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Localization of Peroxidase in C-type Xylem Transfer Cells from *Lupinus*

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With 1 Figure

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Summary

Ros Barceló A., Ferrer M. A., García-Florenciano E. & Muñoz R. 1991. Localization of peroxidase in C-type xylem transfer cells from *Lupinus*. – Phyton (Horn, Austria) 31 (1): 137–142, 1 figure. – English with German summary.

The cytochemical localization of peroxidase was studied in C-type xylem transfer cells from *Lupinus albus*. Both the presence of peroxidase in primary walls and its absence in cell wall ingrowths are sufficient to explain „per se" the differences observed in lignification of both external supra-molecular structures.

Zusammenfassung

Ros Barceló A., Ferrer M. A., García-Florenciano E. & Muñoz R. 1991. Lokalisierung von Peroxidase in Xylem-Transferzellen („C-Typ") von *Lupinus*. – Phyton (Horn, Austria) 31 (1): 137–142, 1 Abbildung. – Englisch mit deutscher Zusammenfassung.

Xylem-Transferzellen („C-Typ") von *Lupinus albus* wurden cytochemisch auf das Vorkommen der Peroxidase untersucht. Aufgrund des Vorkommens von Peroxidase in den Primärwänden und des Fehlens dieses Enzyms in den Zellwandprotuberanzen lassen sich die bereits festgestellten Unterschiede hinsichtlich der Lignifizierung dieser beiden äußeren supramolekularen Strukturen erklären.

Introduction

The elaborate cell wall in-growths characteristic of transfer cells is one of the several specializations related to intensive cell-to-cell transport in

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plants. In these cells, the inner surface of the cell wall develops irregular protuberances which yield a wall labyrinth that leads to the amplification of the plasma membrane surface area and which provide special transport properties.

Since they were described for the first time in the thirties (see GUNNING & PATE 1969), transfer cells have been found in many anatomical locations in plants (BEHNKE 1975). For instance, cell wall in-growths are known to develop in cells closely associated to sieve elements (A and B-type transfer cells), or in cells located in the immediate neighbourhood of vessels and tracheids (C-and D-type transfer cells) (BEHNKE 1975). D-type cells, as derived from bundle sheath cells, exclusively follow minor leaf veins (PATE & GUNNING 1969), while C-type cells, which are modified xylem-parenchyma cells, are present in stems (BEHNKE 1975). Both C- and D-type transfer cells show a polar distribution of their wall in-growths, these being confined to wall parts contiguous to xylem elements (BEHNKE 1975).

Although wall in-growths of transfer cells can be considered as a secondary cell wall, in analogy with the secondary cell wall thikening observed in their adjacent xylem members, wall in-growths remain unlignified for the whole life span. This differs too from the primary cell wall of the transfer cells which also lignify (YEUNG & PETERSON 1974, CZANINSKI 1977, PEREZ-RODRIGUEZ 1981).

There are several possible reasons which explain the absence of lignification of the cell wall in-growths in xylem transfer cells: 1) the unavailability of monolignols (i. e. hidroxy-cinnamyl alcohols), 2) the absence of the enzyme involved in the oxidative coupling of hidroxy-cinnamyl backbones (i. e. peroxidase) and, 3) the presence of inhibitors of the peroxidase-catalyzed coupling reaction. Since the endogenous inhibitors of the peroxidase-catalyzed oxidative coupling of cinnamyl alcohols are located exclusively in phloem and cambial tissues of lupin hypocotyls (FERRER & al. 1990), a role for these compounds of an isoflavone nature in the control of lignification of cell wall in-growths in xylem transfer cells is improbable.

In this report, we study the location at electron microscope level of peroxidase in C-type xylem transfer cells from *Lupinus* in order to explain the absence of lignification of the cell wall in-growths of these xylem cell derivatives.

Materials and Methods

Exponentially growing lupin (*Lupinus albus* cv. multolupa) hypocotyls were grown in darkness as previously described (FERRER & al. 1990). For peroxidase cytochemistry, the most basal 2.0 cm zone of the hypocotyl was chosen because of the high lignifying capacity of the xylem tissues (ROS BARCELÓ & al. 1989b). Vascular tissue cores were obtained by pushing a thin-walled glass tube, 1.5 mm in internal diameter, through the centre of the hypocotyl specimen from the cut end, isolating the vascular tissues and pith (ROS BARCELÓ & al. 1988).

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Tissue cores were fixed in 2.5% glutaraldehyde in 0.1 M Hepes (N-2-hydroxyethylpiperazin-N'-2-ethane sulfonic acid) buffer, pH 7.4, 1% with respect to sucrose, for 1.5 h at 4°C. Then they were washed in the same buffer and incubated for 20 min at 25°C, in the dark, in a solution containing 1 mg/ml 3,3-diaminobenzidine (Sigma) treated with activated charcoal, as previously described (ROS BARCELÓ & al. 1989a), in the presence of 1 mM of H_2O_2. Controls were carried out using 5 mM KCN and in the absence of H_2O_2 or DAB. After cytochemical staining, tissue cores were post-fixed, dehydrated and embedded as already described (ROS BARCELO & al. 1988). Ultrathin sections were cut on a Reichert Jung ultramicrotome using a diamond knife. After mounting on copper grids, the sections were examined, without further staining, with a Zeiss EM 10 electron microscope operated at 60 kV.

Results and Discussion

Ultrastructure of C-type xylem transfer cells in *Lupinus*

The diagnostic morphological feature of transfer cells is their complement of irregular wall in-growths (Fig. 1). In C-type transfer cells from lupin, they may branch and even anastomose to form a veritable labyrinth of wall material, in which the plasma membrane follows faithfully the contours of the wall and the cytoplasm penetrates between the individual in-growths or into the interstices of the labyrinth (Figs. 1A, 1B).

In all of the observed cells, the in-growths are restricted to the surface, or surfaces, of the transfer cells contiguous with, or close to, xylem element(s) (Figs. 1C, 1D). Consequently, where contact with the xylem is limited, the transfer cells exhibit a highly polarized distribution of their in-growths. This strongly suggests a specific role for these cells in exchanging materials with the vessels or tracheids, and the available experimental evidence fully supports this reasoning, highlighting the activity of these types of cells in solute uptake (PATE & GUNNING 1969, 1972, GUNNING & al. 1970, LÖRCHER & al. 1987). Thus, apparently, the presence of wall in-growths not only increases the surface-to-volume ratio of the protoplast but also ensures that no part of its interior is far removed from its plasma membrane (Fig. 1B).

Transfer cell mitochondria (M) frequently lie closely juxtaposed to the plasma membrane and often between the in-growths or in the interstices of the wall labyrinth (Fig. 1B). In contrast with the numerous mitochondria, plastids, which if present are poorly developed (detail not shown), do not appear to be the energy source for transport phenomena in these cells.

The Golgi apparatus of the mature transfer cells is not hypertrophied and does not appear to contribute large populations of vesicles to the cytoplasm, showing a more or less quiescent state (detail not shown), probably due to the highly developed end state reached by these cells. Similarly, the endoplasmic reticulum (ER), which is a very conspicuous feature of all transfer cells, and which is spectacularly well developed in *Cuscuta* haustoria (GUNNING & PATE 1969), is present in lupin C-type

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transfer cells as short rough cisternae (arrow, Fig. 1A). These short rough cisternae are probably, as in other cells derived from the lupin vascular bundles (FERRER & al. 1989), the end products of differentiation of the endomembrane system.

Another distinctive feature of C-type transfer cells is the characteristic presence of osmiophilic bodies (OB) widely distributed along the peripheral cytoplasm and/or trapped within the cell wall labyrinth (Figs. 1A–1C).

Location of peroxidase in C-type xylem transfer cells

The application of the DAB technique to study the location of peroxidase activity in C-type xylem transfer cells reveals that peroxidase activity is high in the lignifying primary wall (PW) contiguous to xylem elements (Fig. 1A), but is absent in the wall in-growths (Fig. 1A). This location is in contrast with that observed at the level of primary and secondary cell walls of tracheary xylem cells which both show a positive peroxidase stain (ROS BARCELÓ & SABATER 1986). Since both primary walls and wall in-growths of transfer cells are supramolecular structures containing pectic substances (CZANINSKI 1977), the differential localization of peroxidase in primary walls suggests that lignifying peroxidases are not bound to pectins (ROS BARCELÓ & al. 1989b), but that they are soluble in the wall matrix. Traces of DAB positive stain were also observed in membranes of the endoplasmic reticulum (arrow, Fig. 1A) and in the granulated substance of the cytoplasm (Fig. 1A). These reactions were due to peroxidase activity, since no staining of these structures was seen on control sections incubated without DAB (Fig. 1B), H_2O_2 (Fig. 1C) or on material treated with KCN (Fig. 1D).

In conclusion, and since monolignols are normally viewed as substances that diffuse through the wall matrix (LEWIS & YAMAMOTO 1990), the main factor in controlling the absence of lignification of cell wall in-growths of xylem transfer cells appears to be the absence of peroxidase, the enzyme that catalyzes the oxidative coupling of monolignols to lignins (LEWIS & YAMAMOTO 1990). This constitutes a distinctive characteristic of these specialized cells.

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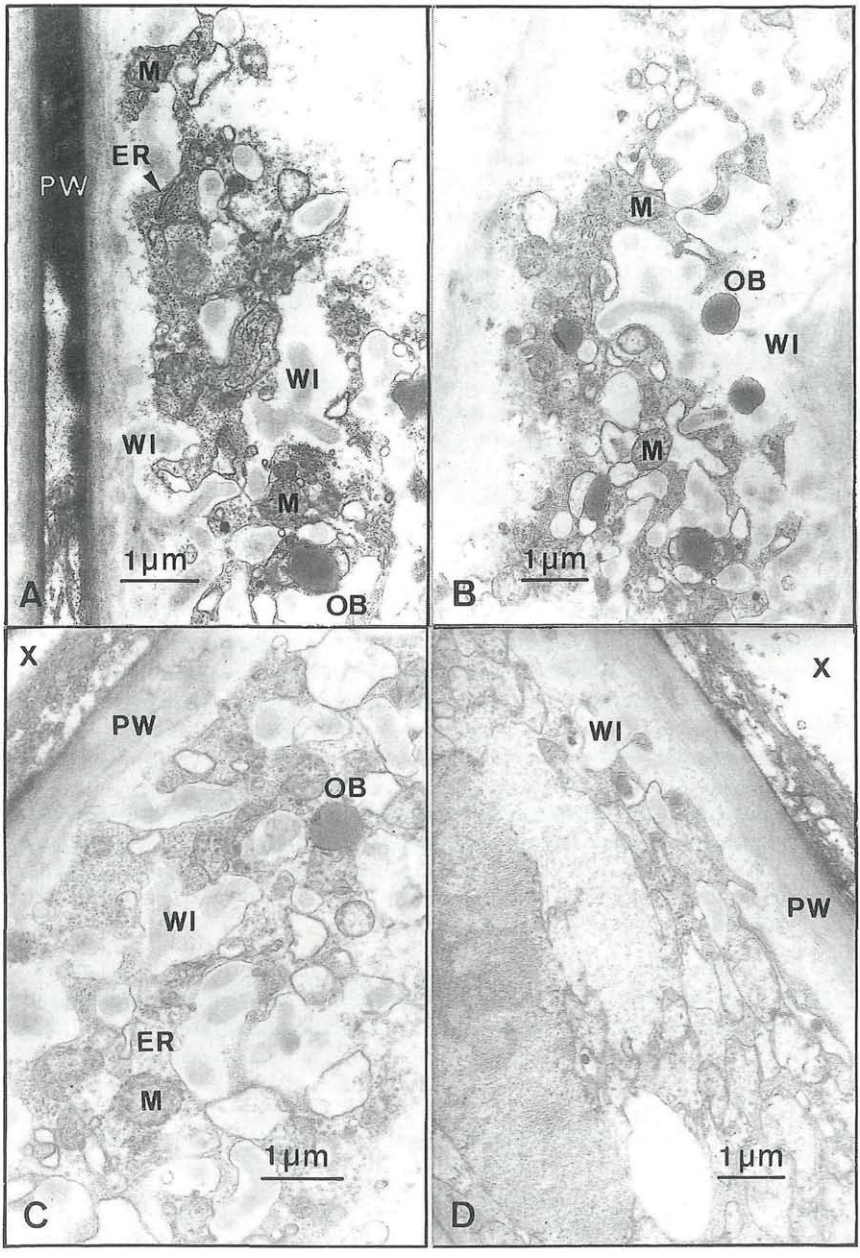

Fig. 1. Ultrastructural view of C-type xylem transfer cells stained with DAB and H_2O_2 (A), and controls in the absence of DAB (B), H_2O_2 (C), and treated with KCN (D). ER = endoplasmic reticulum, M = mitochondria, PW = primary wall, OB = osmiophilic bodies, WI = wall in-growths, X = xylem.

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