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Cell-wall separation during the outgrowth of lateral roots in *Allium porrum* L.

P. BONFANTE and R. PERETTO

Dipartimento di Biologia Vegetale dell'Università, Viale Mattioli 25, I-10125, Torino, Italy

SUMMARY

Cell-wall modifications in the root tissues surrounding the lateral emerging roots of leek were investigated using morphological and *in situ* techniques. Primordium meristem cell walls were thin with a weak gold labelling after treatments for pectin and cellulose localization, as already described in other plant meristems. By contrast, the surrounding tissues of the mother roots displayed deep changes: their walls were swollen, thickened, with large intercellular spaces. Specific probes revealed a distribution of cellulose molecules comparable to the undisturbed root and a substantial increase in pectic material. Cell-wall remnants were at the interface between the emerging roots and the mother root. They were rich in pectic material, but not in cellulose. Other immunogold experiments located a polygalacturonase over the primordium cells and its interface with the mother root.

It is suggested that lateral root morphogenesis involves controlled cell separation, thanks to a specific interaction between pectinolytic enzymes and pectins.

Key-words: cell separation, cellulose, lateral root, leek, morphogenesis, pectin, polygalacturonase.

INTRODUCTION

Lateral roots develop following strategies unique to roots: they form from differentiated cells in the pericycle and emerge by growing through the tissues of the mother root (Schiefelbein & Benfey 1991). The way this is done is mostly unknown. Expression of specific enzymes or proteins changes dramatically: the enzyme hyoscyamine 6-β-hydroxylase, located on pericycle cells, decreases on induction of lateral roots (Hashimoto *et al.* 1991), while a hydroxyproline-rich glycoprotein gene (HRGn3) is expressed in emerging roots (Keller & Lamb 1989). In *Allium porrum*, production of lateral roots has been associated with increased polygalacturonase activity located just over their meristem (Peretto *et al.* 1992). These results suggest that structural (HRGP) and/or enzymic (polygalacturonase) components of cell walls may play important roles in the lateral root outgrowth.

The anatomy of lateral roots from the initiation of primordia to their outgrowth has been the object of extensive and detailed studies (Peterson & Peterson 1986; Charlton

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This paper is dedicated to Professor Dr M.M.A. Sassen on the occasion of his retirement.

1991). Some points, however, including cell-wall ultrastructure of the tissues involved, require further investigation (Charlton 1991).

This paper describes the cell-wall interactions between the emerging root and the surrounding tissues in *A. porrum*, extending preliminary morphological evidence, which has already been presented (Peretto *et al.* 1992). Here we have used different *in situ* techniques to demonstrate that (1) deep ultrastructural modifications occur in the cortex of the mother root, (2) pectic materials, but not cellulose molecules, are released from the cortical cell walls into a newly formed space and (3) a polygalacturonase is located at the periphery of the primordium and at its interface with the mother root tissues.

MATERIALS AND METHODS

Plant material

Seeds of *A. porrum* L. 'Mostruoso di Carentan' (Sementi Dotto, Mortegliano-Udine, Italy) were sown in pots of sterilized quartz sand. A nutrient solution (0·75 mM $MgSO_4$, 1 mM $NaNO_3$, 1 mM K_2SO_4, 2 mM $CaCl_2$, 3·2 μM Na_2HPO_4 plus micronutrients) was applied every other day. The seedlings were maintained in a growth chamber at 22 °C with a 13 h photoperiod.

Microscopy

Root segments were fixed in 2·5% glutaraldehyde in 10 mM Na-phosphate buffer (pH 7·2) for 2 h at room temperature. After rinsing with the same buffer, they were postfixed in 1% OsO_4 in distilled H_2O for 1 h, washed three times with distilled H_2O and dehydrated in an ethanol series (30, 50, 70, 90, 100%; 10 min each step) at room temperature. The root segments were infiltrated in 2:1 (v/v) ethanol/LR White resin (Polysciences Inc., Warrington, PA) for 1 h, 1:2 (v/v) ethanol/LR White for 2 h and 100% LR White overnight at 4 °C, according to Moore *et al.* (1991).

Semi-thin sections were stained with 1% toluidine blue for morphological observations. Thin sections were subjected to the periodic acid–thiocarbohydrazide–silver proteinate (PATAg) reaction for a general visualization of polysaccharides (Roland 1978).

Enzyme–gold affinity labelling

A purified enzyme (cellobiohydrolase I, CBH I, EC 3.2.1.91) linked to colloidal gold and capable of binding cellulose (Berg *et al.* 1988) was used to reveal β-1,4-glucans. The complex enzyme–colloidal gold was obtained as described by Bonfante *et al.* (1990).

Immunogold labelling

An anti-pectin monoclonal antibody (JIM 5, kindly provided by Dr J. P. Knox, J. Innes Institute, Norwich, UK), and a polyclonal antibody raised against the purified polygalacturonase of *Fusarium moniliforme* (kindly provided by Prof. F. Cervone, Università "La Sapienza", Roma, Italy) were used. For a detailed description of their specificity and characteristics, see Knox *et al.* (1990), Peretto *et al.* (1992).

To reveal pectins, thin sections were incubated in undiluted JIM 5 antibody, followed by goat anti-rat gold conjugate (15 nm particles, Bio Cell, Cardiff, UK) according to Bonfante *et al.* (1990).

To detect polygalacturonase antigens, sections were incubated in the presence of the anti-polygalacturonase antibody (1:500 dilution), followed by goat anti-rabbit gold conjugate (15 nm particles, Bio Cell, Cardiff, UK).

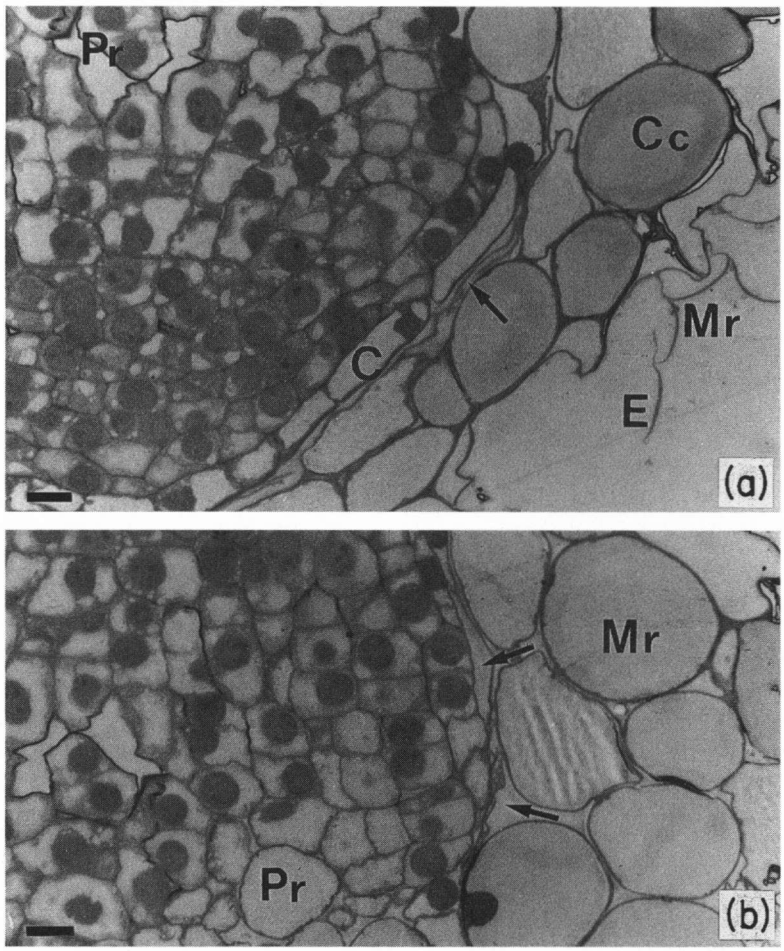

Fig. 1. (a, b) Sections from resin embedded roots showing the contact area between a primordium (Pr) and the mother root (Mr). A space (arrows) is formed between the cap cells (C) of the primordium (longitudinal section) and the cortical cells (Cc) of the mother root (transverse section). Some epidermal cells (E) are broken. Bars correspond to 10 μm.

RESULTS

Lateral roots of leek arise in the parent root pericycle. A detailed description of all the developmental stages was outside the main purposes of this research, so only well-recognizable emerging primordia were analysed. They consisted of a mass of meristematic cells surrounded by a single cell layer forming a sort of root cap (Figs 1a and b). Going towards the base (Fig. 1a), the cells became more vacuolated. Mother root tissues were rounded with large intercellular spaces and sometimes with irregular outlines. In the proximity of the primordium root tip, mother root cortical cells were flattened, while epidermal and hypodermal cells were often broken.

Lateral roots were constantly separated from the mother roots by a temporary structure called the *poche digestive* (van Thieghem & Douliot 1888; Peterson & Peterson 1986; Peretto *et al.* 1992) extending from their base to their tip (Figs 1a and b).

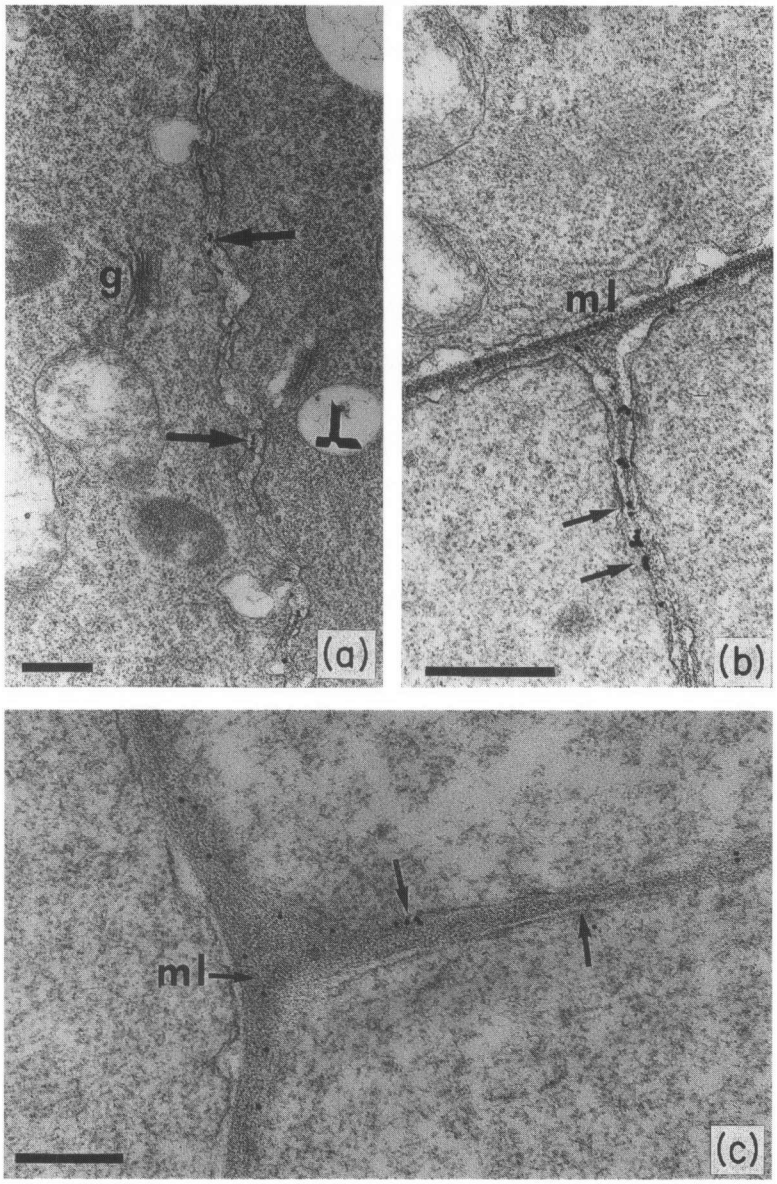

Fig. 2. Ultrastructural features of the primordium meristem. Bars correspond to 0·5, 0·5 and 0·25 μm, respectively. (a) Gold granules are only present over the thin electron-transparent wall after treatment with the CBH I–gold complex (arrows). Golgi bodies (g) are present along the cell periphery. (b) Gold granules, after the CBH I–gold treatment, are evident over the young cell wall (arrows) and not over the wall material present at the cell junction (ml). (c) Gold granules are present over the wall and at the cell junction (ml) after treatment with the antibody JIM 5 for pectin localization (arrows).

Cell-wall architecture in the emerging roots

The cell walls of all root tissues showed distinct ultrastructural features. The meristematic cells possessed thin walls (Fig. 2) where the CBH I–gold complex revealed loose gold

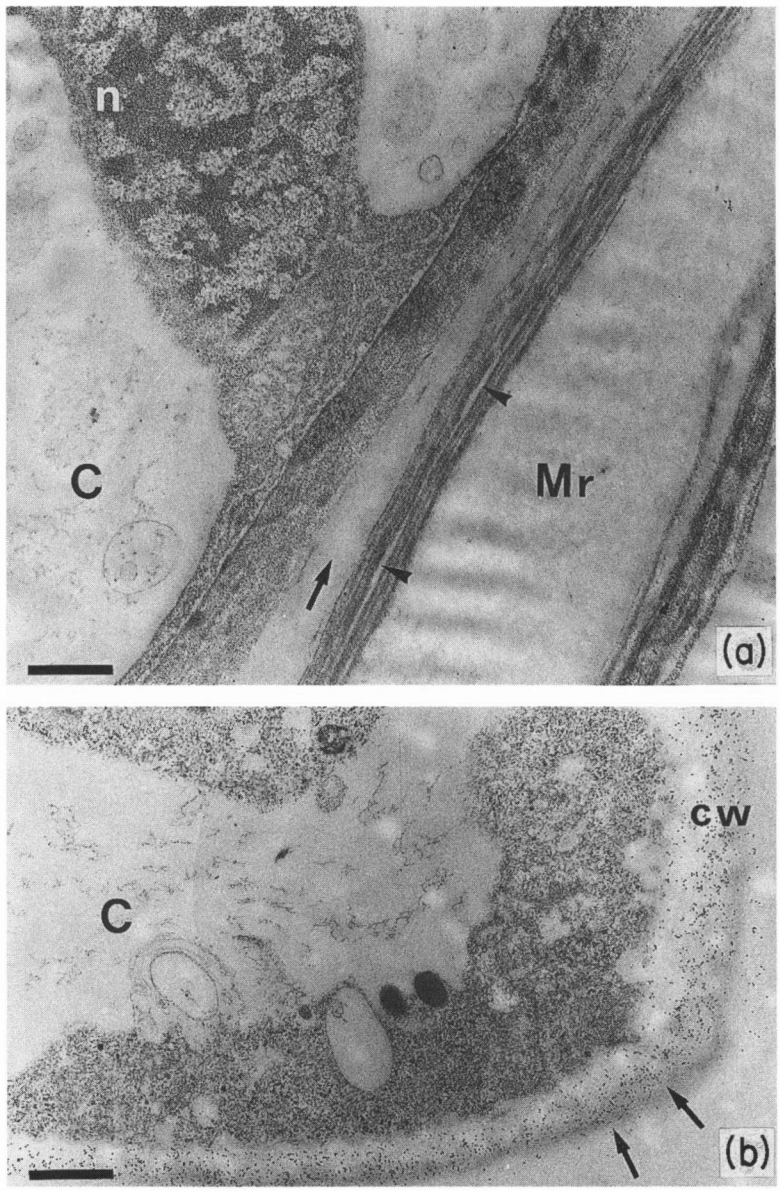

Fig. 3. (a) Ultrastructural features of the contact area between a nucleated (n) cap cell (C) of a primordium and the mother root (Mr) tissues after PATAg reaction. A space is present between the two tissues (arrow). The cap cell wall is thickened, while the walls of the mother root are degraded, consisting in electron dense laminae and non-reactive regions (arrow heads). Bar corresponds to 1 μm. (b) Magnification of the outer wall (cw) of a primordium cap cell (C) after treatment with the antibody JIM 5 for pectin localization. A heavy labelling is evident (arrows). Bar corresponds to 1 μm.

granules (Fig. 2a). No labelling was observed over the cytoplasm or over the intercellular material at the junction between a longitudinal wall and the newly laid down anticlinal wall (Fig. 2b). Loose labelling was also observed after treatment with the antibody JIM 5 (Fig. 2c).

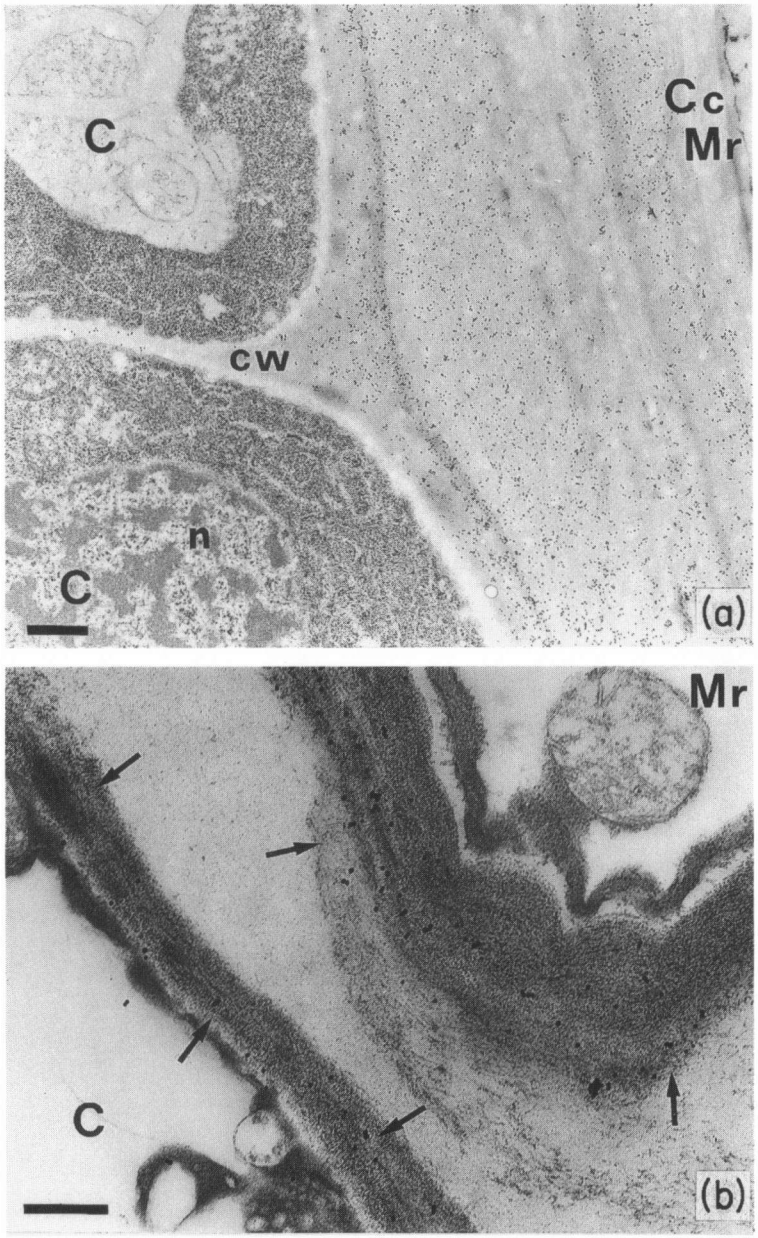

Fig. 4. Pectin and cellulose localization at the contact area between the primordium and the mother root tissues. Bars correspond to 1 and 0·5 μm, respectively. (a) Contact area between the nucleated (n) cap cells (C) and the mother root (Mr) tissues after treatment with the antibody JIM 5 for pectin localization. A heavy labelling is evident at the junction between the cap cell walls (cw) and over the outer walls, as well as over the material joining the primordium with the mother root cortical cells (Cc). (b) Magnification of the contact area after CBH I–gold. The labelling is present over both the walls (arrows), but not in the space.

Labelling was present over the walls, mostly over the electron-dense area corresponding to the middle lamella. Vesicles connected to Golgi bodies were labelled too (not shown).

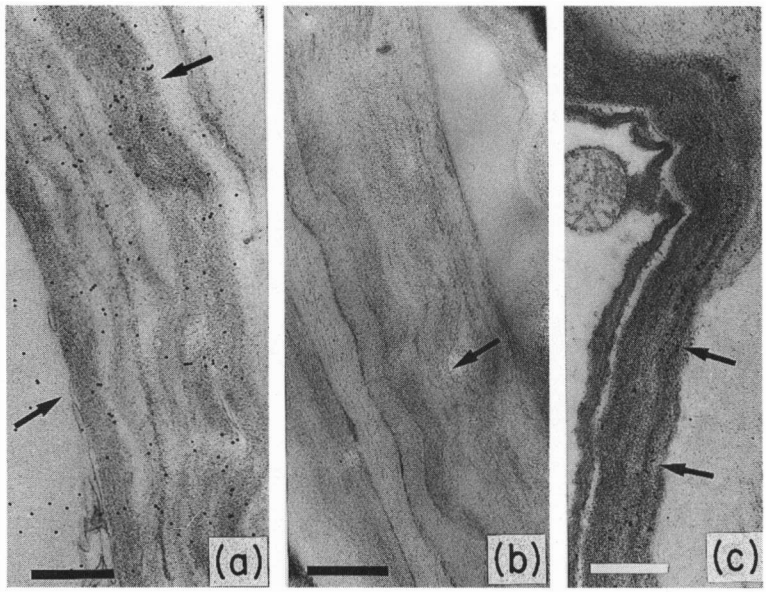

Fig. 5. Details of the cortical cell wall in the mother root. Bars correspond to 0·5, 0·5 and 1 μm, respectively. (a) JIM 5 treatment. (b) Control experiment, where the primary antibody was omitted. (c) CBH I treatment.

Cap cells were irregularly vacuolated with prominent nuclei and thickened walls, particularly the tangential outer ones (Figs 3a, b and 4a). Here a layered fibrillar organization was seen (Fig. 3a). Intense labelling appeared after treatment with JIM 5, mostly over the outer part of the wall and the material joining two different cap cells (Figs 3b and 4a). CBH I revealed a loose gold granule distribution (Fig. 4b).

Mother root: cell-wall architecture of the tissues surrounding the primordium

Highly vacuolated cells were found along the flanks of the primordium, just opposite to its cap cells (Figs 1a and b). Their walls were thick with a wavy outline (Figs 4b–5c) in contrast with the thin walls of the undisturbed cortical root cells (see Bonfante *et al.* 1990, for a full description). Layering with a loose fibrillar organization was seen and PATAg reaction showed large unreactive spaces (Fig. 3a). Intense labelling was found after JIM 5 treatment (Figs 4a and 5a) and no gold granules were present in the control sections (Fig. 5b). By contrast, CBH I led to weak labelling. This was well evident in fully degraded walls, consisting of separated laminae (Figs 4b and 5c).

The cortical cells of the mother root were separated from the primordium by a loose fibrillar material, initially continuous with the two tissues (Figs 4a and 6a). CBH I–gold complex reaction was negative over this material (Fig. 4b), whereas intense deposition appeared after JIM 5 (Figs 4a and 6a). No labelling was seen in the control experiments (Fig. 6b). When the primordium was fully separated from the mother root, only labelled remnants were observed in the space (Fig. 6c).

Localization of polygalacturonase

The antibody against a fungal polygalacturonase revealed a weak, but regular distribution of gold granules at the interface between the two tissues. Granules were also seen in the periplasmic space of the meristematic and cap cells in the primordium (Figs 7a and b).

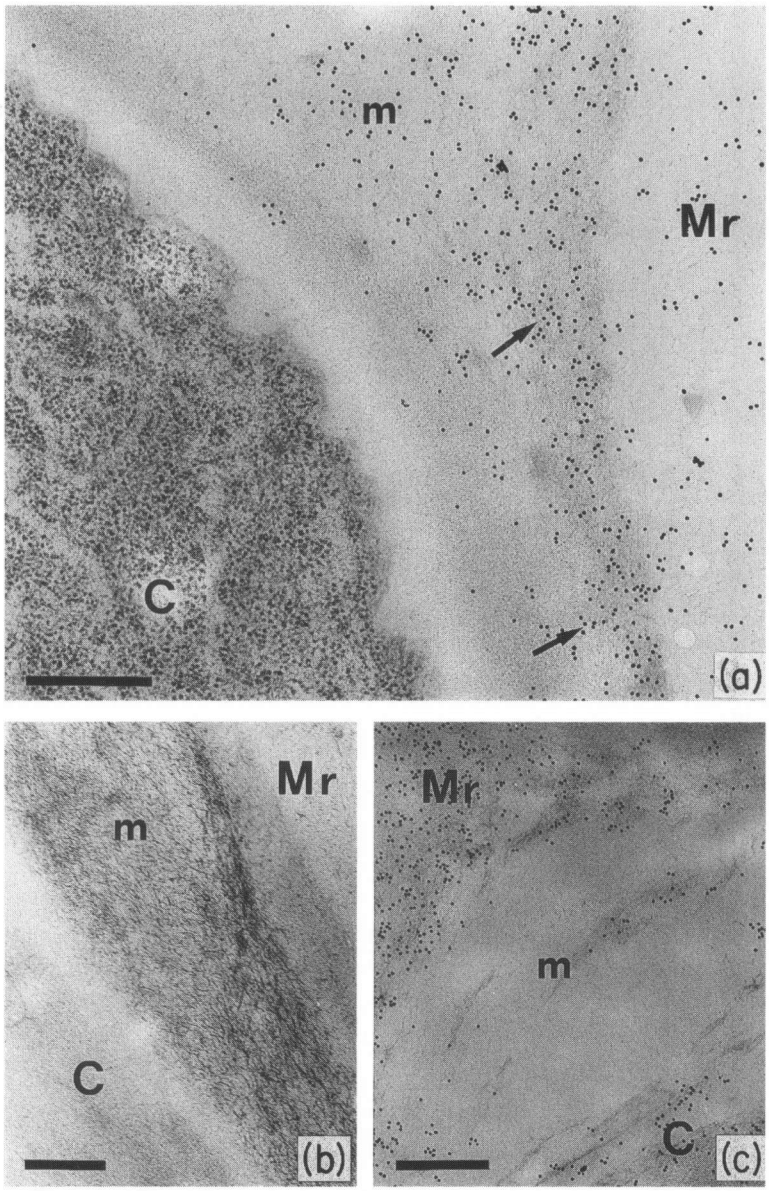

Fig. 6. Details of the material (m) filling up the space after treatment with the antibody JIM 5 (Mr = mother root: C = cap cell). Bars correspond to 0·5 μm. (a) The loose fibrillar material is heavily labelled by the gold granules (arrows). (b) In the control experiment, where the primary antibody was omitted, no labelling is seen. (c) When the primordium is fully separated by the mother root, only remnants of the reactive material are seen.

No labelling was observed in control reactions performed by omitting the primary antibody (not shown).

DISCUSSION

Morphological and *in situ* techniques demonstrate that cell separation events involving pectinolytic enzymes and pectins—as the corresponding substrates—are considerable in

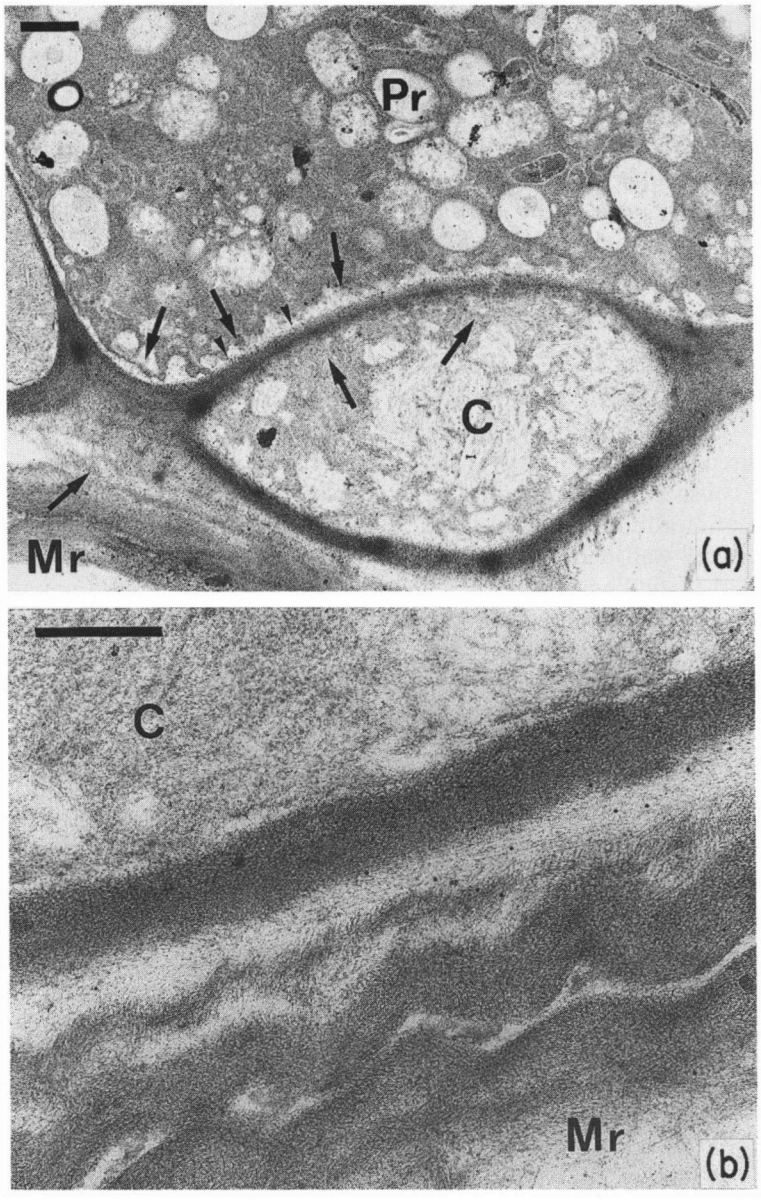

Fig. 7. (a) Localization of polygalacturonase over the primordium (Pr). Gold granules are seen over the meristematic cells as well as over the cap (arrows) (Mr = mother root: C = cap cell). Bar corresponds to 1 μm. (b) Magnification of the contact area showing gold granules at the interface (Mr = mother root: C = cap cell). Bar corresponds to 0·5 μm.

the lateral root morphogenesis. These events take the form of deep structural changes in the walls of the mother root and the primordium, and the creation of an extensive splitting space (the *poche digestive*).

In the primordium meristem, ultrastructural features and distribution patterns of pectins and cellulose were similar to those already described in, for example, *Calluna* or

maize (Peretto *et al.* 1990; Roy & Vian 1991) or *in vitro* cultures of melon cells (Vian & Roland 1991). By contrast, important changes are revealed in the surrounding tissues of the mother root. Unlike the undisturbed roots (Bonfante & Vian 1989; Bonfante *et al.* 1990), cortical cell walls of the mother root are thickened and swollen, particularly along the emerging primordium. The antibody which recognizes homogalacturonans with a low degree of esterification (Knox *et al.* 1990) leads to a heavy labelling, whereas the distribution for cellulose seems to be unchanged. These events (round cell shape, large intercellular spaces, increased presence of de-esterified pectins) can be described as events of *controlled cell separation*. Comparison can be made with ripening cherry tomato (Roy *et al.* 1992), where cell separation and cell-wall swelling constitute the biological basis for the fruit softening.

Previous biochemical and immunological investigation (Peretto *et al.* 1992) has demonstrated increased polygalacturonase activity during leek root outgrowth, and has led to the identification of a protein of 75 kDa which strongly reacted in Western blots with an antibody raised against *F. moniliforme* polygalacturonase. After using the same antibody, a weak but consistent ultrastructural labelling was present in the periplasm and in the walls of the primordium cells as well as at the interface with the mother root tissues. In addition, the cell-wall debris at this interface was rich in pectic material, but not in cellulose. All these findings lead to the suggestion that a complex of pectinolytic enzymes, including a polygalacturonase, synthesized by the primordium, is released to the outer surface. Pea cap cells have already been suggested as possessing a pectolytic activity, which allows them to be separated (Hawes & Lin 1990). By contrast, in our experimental system the enzymic complex produced by the meristem swells the walls of the surrounding cells of the mother root, and—with subsequent enzymic attacks—causes loosening of the middle lamella, release of pectic components and, lastly, cellular splitting.

In conclusion, these morphological observations offer new explanations and clues to an intriguing aspect of root development.

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