with minute samples, such as plant embryos. For small specimens common in tissue culture applications, it is suggested to squeeze a drop of glue on a petri dish, and while holding the specimen with a pair of forceps, gently touch the glue with the side of the tissue to be secured to the specimen tube. With a gentle motion, press the specimen onto the base of the plunger. While the glue is still liquid, the position of the tissue can be adjusted to reach the desired orientation. If not properly glued, the tissue will be wobbling during sectioning and, in some cases, can detach from the specimen tube. To prevent this from happening, it is important to ensure that the base of the plunger is clean before gluing the tissue (acetone can be used to remove old glue) and that the glue covers the whole surface area of the specimen. To increase the contact area between the tissue and the base of the plunger, it is recommended, when possible, to cut a flat surface on the specimen before applying the glue.

Once the specimen is secured to the plunger and the glue has cured, retract the plunger while holding the metallic sleeve until the full height of the specimen enters the sleeve. Using a pipette, fill the space created between the plunger and the sleeve with the agarose solution, ensuring that the specimen is fully submerged (Fig. 4.1f). It is important to check the temperature and the consistency of the agarose solution before this procedure to ensure the agarose has not been partially solidified. It is also crucial to dispense the agarose solution very slowly to minimize air bubbles forming that might interfere with sectioning.

Place the cold chilling block over the specimen tube and hold for about 1 min to ensure a rapid and uniform agarose solidification (Fig. 4.1g). Before this, it is recommended to remove any condensation that might have formed on the chilling block when in ice or at −20 °C. Once the agarose solution is fully congealed, remove any residual agarose that might have solidified outside the metal sleeve using a spatula or pair of forceps.

4.2.4 Sectioning

Before starting sectioning, the blade mount, housing a new blade, needs to be reconnected to the vibration head with the Allen key. Clean the reservoir tray with a wet paper towel (do not use acetone unless the tray is acetone-proof). Make sure

not to disturb the agarose block, insert the plunger tube into the reservoir tray opening by gently twisting the tube as you advance. Push the tube as far as it goes into the tray; a small knob in the tray will prevent the tube from sliding through. Align the manual dial with the specimen tube, and by turning the knob on the dial, advance the micrometer until it touches the plunger tube. Fill the reservoir tank with the desired buffer making sure the agarose block is fully submerged. In the operation box, select the desired speed and oscillation of the blade. Although some instruction manuals suggest optimal values for both speed and oscillation depending on the tissue to be sectioned, from our experience, these parameters should be selected empirically based on the characteristics of the specimen. For better results, and if the dimensions of the tissue allow, it is recommended to start sectioning several microns above the desired thickness and then gradually reduce the thickness while sectioning until the desired microns are reached. As the blade advances, the sections will be released into the buffer within the reservoir tank. Using a small paintbrush, gently extract the sections from the solution and place them on a microscope slide for further treatments (Fig. 4.1h). To obtain a serial sequence, the sections can be arranged in order on labeled microscope slides. For hard-to-section samples, it might be necessary to replace the blade several times during sectioning.

4.2.5 Staining

Staining can be performed directly on the slides using the most appropriate dye, depending on the applications. For general histological analyses, toluidine blue O (TBO) is routinely used due to its polychromatic characteristic. TBO binds to anionic groups imparting unique colors to diverse cellular components, ranging from pink for carboxylated polysaccharides, green-blue for phenolics, and purple for nucleic acids (O'Brien et al. 1964). After incubation in 0.1% TBO for 1 min, use a small filter paper to remove the dye. Wash the sections with water several times, add a drop of water, and place a coverslip on top of the section for examination. More detailed information on dyes and staining procedures is available elsewhere (Chaps. 2 and 3).

4.2.6 Troubleshooting

Sectioning with a compresstome seldom results in immediate successful results. Optimizations in the procedure, depending on the characteristics of the specimens and the overall objective of the analyses, are often necessary. Thus, the user needs to have a proper understanding of the function of the equipment and be able to address possible pitfalls throughout the procedure, from the preparation of the tissue to its visualization. The table below outlines simple suggestions that should be considered when initiating a new project.

1	Uneven sectioning	Mechanical problems with the vibrating head of the microtome or loose blade. Tighten the blade mount to the unit and ensure the blade is glued firmly to the mount The concentration of the agarose might be too low, thus not providing enough support during sectioning. The firmness of the agarose block should be as close as possible to that of the tissue. Empirical adjustments in concentration are required
2	Sample and agarose block wobble during sectioning	Agarose might not have polymerized enough. Allow the chilling block to solidify the agarose solution for a longer period of time Agarose was partially congealed before it was poured onto the sample. Make sure the agarose is fully dissolved during microwaving and check the temperature of the water bath. Reduce the time needed to pipette the agarose on the specimen. The glue might be old or of poor quality The height of the block and/or specimen exceeds the width. Trim the specimen, ensure a larger area of contact with the plunger, and reduce the block's height as much as possible during its preparation
3	Marks on sections	The razor blade might be scratched, or glue residues might be present on the blade. Replace the blade making sure to apply the minimum amount of glue needed Residual glue on the specimen. This is a very common problem when gluing minute specimens that can be overcome by applying a drop of glue on a petri dish and then gently touching the glue with the specimen before securing it onto the plunger of the specimen tube Improper selection and speed and/or oscillation parameters. Start reducing both and then increase gradually. Speed ad oscillation parameters need to be determined empirically Air bubbles in the block. This is due to poor polymerization of the agarose or agarose solution that might be partially solidified when it is poured onto the sample
4	Artifacts when visualizing the sections	Residual glue on the sections interferes with many visualization procedures. Specimens need to be reglued to the specimen tube; use less glue Debris in the reservoir tank. Make sure the tank is cleaned properly as debris can get trapped within the section
5	Agarose cannot be removed from the sectioned specimen	Some procedures require the removal of agarose after sectioning. To facilitate this process, reduce the concentration of agarose when preparing the block

4.3 Equipment and Supplies

Compresstome VF-300 (Precisionary Instruments Inc., San Jose, California).
Microwave oven.
Water bath.
Low melting point agarose.
Petri dishes.
Spatula.
Forceps.
Double edge razor blades.
Pipette.

4.4 Case Studies

4.4.1 Visualization of Nitric Oxide

Nitric oxide (NO) is an endogenous signaling molecule participating in several biological processes related to plant development or response to stress conditions. The brief description below outlines a simple procedure to localize NO in corn roots.

Corn seeds can be germinated on moist filter paper for 5 days at a high relative humidity (95%). Visualization of NO was performed using the fluorescent stain diaminofluorescein (DAF) (Mira et al. 2020). The whole root was incubated in a buffer (50 mM Tris and 50 mM KCl, pH 7.2) containing 1% (v/v) Triton X-100 and 50 μM DAF-FM DA for 1 h at 37 °C with gentle agitation in the dark. Roots were washed twice in the same buffer devoid of DFA and cut in segments of 0.5 cm thick. Following the procedure outlined in Sects. 4.2 and 3.4, root segments were then oriented vertically and glued onto the plunger, and sectioned (10 μm) using the compresstome VF-200. Nitric oxide visualization was performed with confocal microscopy (excitation 495 nm; emission 515 nm) (Fig. 4.1i).

4.4.2 Visualization of PIN1

The localization of the Zea mays PIN1 (ZmPIN1), an auxin efflux transporter, was performed using a PIN1:GFP reporter line (Mira et al. 2020). Germination of corn and tissue collection and processing were identical to those described in Sect. 4.4.1, excluding the use of the buffer and washes. Dissected root segments were directly glued onto the plunger without any treatment. GFP signal was localized by confocal microscopy (excitation 530 nm; emission 580 nm) (Fig. 4.1j).

4.5 Concluding Remarks

The analysis of fresh tissue is crucial in plant research to help to address fundamental biological questions. The conventional use of free-hand sectioning or hand microtomes poses some serious limitations to those new to sectioning procedures, as well as more experienced users dealing with minute specimens often used in tissue culture practices. While still underutilized in plant research, the use of the compresstome represents a better alternative as it can generate uniform sections suitable for many downstream applications. Compresstomes can section fresh or fixed material of various dimensions, and compared to vibratomes, they offer several advantages, including a faster cutting speed, the ability to section larger specimens without chatter marks, and improved section quality to a reduction in tissue displacement during sectioning. The ability to immobilize the tissue in agarose is another desirable feature for processing minute samples or difficult-to-handle specimens. Like any other procedure, the use of compresstomes requires some practice and optimizations that need to be geared towards the characteristics of the sample and the final application.

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