An Ultrastructural and Cytochemical Study of the Wall-Membrane Apparatus of Transfer Cells Using Freeze-Substitution

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Summary

A freeze-substitution technique is described which enables the ultrastructure of certain types of plant transfer cells to be preserved with minimal ice crystal damage. The ultrastructure of transfer cells from Funaria, Lonicera and Senecio after freeze-substitution has been compared with that of glutaraldehyde-osmium fixed material. The irregular clear zone between wall and plasma membrane, present in conventional preparations, is absent in freeze-substituted tissue. It is proposed that this interfacial zone is an artefact caused by expansion of wall ingrowth material during conventional fixation procedures. In transfer cells with a complex wall labyrinth the swelling of wall material severely disrupts the true structure of the wall-membrane apparatus and results in a large decrease in the surface to volume ratio of the protoplast. These findings are supported in the case of Funaria by a freeze-fracture study. The reactivity of the plasma-membrane to the PTA/chromic acid stain is enhanced in freeze-substituted material. Use of the Thiényl silver proteinate reagent in conjunction with freeze-substitution has revealed marked differences between the wall ingrowths of Funaria sporophyte haustorium transfer cells and those of Lonicera nectary trichomes.

1. Introduction

The distinguishing feature of transfer cells is a wall-membrane apparatus, in which ingrowths of secondary wall material increase the local surface area of the protoplasts, the surface amplification depending on the degree of complexity of the wall labyrinth (GUNNING and PATE 1974). In order to study the ultrastructural basis of solute transport in this and other systems it is preferable to use methods which simultaneously preserve cell ultrastructure and prevent the movement of soluble substances. One such method is freeze-substitution (LÄUCHLI et al. 1970, FISHER and HOUSLEY 1972, HARVEY et al. 1976). In plant material the quality of ultrastructural preservation after freezing is however quite variable and is mainly dependent on the speed of
freezing, the distance from the tissue to the surface of the block (Dempsey and Bullivant 1975) and the degree of natural cryoprotection conferred by the cellular constituents (Fisher 1975). Transfer cells usually have small vacuoles and dense cytoplasm rich in organelles and may also be expected to contain high concentrations of natural cryoprotectants. These factors should minimise freezing damage and make transfer cells favourable objects for freeze-substitution. In the present investigation a variety of transfer cells from different anatomical locations was examined to determine which types are best suited to freeze-substitution. The ultrastructure and cytochemistry of transfer cells after freeze-substitution was compared with that in material conventionally fixed in glutaraldehyde and osmium tetroxide, and the validity of some of the results was checked by examining one type of transfer cell by means of freeze-fracturing.

2. Materials and Methods

2.1. Plant Material

Lonicera japonica L. (honeysuckle) flowers actively secreting nectar and root nodules of Pisum sativum L. were obtained locally as were leaves from Senecio vulgaris L. (groundsel), and the water plants Ranunculus fluitans Lam., Elodea canadensis Michx., and Vallisneria spiralis L. Mature sporophytes of Funaria hygrometrica Hedw. and secreting extra-floral septal nectaries of Vicia faba L. were obtained from plants growing in soil in a greenhouse.

2.2. Conventional Electron Microscopy

For conventional electron microscopy the tissues were fixed for 4 hours in 2.5% glutaraldehyde plus 1.5% formaldehyde in phosphate buffer (0.025 M pH 7.0). They were post-