Water Permeability of Hypodermis Isolated from *Clivia miniata* Roots

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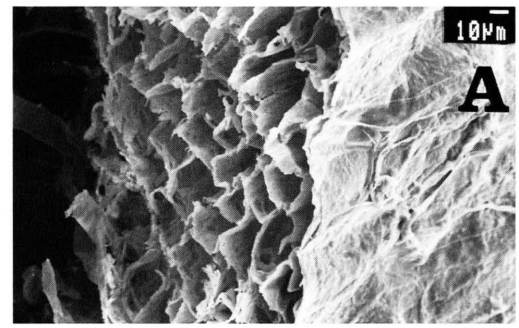
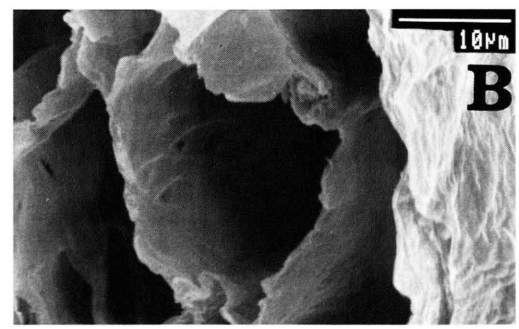
The fine structure and water permeability of hypodermis isolated from roots of *Clivia miniata* have been studied. The hypodermis is composed of five layers of cells arranged in radial rows. The cell walls of these layers consist of primary and tertiary walls and suberized secondary walls which are lamellated. Water permeability of the isolates was low, around the value of 10^{-9} m s^{-1}. This value was found independent of the pH solution and of the ionic exchange capacity of the isolates. Suberin extraction increased water permeability one order of magnitude.

Living organisms are packaged in envelopes that consist of polymeric structural components. In higher plants the main structural component is a biopolyester, cutin, in the aerial parts, and the biopolymer suberin in the underground parts and at wound plant surfaces. These polyesters constitute the major protective barrier between the plant and its environment (Kolattukudy, 1980).

From a structural point of view, the suberin consists of an aliphatic and aromatic domain. The major aliphatic constituents are ω-hydroxyfatty acids, dicarboxylic acids and very long-chain acids and alcohols, and they are covalently attached to a lignin-like phenolic matrix so-called the aromatic domain. Suberin is deposited in an extracellular location, on the plasma membrane side of the cell wall and its present in the endodermis (Casparian bands), hypodermis and exodermis of roots and the bundle sheaths of grasses (Kolattukudy, 1980). Electron microscopy studies of the suberized regions have shown a lamellar structure consisting of alternating light and dark bands: the light bands wax layers, mainly formed by *n*-alkanes and fatty alcohols, and the dark bands constitute the polyester molecular domain (Soliday *et al.*, 1979). Root endodermis forms a uniform layer of cells separating the root cortex from the central cylinder (Essau, 1977). The radial walls of the endodermal cell layer are encrusted with a Casparian band which physiological function is to constitute an apoplasmatic transport barrier. Casparian bands have been observed also in the radial walls of the exodermal cell of root. The exodermal cells lay down superficial suberin lamellae soon after the Casparian bands have been deposited (Peterson, 1988). Thus, this author uses the term exodermis to denote a hypodermis with a Casparian band.

Two important functions of barrier biopolymers are assigned to suberin: the control of ion uptake and the minimization of water loss from plant tissues. In this sense, the water permeability of cuticles and periderm membranes have been investi-





Figs 1A and 1B. Scanning electron micrographs of isolated root hypodermis from *Clivia miniata*. The micrographs show oblique view (1A) and details of the cell walls (1B).

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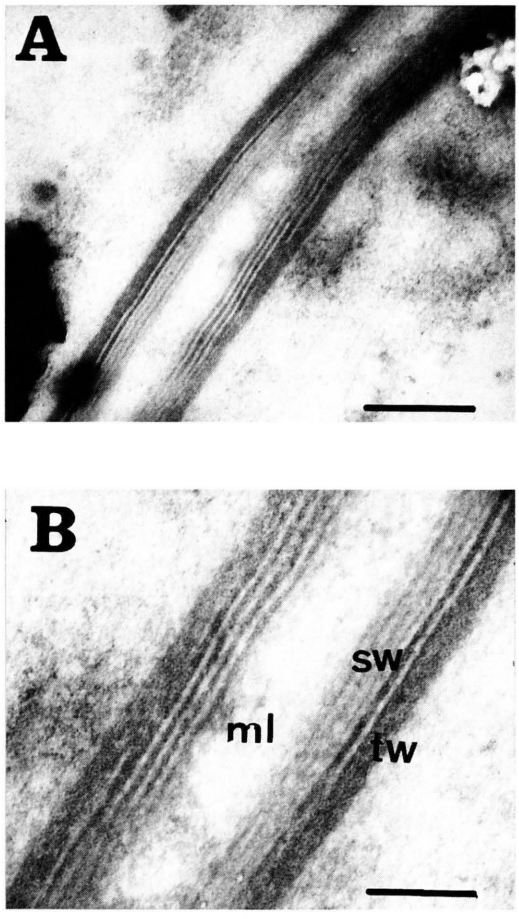
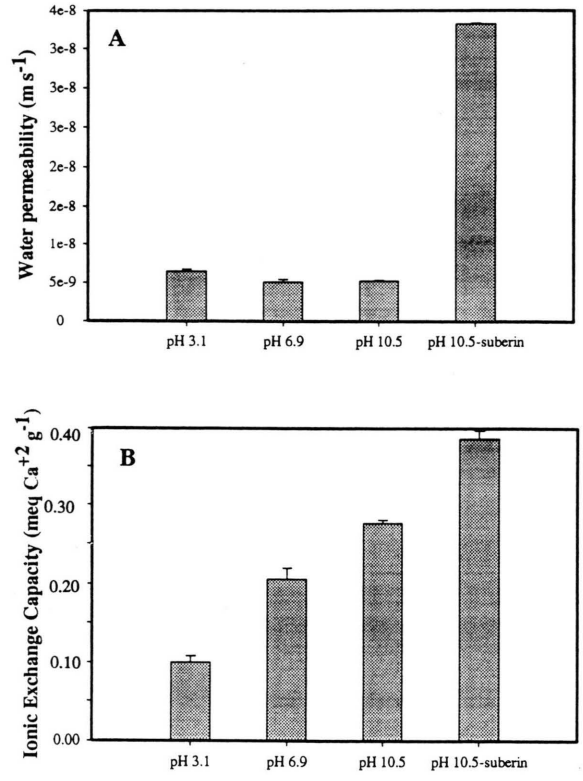
gated by several authors (Schönherr, 1982) but very low attention has been focused on root exodermis and endodermis. Zeier and Schreiber (1997) have recently reported the occurrence and chemical composition of the biopolymers lignin and suberin in hypodermal and endodermal cell walls of *Clivia miniata* roots. As a complement to this research, in this communication we report significant data on water permeability of isolated hypodermal cell walls from *Clivia miniata* roots.

Scanning electron microscopy (SEM) showed that the isolated hypodermis of *Clivia miniata* root consists of five cells layers packed in radial rows with a thickness of approximately 50–60 μm (Fig. 1A). Enzymatic isolation, using standard mixtures of cellulase and pectinase, does not destroy the integrity of the membranes and the inner surfaces appeared smooth and clean (Fig. 1B). The ultrastructure of the hypodermis was also investigated by transmission electron microscopy (TEM). Figure 2 shows a transversal section of root hypodermis in a mature growth state. The micrograph shows the highly contrasted tertiary wall (Fig. 2A) with a thickness of about 200 nm. Figure 2B exhibits in great detail the suberized secondary wall, highly contrasted, with the characteristic lamellar structure. The adjacent suberized walls are sepa-



Figs 2A and 2B. Transmission electron micrographs of root hypodermis fixed with osmium tetroxide followed by section staining with lead citrate. For micrograph 2A, bar indicates 200 nm; for micrograph 2B, bar indicates 100 nm. Figure 2B: ml = middle lamella; sw = secondary (suberized) wall; tw = tertiary wall.



Figs 3A and 3B. Effect of pH on the external solution and suberin extraction (pH 10.5-suberin) on water permeablity (3A) and exchange ionic capacity (3B) of isolated root hypodermis. The different pH values of the solutions were obtained with citrate buffer (pH 3.1) and Tris (Tris(hydroxymethyl)aminomethane) buffer (pH 6.9 and 10.5). Ionic exchange capacity (in equivalents of calcium ion per gram of tissue) was determined by atomic absorption spectrometry, after putting the isolates in the H^+ form. Water permeability values ranging from about 5×10^{-9} (5e-9) until 4×10^{-8} (4e-8) m s^{-1}. Bars indicate the standard deviation for three different membranes. For details on suberin extraction, see text.

rated by a middle lamella and two primary walls wich could not clearly be discriminated (Figs 2 A and 2B).

Water permeability across the isolated hypodermis was measured using tritiated water as tracer in a water/membrane/water system as previously described for plant cuticle water permeability determinations (Luque *et al.*, 1995). The water difussion across the isolated hypodermis was determined at three different pH solutions according to the different ionic exchange capacity showed by the isolates (Figs 3A and 3B, respectively). The flux of water was always linear and the permeability coefficients could be calculated in all cases (Luque *et al.*, 1995). Figure 3A shows the pH effect on the permeability coefficient (in $m\ s^{-1}$). The results indicate that water permeability was almost constant for the three pH values investigated. Suberin extraction increased the water permeability one order of magnitude, showing the barrier characteristic of this biopolymer. Suberin extraction was carried out following two steps (Soliday *et al.*, 1979). First, the plant material was refluxed during 24 h in a mixture of chloroform/methanol (2:1) in order to remove wax components. Secondly, the root hypodermis were partially depolymerized in a methanolic solution containing 1% of potassium hydroxide under reflux conditions during 12 hours. Gas chromatography combined with mass spectrometry analysis of suberin extracts showed the presence of *n*-alkanes, *n*-alcohols and unsaturated fatty acids as major components. Detailed identification of these compounds gave a suberin chemical composition similar that the recently reported by Zeier and Schreiber (1997). Thus, we can afirm that the selective extraction of this aliphatic-aromatic molecular barrier in the hypodermis produces, at the structural level, holes that will increase water difussion across hypodermis.

The fact that water permeability of the root isolates is neither dependent of the solution pH and the ionic exchange capacity (Figs 3A and 3B) is a noticeable difference, non previously reported, between plant isolated cuticles and the isolated root hypodermis and allows to stablish a hypothesis about the distribution of the dissociable functional groups in the cell walls of root hypodermis. The biopolymer swelling, that permits the water permeability, must be very low and the fixed dissociable functional groups, which increased at differents pH as indicate the data of ionic exchange capacity showed in Fig. 3B, should be located into a rigid macromolecular matrix where the electrostatic repulsion between neighboring fixed charged of equal signs, the volume occupied by the couterions and their hydration shells should be minimised (Schönherr, 1976, 1982; Luque *et al.*, 1995).

Assuming water moves primarily along the transcellular path, and using the ultrastructural arrangenment showed in Fig. 1, one would expect that the development of suberin lamellae in *Clivia miniata* root hypodermis would increase the root's resistence to water flow (Peterson and Enstone, 1996). In an exodermis isolate that consists of five layers of cells (Fig. 1), ten tangential suberized walls are arranged in series. Since the average thickness of the suberized cell wall is about 200 nm (see Fig. 2), the total thickness of all suberized walls is around 2 μm. Using the water permeability values of Fig. 3 and the above mentioned data, the specific water permeability of this pathway is 1.04×10^{-14} $m^2\ s^{-1}$. This value is very similar to the specific permeabilities calculated for cuticular membranes which ranges from 1×10^{-14} to 2×10^{-16} $m^2\ s^{-1}$ (Schönherr, 1982) and it is an indication that suberin incrusted into the secondary cell wall of *Clivia miniata* hypodermis represents an excellent water barrier.

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