

Differential Localization of Carbohydrate Epitopes in Plant Cell Walls in the Presence and Absence of Arbuscular Mycorrhizal Fungi¹

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Two monoclonal antibodies (McAbs) generated against rhamnogalacturonan I and characterized as specific for a terminal α -(1 \rightarrow 2)-linked fucosyl-containing epitope (CCRC-M1) and for an arabinosylated β -(1,6)-galactan epitope (CCRC-M7) were used in immunogold experiments to determine the distribution of the epitopes in four plants. *Allium porrum*, *Zea mays*, *Trifolium repens*, and *Nicotiana tabacum* plants were chosen as representatives of monocots and dicots with different wall structures. Analyses were performed on root tissues in the presence and absence of arbuscular mycorrhizal fungi. A differential localization of the two cell wall epitopes was found between tissues and between species: for example, in leek, CCRC-M1 labeled epidermal and hypodermal cells, whereas CCRC-M7 labeled cortical cells only. Clover walls were labeled by both McAbs, whereas maize and tobacco were only labeled by CCRC-M7. In the presence of the arbuscular mycorrhizal fungi, labeling was additionally found in an apoplastic compartment typical of the symbiosis (the interface) occurring around the intracellular hyphae. Epitopes binding both McAbs were found in the interfacial material, and their distribution mirrored the pattern found in the host cell wall. These findings demonstrate that the composition of the interface zone in a fungus-plant symbiosis reflects the composition of the wall of the host cell.

The plant extracellular matrix regulates plant cell behavior. It controls cell expansion, a complex process that requires the integration of wall loosening and the deposition of new wall molecules (Roberts, 1994). In addition to being a flexible box for the cytoplasm, the cell wall is crucial in

the interactions of plants with the environment and other organisms, in particular with microorganisms (Hahn et al., 1989). Substantial advances have been made in elucidation of the chemical structure of wall components, of which polysaccharides, glycoproteins, and phenolics are the most important (Carpita and Gibeaut, 1993). Current models indicate that this complex array of macromolecules produces a matrix microheterogeneity not only in organs but also in tissues and individual cells (Roberts, 1994). Of the methods for investigation of this heterogeneity, Fourier transform IR microspectroscopy can reveal dynamic changes in the wall (McCann et al., 1993; McCann and Roberts, 1994), and specific antibodies are of great value in locating matrix components, as suggested by Roberts et al. (1985) and many others (review in Knox, 1992).

Localization studies are enhanced significantly by knowledge of antibody specificities. Generation of a panel of McAbs recognizing different epitopes in plant cell wall polysaccharides and characterization of the structure of the epitopes recognized by some of these antibodies provide new, well-characterized probes for investigating wall biosynthesis and structure (Puhlmann et al., 1994; Steffan et al., 1995). Three groups of McAbs were identified: the first group binds mostly to rhamnogalacturonan and arabinogalactan protein epitopes, the second recognizes only the former, and the third exhibits a strong affinity for xyloglucans. Even if McAbs recognize a carbohydrate epitope present on more than one polysaccharide in the wall (Puhlmann et al., 1994), their specificity allows their use to study how wall components are organized in the matrix and whether their localization patterns change with time and in response to different stimuli (Freshour et al., 1996).

We have used two McAbs, CCRC-M1 and CCRC-M7, from this panel (Puhlmann et al., 1994; Steffan et al., 1995) in immunogold experiments in root tissues from four plants (leek [*Allium porrum* L.], maize [*Zea mays* L.], tobacco [*Nicotiana tabacum* L.], and clover [*Trifolium repens* L.]) representative of monocots and dicots, which are expected to have different patterns of wall organization (Carpita and

¹ The research was supported by the Italian Ministero Università Ricerca Scientifica e Tecnologica (40%) and National Council for Research, Special Project Ricerche Avanzate per Innovazioni nel Sistema Agricolo, subproject no. 2, paper no. 2540. Exchanges between the Universities of Konstanz and Torino occurred in the frame of COST Action 821. Work on monoclonal antibodies against plant cell wall polysaccharides at the Complex Carbohydrate Research Center, University of Georgia, is supported by a grant from the U.S. Department of Energy (DE-FGO5–93ER20115) and also by the U.S. Department of Energy-funded Center for Plant and Microbial Complex Carbohydrates (DE-FGO5–93ER20097).

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Abbreviations: AM, arbuscular mycorrhizal; McAb, monoclonal antibody; RG I, rhamnogalacturonan I.

Gibeaut, 1993). The following questions were addressed: (a) what is the location of the epitopes recognized by CCRC-M1 and CCRC-M7 in these plants? and (b) do the epitope distribution patterns change following the establishment of a symbiosis with mycorrhizal fungi? The labeling pattern of roots obtained from sterile, germinated seeds was compared with that of roots inoculated with symbiotic AM fungi. Previous experiments have in fact demonstrated that some fungal endosymbionts cause the deposition of cell wall molecules in an apoplastic compartment, the interface, which is the result of the symbiotic interaction (Bonfante, 1994; Bonfante and Perotto, 1995). We were interested in obtaining additional evidence for the host origin of the interfacial material.

MATERIALS AND METHODS

Plant Material

Seeds of *Zea mays* L. cv W64A, *Nicotiana tabacum* L. (kindly provided by Dr. Puigdomenech, Departamento de Genetica Molecular, Centro de Investigacion y Desarrollo-Consejo Superior de Investigaciones Cientificas, Barcelona, Spain), *Trifolium repens* L. cv Grassland HUIA (from Societa Agricola Italiana Sementi, Cesena, Forli, Italy), and *Allium porrum* L. cv "Mostruoso di Carenta" (from Sementi Dotto, Mortegliano, Udine, Italy) were surface sterilized and sown in sterilized quartz sand. The germinated seedlings were watered three times a week with low phosphorus ($\text{HPO}_4^{2-} = 6 \text{ mg L}^{-1}$) Long Ashton solution (Hewitt, 1966). Mycorrhizal plants of maize and leeks were obtained by inoculating seeds with a spore suspension collected from *Glomus versiforme* (Karst) Berch fruit bodies, and clover and tobacco seedlings were inoculated with germinating spores of *Gigaspora margarita* (Becker & Hall). Original spores were provided by Dr. J. Trappe (Oregon State University, Corvallis) and by Dr. V. Gianinazzi-Pearson (Institut National de la Recherche Agronomique, Dijon, France), respectively (HC/F-E01, HC/F-E10). All plants were maintained in a growth chamber at 24°C with a RH of 75% and a 16-h daylength. Infected and uninfected root samples were collected 2 months after mycorrhizal fungus inoculation.

Electron Microscopy

Differentiated segments from uninfected and mycorrhizal roots were fixed in 2.5% (v/v) glutaraldehyde in 10 mM Na-phosphate buffer (pH 7.2) for 2 h at room temperature. After rinsing with the same buffer, they were postfixated in 1% (w/v) OsO_4 in H_2O for 1 h, washed three times with H_2O , and dehydrated in an ethanol series (30, 50, 70, 90, and 100%; 10 min each step) at room temperature. The root segments were infiltrated in 2:1 (v/v) ethanol/London Resin white resin (Polysciences, Warrington, PA) for 1 h, 1:2 (v/v) ethanol/London Resin white for 2 h, and 100% London Resin white overnight at 4°C according to Moore et al. (1991). Semithin sections (1 μm) were stained with 1% toluidine blue for morphological observations.

Some clover root segments were cryofixed and freeze-substituted. In brief, the segments were vacuum-infiltrated in 20 mM Mes buffer solution (pH 5.5) containing 2 mM

CaCl_2 , 2 mM KCl, and 0.2 M Suc. Single samples were then placed in an aluminium holder as described by Mendgen et al. (1991), immediately frozen at high pressure using the Balzer HPM 010 apparatus (Muller and Moor, 1984), and then stored in liquid nitrogen until further processing. The medium for the freeze substitution consisted of 4% (w/v) OsO_4 in acetone that had been dried. Samples were freeze-substituted in this medium from -90 to 0°C over the course of 3 d, and the medium was changed once at -20°C . Subsequently, the samples were washed three times in dry acetone while the temperature was slowly raised to 10°C .

Samples were infiltrated with Epon/Araldite-acetone mixture (Fluka) from 5 to 100% (v/v) resin over the course of 4 d while the temperature was raised to 20°C . They were then placed in fresh resin and polymerized at 60°C for 10 h.

Antibodies

CCRC-M1 and CCRC-M7 were generated against RG I purified from suspension-cultured sycamore maple (*Acer pseudoplatanus*) cells (Puhlmann et al., 1994). CCRC-M1 recognizes an epitope whose essential part is a terminal α -(1 \rightarrow 2)-linked fucosyl residue. CCRC-M7 binds to RG I and arabinogalactan protein epitopes from various plants. Competition experiments have demonstrated that the minimal epitope recognized by CCRC-M7 is a (1 \rightarrow 6)-linked β -D-galactan containing at least three galactosyl residues with one or more arabinosyl residues attached (Steffan et al., 1995).

Immunocytochemistry

Hybridoma culture supernatants containing CCRC-M1 and CCRC-M7 (Puhlmann et al., 1994, Steffan et al., 1995) were used 1:10 or 1:20 (v/v) to locate, by EM, the cell wall epitopes. Thin sections were incubated for 15 min in normal goat serum diluted 1:30 in 0.05 M Tris-HCl buffer with 500 mM NaCl (TBS, pH 7.6) and 0.2% BSA and treated overnight with the McAbs. After washing, they were incubated for 1 h with 15 nm of colloidal gold-goat antimouse Ig complex (BioCell, Cardiff, UK) containing 1% BSA (diluted 1:20 in TBS). Labeling specificity was determined: (a) by replacing the primary antibody with buffer and (b) by preincubating the antibody for 2 h at room temperature with 1 mg/mL RG I or 100 $\mu\text{g}/\text{mL}$ sycamore xyloglucan. The sections were poststained with uranyl acetate and lead citrate before observation with a Philips (Eindhoven, The Netherlands) CM 10 transmission electron microscope operated at 80 kV.

RESULTS

The localization studies were limited to the differentiated root regions because colonization by AM fungi only occurs there. Transverse sections of leek roots (Fig. 1, a and b) give an example of the cell layers examined: epidermal, hypodermal, cortical, and central cylinder cells. Cortical cell walls were not autofluorescent when seen under UV light.

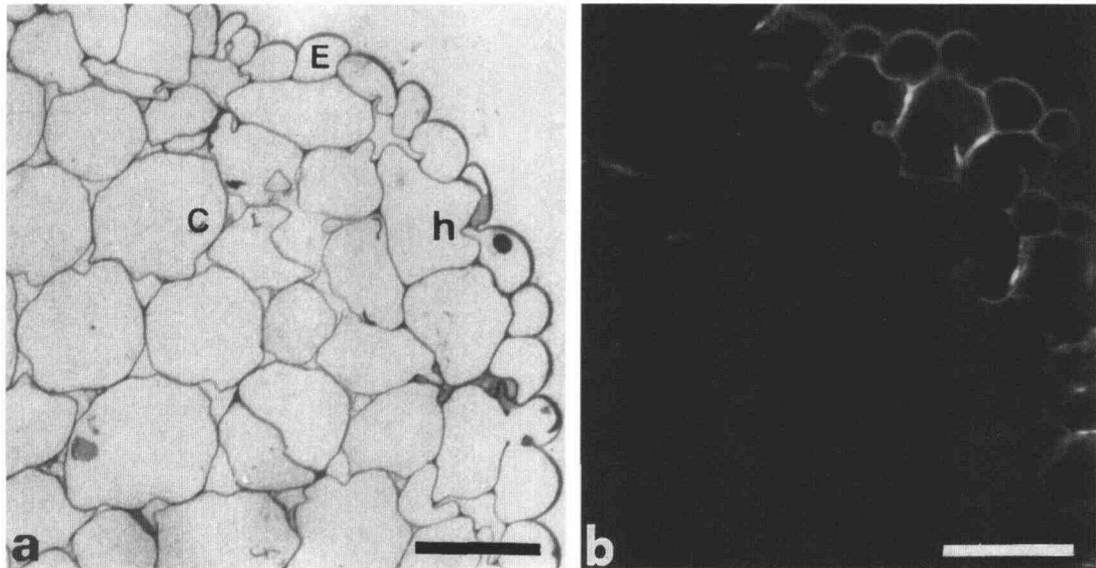


Figure 1. Transverse sections of leek roots in the differentiated region. a, The light microscopy picture shows the following tissues: epidermis (E), hypodermis (h), and cortex (C). b, A similar section is seen under UV light. Only epidermal and hypodermal cell walls are autofluorescent. Bars = 100 μm .

Leek Root

The fucosyl-containing epitope recognized by CCRC-M1 was localized to microdomains of epidermal and hypodermal cell walls. Epidermal cells display a layered organization: only the outer layers and the mucilage were labeled (Fig. 2, a and b). The cell walls around the junction zone, between epidermal cells and hypodermal cells, were also rich in epitopes that bind CCRC-M1 (Fig. 2c). The cortical cell walls were usually not labeled (Fig. 2d), in contrast with those of the nearby hypodermal cells.

The arabinogalactan epitope recognized by CCRC-M7 showed a very different distribution than the CCRC-M1 epitope. Epidermal and hypodermal cell walls were never labeled (Fig. 3a), whereas gold granules were mostly found in the cortical cell wall (Fig. 3b). Labeling was present over the whole wall, except the middle lamella and the innermost wall layer.

Upon colonization by the AM fungus *G. versiforme*, the differential distribution of the epitopes recognized by the two McAbs was maintained not only in the peripheral wall but also in the interface space around the hyphae (Fig. 4), that is the space bounded by the perifungal membrane of the host origin and the fungal wall (see Bonfante and Perotto, 1995, for a full description). The material present around the large fungal coils in epidermal cells was labeled with CCRC-M1 (Fig. 4a) and was not labeled with CCRC-M7 (Fig. 4b), whereas that around the arbuscular branches in the cortical cells was not labeled by CCRC-M1 (Fig. 4c) and was labeled by CCRC-M7 (Fig. 4d).

Maize Root

Substantial labeling of the cell walls was found after treatment with CCRC-M7, especially in epidermal and cor-

tical cells. Labeling was usually localized close to the plasma membrane (Fig. 5a). The middle lamella was not labeled nor was the material occurring at cell junctions. Secondary thickenings, such as those of xylematic or endodermal cells, were not labeled (not shown). In the presence of the AM fungus *G. versiforme* (for further details, see Balestrini et al., 1994), substantial labeling was found in the walls of infected cells and also extended into the interface space. Labeling was particularly abundant at the penetration point (Fig. 5b), where there was an accumulation of electron-dense material (Fig. 5, b and c). The interface space was consistently labeled, irrespective of the size of the fungal branches (Fig. 5d). Labeling was mostly associated with the host membrane and was never found over the fungal wall (Fig. 5, c and d). Many vesicles, closely connected to the proliferating perifungal membrane, were labeled in the infected cells (Fig. 5c). By contrast, gold granules were not detected when CCRC-M1 was used on the thin sections of maize roots (Fig. 5, inset).

Clover Root

Both antibodies labeled cells in clover roots. CCRC-M1 labeled the walls of epidermal, cortical, and central cylinder cells, irrespective of the procedure used for fixation (not shown). With CCRC-M7, labeling was observed in the cortical cell walls, primarily localized near the plasma membrane (Fig. 6a). Cell walls around the junction zone were also labeled with CCRC-M7 (Fig. 6a). In the presence of the mycorrhizal fungus *G. margarita*, substantial labeling was found with both McAbs. The intensity of CCRC-M7 labeling was particularly noteworthy. All of the fungal branches were surrounded by interfacial material that contained arabinogalactan epitopes that are recognized by CCRC-M7 (Fig. 6, b and

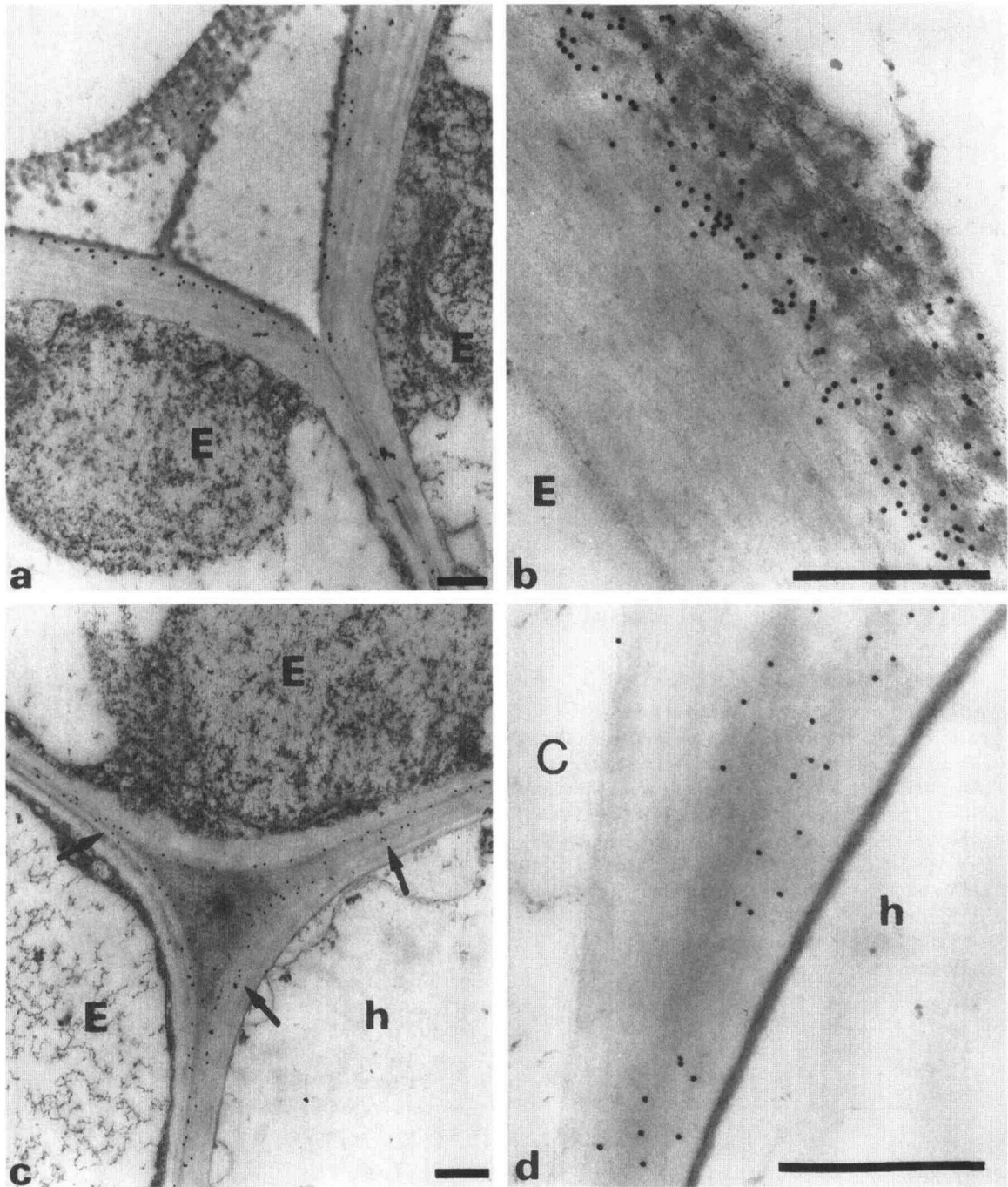


Figure 2. CCRC-M1 McAbs labeling on ultrathin sections of a leek root. a and b, Gold granules are present on the outer layers of the epidermal cell wall and on the mucilage. c, Labeling (arrows) is present on the outer layer of epidermal and hypodermal cell walls. The wall material occurring at the junctions is not labeled. d, Magnification of the contact zone between hypodermal and cortical cells. Gold granules are present only in the hypodermal cell wall. Bars = 0.5 μm ; C, cortical cell; E, epidermal cell; h, hypodermal cell.

c). Labeling was particularly intense around the active fungal hyphae and less substantial around the collapsed ones (Fig. 6c). In the chemically fixed samples, the labeling appeared mostly over the host membrane. CCRC-M1 labeling was detected at the penetration point and around the intracellular fungus (Fig. 6d), whereas it was absent deeper in the cell around the thinner branches.

Tobacco Root

CCRC-M1 did not recognize any epitopes in tobacco roots. CCRC-M7 led to a weak labeling, mostly associated with the plasma membrane. Fungal colonization resulted in substantial labeling over all of the membranes surrounding the fungus (Fig. 7, a and b). In all of

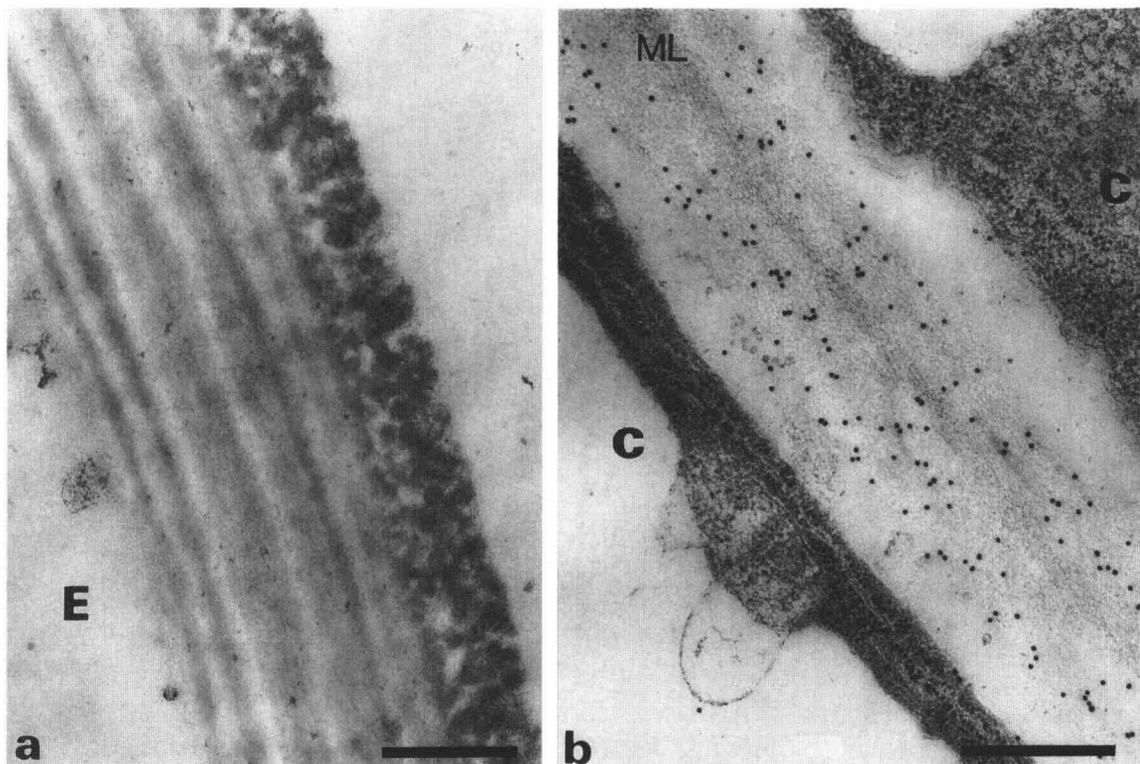


Figure 3. CCRC-M7 McAb labeling on ultrathin sections of a leek root. a, No labeling is present on the epidermal cell wall. b, Labeling is present on the cortical cell wall. The middle lamella is not labeled. Bars = 0.5 μm ; C, cortical cell; E, epidermal cell; ML, middle lamella.

the samples, no labeling was observed when the primary antibody was omitted or when the primary antibody was preincubated with free antigens. The results are summarized in Table I.

DISCUSSION

By using two McAbs that recognize different carbohydrate epitopes in plant extracellular matrix complex carbohydrates (Puhlmann et al., 1994), we were able to show that: (a) there is pronounced heterogeneity in both the cell walls of roots from four plants and in tissues from the same plant, and (b) this heterogeneity is maintained in the new apoplastic compartment formed when the roots are colonized by mycorrhizal fungi. Epitopes recognized by CCRC-M1 and CCRC-M7 vary their location from one plant and from one tissue to another.

The notion that the composition of primary and secondary walls changes in different taxa dates back many years: Bacic et al. (1988) summarized the knowledge at that time by comparing the composition of primary and lignified walls in gymnosperms, dicots, and monocots, where Gramineae were separated from the other monocots. The use of two McAbs that specifically bind to either a terminal α -(1 \rightarrow 2)-linked fucosyl-containing epitope (present primarily in xyloglucan and to a lesser extent in RG I) or to an arabinosylated β -(1,6)-galactan epitope (present in RG I and in arabinogalactan proteins) permitted us to examine

the distribution of these specific structures in the walls of four taxonomically diverse plants. Xyloglucans with a terminal fucosyl residue are present in leek and clover cell walls, showing that the xyloglucans in Liliaceae, e.g., onion and leek, are structurally more closely related to the xyloglucans of dicots than to other monocots, such as the Poaceae (Carpita and Gibeaut, 1993). The absence of CCRC-M1 labeling in maize and tobacco is consistent with structural studies showing that Fuc is absent in xyloglucans from those plants (McNeil et al., 1984). Labeling over the wall is similar in distribution to that observed in clover when a polyclonal antibody against xyloglucans is used (Moore and Stahelin, 1988). Here, too, the middle lamella is not labeled. The labeling pattern is also similar to that described for cellulose in leek and clover and in the same cell types (Bonfante et al., 1990; P. Bonfante, unpublished results). The presence of xyloglucan epitopes in the cellulose region is not surprising because of the interaction of these two polysaccharides in the xyloglucan-cellulose framework (Carpita and Gibeaut, 1993).

The epitope recognized by CCRC-M7 is present in all four plants, but the epitope distribution pattern varies. The strong reactivity of CCRC-M7 with maize root cells is in agreement with ELISA experiments, in which CCRC-M7 binds strongly to maize RG I (Puhlmann et al., 1994). With the exception of leek, the epitope is located in the inner part of the wall, in close proximity to the plasma membrane. It

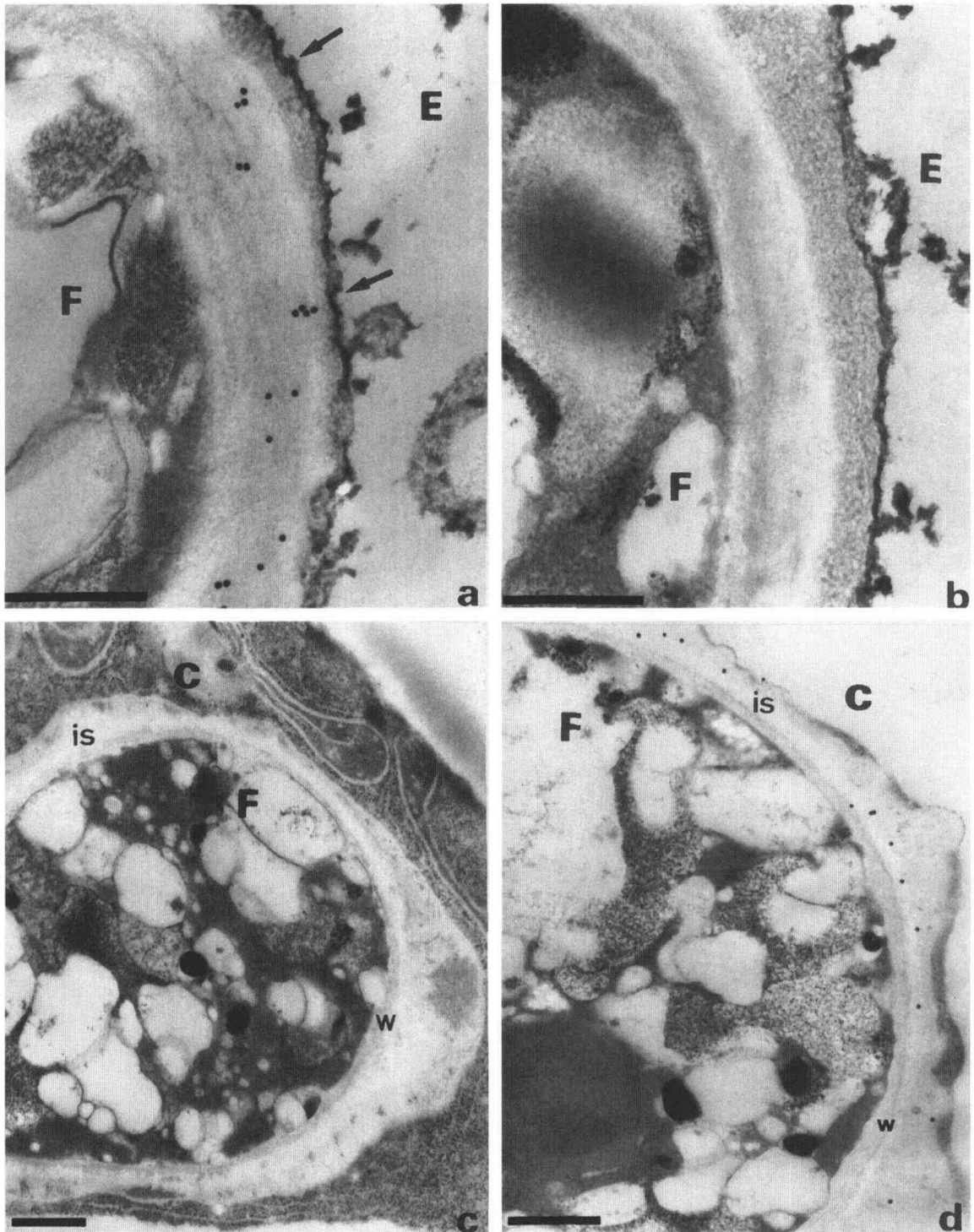


Figure 4. CCRC-M1 (a and c) and CCRC-M7 (b and d) labeling on ultrathin sections of a mycorrhizal leek root. Detail of the interface zone occurring between the fungus and the host membrane (arrows) in the epidermal or cortical cells. The fungal wall is thick in the epidermal cells and thin in the cortical ones. a and b, The interface space around the fungal hyphae, in the epidermal cells, is labeled after CCRC-M1 (a) and is negative after CCRC-M7 (b). Bars = 0.25 μm . c and d, The interface space around the arbuscular branches, in the cortical cells, is not labeled by CCRC-M1 (c) and is labeled by CCRC-M7 (d). Bars = 0.5 μm ; C, cortical cell; E, epidermal cell; F, fungus; is, interface space; w, fungal wall.

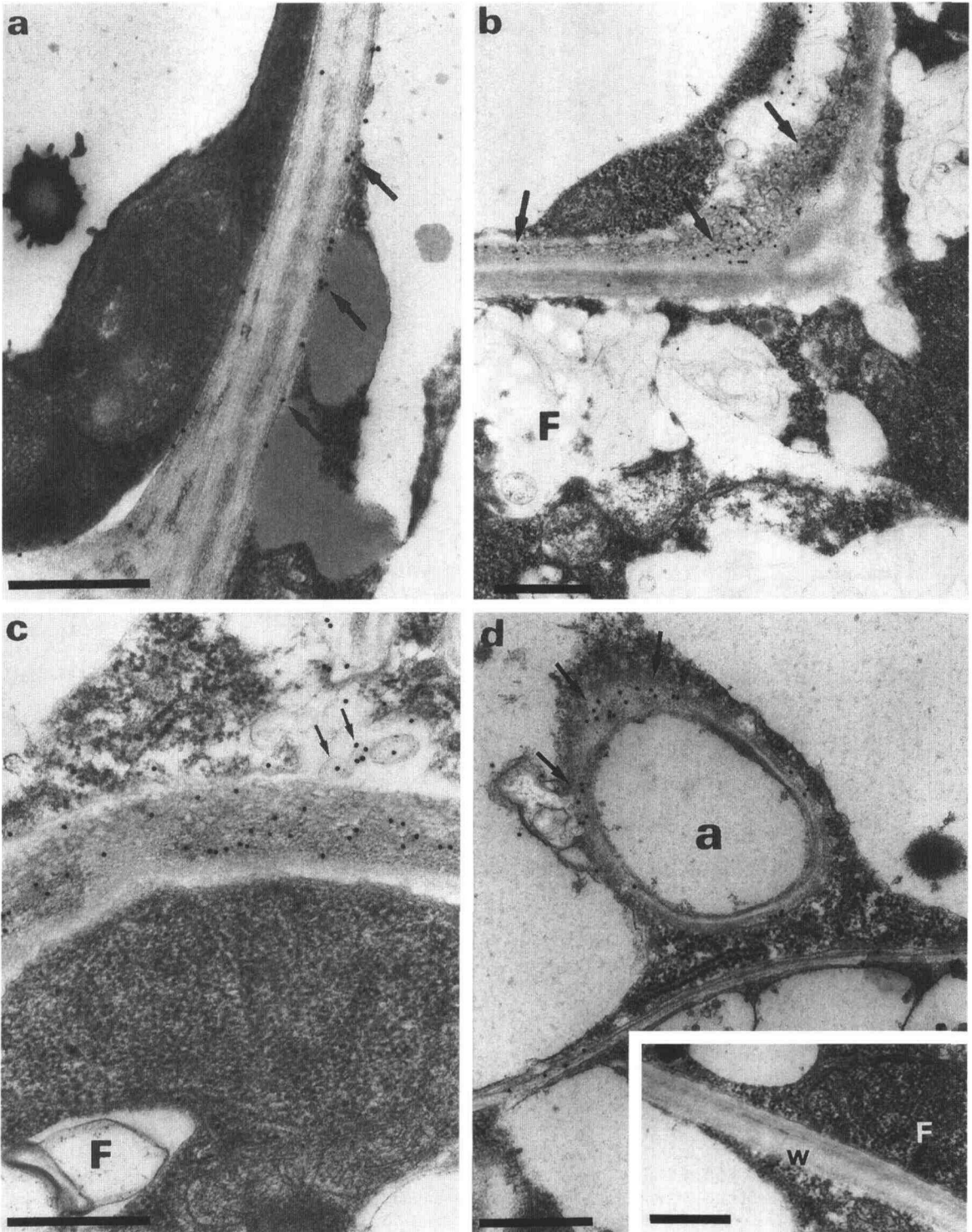


Figure 5. CCRC-M7 (a-d) and CCRC-M1 (inset) labeling on ultrathin sections of maize roots. a, After CCRC-M7, labeling (arrows) is present over the cortical cell walls close to the plasma membrane. b and c, In the presence of the mycorrhizal fungus (*G. versiforme*), gold granules (arrows) are present on the electron-dense material occurring at the penetration point. Labeling is also present on the vesicles connected to the proliferating perifungal membrane (c). d, The interface space around the hyphae is labeled, irrespective of the size of the fungal branches. Labeling (arrows) is associated mostly with the host membrane and is not present on the fungal wall. Inset, No labeling is present after CCRC-M1. Bars = 0.5 μm ; a, arbuscule branches; F, fungus; w, host wall.

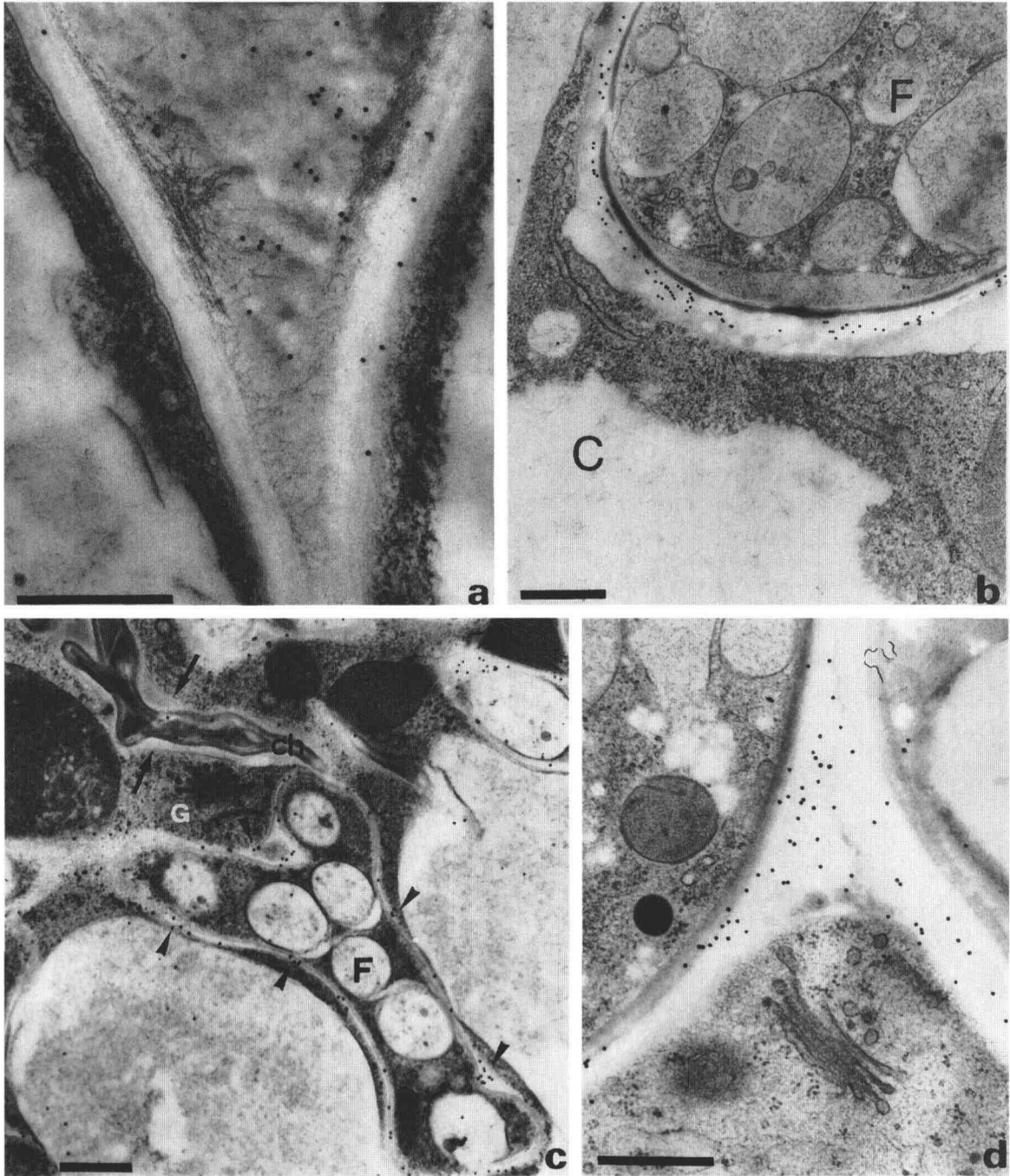


Figure 6. CCRC-M7 (a–c) and CCRC-M1 (d) labeling on ultrathin sections of clover roots. a, After CCRC-M7, labeling is present over the cortical cell walls and at the corner material among cells. b, In the presence of the mycorrhizal fungus (*G. margarita*), substantial labeling is present on the interface space around the fungal branches. c, Labeling (arrows) is less substantial around the collapsed hyphae than around the living fungal branches (arrowheads). Bar = 0.25 μm . d, After CCRC-M1, labeling is present around the intracellular fungus. Bars = 0.5 μm ; C, cortical cell; ch, collapsed hyphae; F, fungus; G, Golgi.

is not possible to tell whether the observed labeling pattern reflects localization of RG I or of membrane arabinogalactan glycoproteins because the epitope recognized by CCRC-M7 is present in both molecules (Puhlmann et al., 1994). As previously seen in *Arabidopsis* roots (Freshour et al., 1996), the epitope recognized by CCRC-M7 is never

found in the middle lamella or in the material occurring at cell junctions. These observations suggest that, in leek, clover, and tobacco, this arabinosylated galactan epitope does not share the location of the epitope in nonesterified galacturonans recognized by the McAb JIM 5 (Bonfante et al., 1990; P. Bonfante, unpublished results).

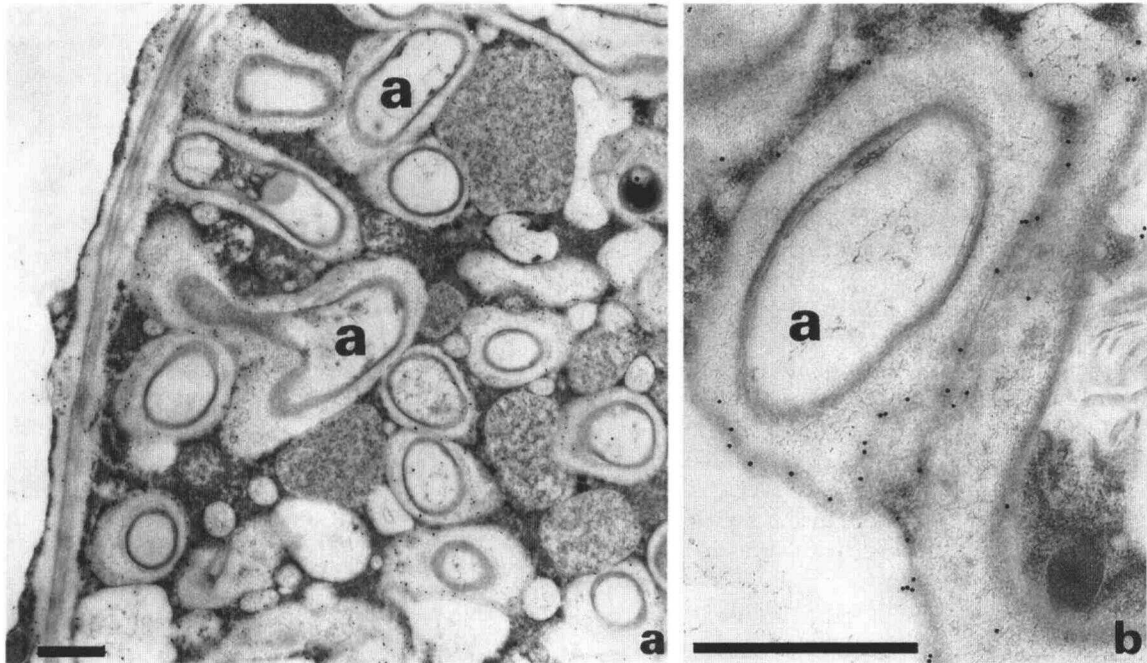


Figure 7. a and b, CCRC-M7 labeling on ultrathin sections of a cortical cell in a mycorrhizal tobacco root. Gold granules are present over all the membranes surrounding the mycorrhizal fungus (*G. margarita*). Bars = 1 μm ; a, arbuscular branches.

The locations of the epitopes recognized by CCRC-M1 and CCRC-M7 vary from one tissue to another in the same plant. This result is particularly evident in leek, where the morphological heterogeneity of epidermal, hypodermal, and cortical cell walls (Fig. 1) is mirrored by a differential distribution of molecular components in the walls (Figs. 2 and 3). Hypodermal cell walls are rich in cellulose, xyloglucans, and phenols; have irregular suberin layers; and lack the nonesterified pectins and arabinosylated galactans present in the cortical cell walls (Bonfante et al., 1990, and present results). Cell-specific location of CCRC-M7 epitopes is also observed in maize roots (Fig. 5). These arabinogalactan epitopes are present in epidermal and cortical cells but are not present in the closely related endodermal cells (not shown). This suggests that the biosynthesis of extracellular glycoconjugates is carefully regulated, cell by cell, inside tissues that have a common origin, such as cortical and endodermal cells.

The Deposition of Epitopes Recognized by CCRC-M1 and CCRC-M7 Is Affected by Mycorrhizal Fungi

One of the main ultrastructural features of successful colonization of roots by AM fungi is the formation of an interface space at the point of contact between plant and fungal cell surfaces (Bonfante and Perotto, 1995). Different types of interfaces are created, depending on whether the fungus does or does not penetrate the host. When the fungus penetrates the host, the interface space is bounded by the fungal wall and by the host membrane and contains the so-called "interfacial material." Affinity probes, such as enzymes, lectins, and antibodies, demonstrate that host cell-wall macromolecules are present at the interface and interact to produce a cell wall-like tunnel. β -1,4-Glucans, nonesterified polygalacturonans, and proteins rich in Hyp have been found in the interfacial material in many different plant/AM fungi com-

Table 1. Summary of the labeling experiments

Plant	CCRC-M1		CCRC-M7	
	In the absence of the fungus	In the presence of the fungus	In the absence of the fungus	In the presence of the fungus
Leek	+ ^a	+ ^a	- ^a	- ^a
Maize	-	-	+ ^b	+ ^b
Clover	+	+	+	+
Tobacco	-	-	+	+

^a With the exception of cortical cells.

^b With the exception of endodermal cells.

binations. Irrespective of the function of the interface in the plant-fungus interaction, morphological changes in its material are mirrored by changes in its composition and are developmentally regulated (Balestrini et al., 1994). These changes in the interfacial material suggest that new cell wall deposition requires a specific metabolic activation in the host.

This study offers new information about the deposition of xyloglucans and rhamnogalacturonans in the interface and provides further evidence that the interfacial material originates mostly from the host plant. Our data also suggest that the interfacial material is a zone of high molecular complexity that is closely related compositionally to the plant wall. In maize, whose cell walls are rich in the arabinogalactan epitope recognized by CCRC-M7 (Puhlmann et al., 1994), the interfacial material is rich in this same epitope. The arabinogalactan epitope is mostly found closely associated with the limiting membrane of host origin (Fig. 5), in accordance with previous observations that CCRC-M7 binds to both RG I and to membrane glycoproteins (Puhlmann et al., 1994; Freshour et al., 1996).

The specific distribution of epitopes recognized by CCRC-M1 and CCRC-M7 in leek roots indicates that the interfacial material not only mirrors the composition of the plant cell wall but more specifically that of the wall of the cell most closely associated with the symbiotic fungus at a particular location. Thus, in leek, an infected epidermal cell possesses an interface with epitopes recognized by CCRC-M1, whereas cortical cells have an interface with epitopes recognized by CCRC-M7 (Table I). In other plants, such as clover, both epitopes are present in the cell walls of uninfected roots and again around the intracellular fungus in the interface material in infected roots. In these metabolically active cells, many Golgi bodies were located in close proximity to the fungal branches. Some of the Golgi were labeled with CCRC-M7 (data not shown), which is consistent with previous findings in *A. pseudoplatanus* showing that arabinogalactan epitopes are synthesized in the Golgi (Zhang and Staehelin, 1992). Our results clearly demonstrate that the establishment of a symbiotic interaction involves new deposition of extracellular macromolecules in differentiated cells (Bonfante and Perotto, 1995). A comparable modulation of cell wall synthesis, and in particular of xyloglucans, has been demonstrated in cotton roots after infection with *Fusarium oxysporum* (Rodriguez-Galvez and Mendgen, 1995), and of RG I in differentiating epidermal cells of *Arabidopsis thaliana* roots (Freshour et al., 1996).

In conclusion, this investigation supports the current view that the plant extracellular matrix exhibits a high degree of structural heterogeneity. This heterogeneity can, at least in part, be documented for the terminal α -fucosyl-containing epitope present primarily in xyloglucan and to a lesser extent in RG I (recognized by CCRC-M1) and for the arabinosylated β -(1, 6)-galactan epitope (recognized by CCRC-M7). The heterogenous deposition of these two epitopes is carefully controlled at the cellular level, giving rise to walls that are cell-

specific, even inside the same organ. Finally, the cell-specific composition of the extracellular matrix is maintained when the plant's development is altered by an interaction with a symbiotic fungus.

Received September 15, 1995; accepted January 29, 1996.

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