A Simple and Efficient Method for Isolating Trichomes for Downstream Analyses

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Trichomes are useful as a model system to study many processes central to plant development including acquisition of cell fate, cell expansion, endoreduplication, and cell polarity (Hülskamp 2000, Qiu et al. 2002, Schellmann et al. 2002, Schnittger and Hülskamp 2002, Schwab et al. 2000, Szymanski et al. 2000, Walker et al. 2000, Wasteneys 2000). Arabidopsis trichomes afford many advantages for their use in developmental studies. First, trichomes are relatively large, single cells (up to 500 µm long) and can be easily observed using a dissecting microscope. Trichomes differentiate from the plant epidermis so invasive procedures are not necessary to observe their development. Due to the ease of genetic analysis in Arabidopsis, there is a wealth of genetic data on trichome development; mutations affecting each stage of trichome development have been isolated (Hülskamp et al. 1994, Larkin et al. 1994, Luo and Oppenheimer 1999, Oppenheimer 1998, Schellmann et al. 2002). Finally, the Arabidopsis genome has been completely sequenced (The Arabidopsis Genome Initiative 2000), and full-length cDNAs are available for a large percentage of the genes (Seki et al. 2002a, Seki et al. 2002b).

Although a wealth of information has been obtained from both genetic and molecular analyses of trichome development, the biochemical analysis of Arabidopsis trichomes has lagged behind. This is, in part, because trichomes make up only a small fraction of the total number of cells of a leaf. Therefore, if entire leaves are analyzed, trichome-specific biochemical properties are overwhelmed by the contribution of the other cells of the leaf. In addition, immunolocalization of trichome proteins is impeded by the thick, secondary cell walls possessed by trichome cells. Current procedures for immunolocalization require a permeablization step to allow antibody access to the intracellular milieu. Permeablization of mature trichomes using cell wall-degrading enzymes usually results in loss of cell structure, and permeablization of mature trichomes by freeze shattering usually results in broken trichomes. In addition, measurement of the DNA content of trichomes is hindered by their attachment to the leaf. Staining of an intact Arabidopsis leaf with DAPI can result in unwanted fluorescence contribution from the nuclei (and potentially cell walls) of the non-trichome cells.

In this report, we describe a simple and efficient method to isolate intact trichomes from Arabidopsis leaves that overcomes these limitations to trichome analysis. Our method is based on the removal of Ca2+, which ionically cross-links the pectins in the middle lamella that helps hold the trichome cells to the leaf epidermis. The middle lamella lies between the primary walls of adjacent plant cells and originates from the cell plate that forms during cytokinesis. The middle lamella is important in abscission zones, and is responsible for holding cells together. The major component of the middle lamella is the anionic polysaccharide pectin.

In dicots, the primary pectin is rhamnogalacturonan I. The backbone of rhamnogalacturonan I consists of long stretches of α1,2-linked polygalacturonic acid alternating with α1,2-linked rhamnose. Attached to the rhamnose are side chains of mostly arabinose and galactose. The unsubstituted polygalacturonic acid regions of the backbones of different pectin polymers are held together by ionic bridges formed by divalent Ca2+. During some cases of abscission, the ionic groups of the homogalac-
turonan regions are methylated. The methylation decreases the negative charges to which the Ca\(^{2+}\) binds, thus decreasing the number of ionic bridges. This, in turn, decreases the ability of the adjacent cells in the abscission zone to hold together. We exploited this property of pectins to dissociate upon Ca\(^{2+}\) removal from the middle lamella to develop a method to remove trichomes from leaves of Arabidopsis plants.

Generally, trichome removal was carried out as follows. Leaves from plants grown in soil or on plates were collected, fixed and treated with EGTA. Individual leaves or leaf pieces were placed in a Petri dish. Trichomes were removed by gentle rubbing using a small paintbrush. The denuded leaves were discarded and the trichomes were transferred by pipette to a microcentrifuge tube. We found that it was important to keep a wetting agent (such as 0.05% Triton X-100) present at all times to prevent the trichomes from floating and sticking to the sides of the microcentrifuge tube. Low speed centrifugation was used to collect the trichomes and they were washed several times to remove the EGTA solution. Once washed, the trichomes were ready for further analyses. Fig. 1A shows a Toluidine Blue-stained sample of isolated Arabidopsis trichomes demonstrating that the trichome preparation was free of other cell types, and that the trichomes retained their shapes after removal from the leaves.

To determine the efficiency of trichome removal, we examined leaves by SEM before (Fig. 1B) and after (Fig. 1C) trichomes were removed. We found that we routinely removed more than 90% of the trichomes on the leaves. This result establishes that not only is this procedure an efficient means to remove and collect Arabidopsis trichomes, but also that the collected trichomes are a representative sample of the trichomes present on the leaves.

One of our motivations for developing this procedure was to use the trichomes for immunolocalization studies. Microscopic observations of trichomes in our lab indicated that the base of the trichome has a thinner cell wall than the stalk and branches. We reasoned that it would be possible to use mild enzymatic digestion to create an entry point for the antibodies in the trichome base without destroying the trichome structure. To test this, we used anti-actin antibodies to label the actin cytoskeleton in mature trichomes following a mild pectinase treatment. Immunolabeling of the actin cytoskeleton of the trichome was strong and uniform throughout the volume of the trichome cell (Fig. 2A).

We also examined whether the isolated trichomes would be suitable for nuclear DNA content measurements by staining isolated mature trichomes with DAPI. We found that staining of mature trichome nuclei was strong and consistent (Fig. 2B). Because the trichomes are stained and viewed after their removal from the leaves, background fluorescence from other leaf cell nuclei or cell walls is eliminated.

Here we describe a method for easily isolating mature trichome cells from the epidermis of Arabidopsis plants. This method is straightforward and requires no specialized skills or equipment. Furthermore, in principle, this method should be applicable to any plant species that has large, single-celled trichomes. There are numerous applications for which a pure population of a particular cell type is advantageous including biochemical, microarray, and proteomic analyses. This procedure opens the door for comparative studies of trichomes between wild-type Arabidopsis and the myriad trichome

![Fig. 1 Isolation of trichomes from Arabidopsis leaves. (A) Light micrograph of a sample of isolated wild-type Arabidopsis trichomes stained with 0.05% Toluidine Blue. (B) Scanning electron micrographs of Arabidopsis leaves before and (C) after trichome removal. Fully expanded leaves were fixed and dehydrated in 100% methanol, critical point dried, coated with gold/palladium and viewed at 10 kV. Scale bars are: (B) 1.5 mm, and (C) 1.2 mm.](https://academic.oup.com/pcp/article-abstract/45/2/221/1814954)
A method for isolating trichomes mutants that are available. In addition, this method will facilitate comparative studies of trichomes between species.

Wild-type Arabidopsis plants (Columbia and RLD ecotypes) were grown in soilless potting mixture (Metro Mix 360) supplemented with PGP (Pollock and Oppenheimer 1999) under continuous fluorescent illumination at 25°C. For immunolabeling, plants were grown under aseptic conditions on MS medium solidified with 0.8% (w/v) agar. Plates were incubated in a growth chamber under a 16 h : 8 h light : dark cycle at 22°C.

Trichomes were removed from leaves as follows. Fully expanded leaves with mature trichomes were fixed in 1.5% (v/v) formaldehyde, 0.5% (v/v) glutaraldehyde in PEMT (25 mM PIPES, 2.5 mM EGTA, 0.5 mM MgSO₄, 0.05% [v/v] Triton X-100, pH 7.2) or PBST (phosphate-buffered saline containing 0.05% [v/v] Triton X-100, pH 7.2) for 40 min to 3 h at room temperature. Alternative fixatives such as FAE (3.7% [v/v], 50% [v/v] ethanol, 5% [v/v] acetic acid) or 50% (v/v) ethanol, 5% (v/v) acetic acid also have been used successfully. Fixation may be omitted, if needed, depending on particular downstream applications. Following fixation, leaves were washed three times in PEMT (or PBST) for 10 min to remove fixative. Leaves were transferred to 20 ml glass scintillation vials containing approximately 10 ml of PEMT supplemented with 50 mM EGTA, vacuum infiltrated for 10–15 min, and incubated 16-24 h at 4°C. Concentrations of EGTA as high as 250 mM and times as short as 1 h (at 50°C) have been used successfully. Leaves were transferred to Petri dishes and trichomes were removed by gentle rubbing using an artist’s paintbrush (4 mm wide, flat nylon bristles). Trichomes were transferred by pipette (with the aid of a dissecting microscope) to 2 ml microcentrifuge tubes. Trichomes were washed three times with PBST by gently centrifuging (1,600–5,000×g for 4 min) and removal of the supernatant taking care not to disturb the loose trichome pellet. Trichomes were gently resuspended in the desired volume of PBST. Alternatively, trichomes were transferred to 70 μm nylon cell strainers (Falcon, 2350) and further manipulations were carried out by placing the cell strainers in Petri dishes containing the desired solutions.

Immunolocalization of the trichome cytoskeleton was performed essentially as described by Sugimoto et al. (2000). Monoclonal anti-actin antibody clone C4 (ICN Biochemicals, Inc.) was used at a 1 : 400 dilution to localize the actin cytoskeleton. FITC-conjugated goat anti-mouse IgG secondary antibody (Sigma) was used at a 1 : 100 dilution.

For SEM analysis, pubescent or denuded leaves were transferred to anhydrous 100% methanol for at least 1 h. The methanol was changed twice with at least 30 min between changes. Samples were critical point dried, coated with gold/palladium, and viewed at 10 kV.

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A method for isolating trichomes

References


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