FIXATION AND SEPARATION OF ARABIDOPSIS LEAF CELLS

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* This method is an adaptation of the methods of Possingham & Smith (1972, J. Exp. Bot. 23, 1050-1059) and of Boffey, Ellis, Sellden & Leech (1979 Plant Physiol 64, 502-505).

1. SOLUTIONS
   (i) (A) 0.5% (w/v) glutaraldehyde (Sigma, dilute 25% (w/v) stock with water).
   (ii) (B) 0.1M Na2EDTA, (pH 9).

2. PROCEDURE
   (i) Harvest leaf tissue and fix in 3.5% glutaraldehyde (A) for one hour in the dark. Leaves less than c. 25 mm² can be left intact otherwise cut the leaves into 1 mm wide pieces with a new double-sided razor blade. For small leaves, Eppendorf tubes are the best containers to use. If harvesting many samples at once, use 96-well microtitre plates. Samples from larger leaves are best in glass vials with plastic snap-on lids (e.g., Camlab).
   (ii) After fixing, remove the glutaraldehyde carefully with a pipette and replace with 0.1M Na2EDTA (pH 9) (B). Make sure the depth of liquid is sufficient to cover the tissue pieces so that they can move about. Place in a shaking water bath (100 shakes min⁻¹) at 60°C. Make sure both that the lids have holes in to prevent them popping off and that the labels don't float off in the warm water. The length of time of heating necessary to give good cell preparations varies between species and for the first leaves of Arabidopsis two hours is sufficient. For older leaves a slightly longer time may be necessary, but certainly no more than three hours will be required. If samples from small leaves are in Eppendorf tubes, heating them in a heating block works very well.
   (iii) After heating, let the still-green samples cool and store in a fridge at 4°C. Samples will keep for years in these conditions although the chlorophyll will slowly fade over a period of months. To make slides for observation, take a small piece of the prepared leaf tissue, place on a slide with a drop of 0.1M Na2EDTA and tap gently, but firmly with the blunt end of a metal scalpel handle. The tissue should break up easily. Put on a thin cover slip (size 1). On viewing, the cell suspension should contain large numbers of individual isolated mesophyll cells (over tapping will cause excessive cell damage and release of individual chloroplasts). The intact cells can be used for counting chloroplasts in individual cells. By using Differential Interference Contrast Optics (Nomarski), which allows optical sectioning of the cells so chloroplasts on the bottom surface of the cell can be counted independently of those on the top surface, the total number of chloroplasts within individual cells can be determined. Counting is most easily done using an eyepiece grid and counting chloroplasts within each square of the grid at different levels of focus. Mesophyll cell plan areas can also be measured in these cell preparations either following photomicrography or by image analysis. Wheat leaf cells prepared by this method are shown in Pyke and Leech.