ACTA BIOLOGICA CRACOVIENSIA Series Botanica 49/2: 73–80, 2007

VASCULAR ENDODERMIS IN ROOT NODULES OF *LUPINUS LUTEUS* L. (FABACEAE)*

BARBARA ŁOTOCKA**

Department of Botany, Warsaw Agricultural University, ul. Nowoursynowska 159, 02-776 Warsaw, Poland

Received November 20, 2006; revision accepted October 20, 2007

The development of endodermis ensheathing the vascular system of root nodules was examined in *Lupinus luteus* L. Within the system, the vascular endodermis was found to attain different developmental stages. Close to the nodule meristems, nodule vascular bundles were surrounded with endodermis with a Casparian band (first developmental stage). In the oldest part of the nodule vascular system, that is, the nodule vascular trace together with its first range ramifications, vascular endodermis in the third developmental stage (not reported previously for the root nodule vascular system) was observed, with a secondary cell wall.

Key words: *Lupinus luteus* L., yellow lupine, Casparian band, endodermis, root nodule.

INTRODUCTION

Endodermis, defined as a monolayer sheath encircling the root stele, has been widely studied since the 19th century (for review see: Clarkson and Robards, 1975). It occurs in the root of primary structure in every species studied (Clarkson, 1993, and literature cited therein). At present, four stages of its differentiation are commonly recognized: pro-endodermis and the first, second and third stages (van Fleet, 1961; Clarkson and Robards, 1975). Close to the root apical meristem, in the root elongation zone, the pro-endodermis is discernible anatomically and histochemically (van Fleet, 1961; Mueller and Beckman, 1976, and literature cited therein). The pro-endodermis cells are more elongated axially than pericyclic cells (van Fleet, 1961; Scott and Peterson, 1979a). Its characteristic feature is accumulation of phenolic compounds not confirmed in *Pisum sativum* (Kopcińska and Golinowski, 1987), *Triticum aestivum* (Grymaszewska and Golinowski, 1987) or *Ranunculus acris* (Scott and Peterson, 1979a). Ultrastructurally, pro-endodermal cells resemble meristematic cells (Grymaszewska and Golinowski, 1987; Kopcińska and Golinowski, 1987).

In the first developmental stage, the endodermis has a Casparian band formed in its transversal and radial walls (Clarkson and Robards, 1975), usually at the time that the first elements of primary xylem complete differentiation. The Casparian band is a specialized zone of the cell wall, where lipophilic and polyphenolic substances (suberin and lignin) are deposited inside the framework of the existing primary cell wall together with the middle lamella (Bonnett, 1968; Scott and Peterson, 1979b; Zeier et al., 1999). The plasma membrane is slightly thicker where it is juxtaposed to the band, and its characteristic three-layered ultrastructure is particularly distinct there. It adheres to the Casparian band tightly, also in plasmolyzed cells (Robards et al., 1973). Polypeptides of 46, 30 and 20 kDa extractable with SDS (sodium dodecyl sulphate) from isolated pea root endodermis are probably specific to the Casparian band, and therefore may be responsible for the adhesion of the plasma membrane to the Casparian band (Karahara and Shibaoka, 1992). In *Convolvulus arvensis*, the Casparian band exhibits homogenous ultrastructure and slightly higher electron density than the neighboring cell wall (Bonnett, 1968). In pea, numerous Golgi bodies and endoplasmic reticulum cisternae accompany the band (Kopcińska and Golinowski, 1987). It is suggested that these organelles are associated with the production and secretion of Casparian band components. Additionally, exocytosis

**e-mail: lotocka@acn.waw.pl

*This work is part of the author's unpublished doctoral dissertation.

PL ISSN 0001-5296

byproducts – multivesicular bodies or paramural bodies – are observed close to the band in the periplasmic space (Scott and Peterson, 1979a). The structure of the Casparian band prevents apoplastic solute flow to the root stele. Thus, water and solutes cross the endodermal barrier via the active transport pathway, involving the carrier molecules in the endodermal plasma membrane.

In the second stage of endodermis differentiation, a continuous thin layer of suberin is deposited between the primary cell wall and plasma membrane (Clarkson and Robards, 1975). It results in disassociation of the plasma membrane and Casparian band (Robards et al., 1973; Haas and Carothers, 1975). However, remnants of the original plasma membrane tightly bound to the Casparian band can be found to be occluded within the wall (Robards et al., 1973). Suberin lamellae can be thinner at the Casparian band or absent from this part of the cell wall. The suberin layer prevents direct access of solutes to the whole surface of endodermal plasma membranes and thus blocks the intake of water and solutes via this pathway (Robards et al., 1973; Clarkson and Robards, 1975). Still, suberin lamellae are discontinuous at the plasmodesmata, so symplastic continuity remains intact from the root cortex to root stele.

In the third developmental stage, the endodermal cells are characterized by a secondary cell wall deposited on the inner surface of the suberin layer. Between the new wall layer and suberin lamella (also inside the lamella; Robards et al., 1973) membrane fragments which may be remnants of the plasma membrane or paramural bodies are observed (Scott and Peterson, 1979a). The new cell wall layer is often more electron-dense than the primary cell wall. The transition from the second to the third stage is not always sharply delimited; in some species alternate cellulosic and suberin layers are formed, and in others the cellulosic wall is clearly distinct from the suberin layer (Clarkson and Robards, 1975). The secondary cell wall is deposited uniformly around the whole protoplast (O thickenings) or is visibly thinner at the outer tangential wall (C or U thickenings). The formation of the secondary wall is correlated with a significant decrease in water and calcium intake in the respective root zone (Sanderson, 1983).

Additionally, a fourth stage in endodermis differentiation can be distinguished (van Fleet, 1961), consisting in the incrustation of the secondary cell wall formed during the third stage with phenolics and chinones. For example, such incrustation was observed in wheat (Grymaszewska and Golinowski, 1987).

During all the developmental stages, endodermal cells maintain numerous symplastic connections through plasmodesmata with the cells of adjoining root zones (e.g., Helder and Boerma, 1969; Robards et al., 1973; Warmbrodt, 1986). Sometimes, as in pea (Kopcińska and Golinowski, 1987), they are especially abundant in tangential walls. In some species, plasmodesmata also occur within Casparian bands (Haas and Carothers, 1975; Kopcińska and Golinowski, 1987).

Endodermal cells that remain arrested in an earlier developmental stage while the adjacent endodermal cell pass through the second and third stages are termed passage cells (van Fleet, 1961; Clarkson and Robards, 1975). Differentiation of endodermal cells of the same age (i.e., located in the same cross section of the root) is usually asynchronous, and the cells facing the protoxylem are the last to mature (Robards et al., 1973; Scott and Peterson, 1979a; Grymaszewska and Golinowski, 1987; Kopcińska and Golinowski, 1987).

In legume root nodules, the presence of endodermis was identified first in *Vicia faba* (Brenchley and Thornton, 1925) and *Medicago sativa* (Thornton and Rudorf, 1936). In nodules, typical endodermis (also termed vascular endodermis; Abd-Alla et al., 2000; Hartmann et al., 2002) ensheathing the nodule vascular bundles is recognized, and the so-called common endodermis (term first used by Fraser, 1942) or cortical endodermis or nodule endodermis. The latter is in fact rather a passive component of the oxygen diffusion barrier, since after insertion of an O_2 microelectrode into a nodule, the measured pO_2 drops sharply near the region of the common endodermis (Tjepkema and Yocum, 1974; Witty et al., 1987; Dakora and Atkins, 1989). Although the presence of endodermis around nodule vascular bundles has been mentioned by numerous authors, its development or ultrastructure is little studied. In *Vicia faba*, the vascular endodermis appears immediately below the meristematic nodule apex in the first developmental stage, and attains the second stage at the base of the nodule (Hartmann et al., 2002). The appearance of the second developmental stage was also noted in the oldest vascular bundle of *Trifolium repens* root nodules (Łotocka et al., 1997). The ultrastructure of nodule vascular endodermis was briefly described in *Pisum sativum* and *Trifolium repens* (Pate et al., 1969); in these species it reached the first developmental stage, which did not differ from its counterpart in the root. The vascular endodermal cells had numerous connections via plasmodesmata with nodule cortex cells and cells of the bundle pericycle.

This work summarizes a study of endodermis development in the nodules in *Lupinus luteus* L., undertaken to gain greater understanding of root nodule structure in this species.

MATERIALS AND METHODS

GROWTH CONDITIONS

Seeds of *Lupinus luteus* L. cv. Ventus (Polish commercial cultivar, Plant Breeding Station in Wiatrowo) were surface-sterilized with 3.5% calcium hypochlorite for 20 min and then pregerminated on moist filter paper in sterile Petri dishes at 24°C for 48 h. Seedlings with roots ~2 cm long were examined under a dissecting microscope, and the zone of emerging root hairs was marked, since it is known from preliminary experiments that lupine's nodule primordia are induced just below this zone. Seedlings were carefully transferred to pots filled with sterile perlite and inoculated with a suspension of *Bradyrhizobium* sp. (*Lupinus*), wild-type strain 3045USDA (*Rhizobium* Culture Collection, Beltsville, U.S.A.). The plants' growth conditions and bacterial or plant media were as described previously (Łotocka et al., 2000).

For microscopic observations, three stages of nodule development were chosen: the juvenile stage, 10 days after inoculation (DAI) as determined by Łotocka et al. (1995); the stage of full metabolic activity, 25 DAI; and the stage with a wide zone of senesced bacteroid tissue, 60 DAI.

PROBE SAMPLING

For examination of juvenile nodules (10 DAI), root fragments below the mark were cross-cut into several slices ~1 mm thick. From older roots, only nodules from the upper part of the taproot were taken, and only nodules initiated at a distance from other nodules; this was done to exclude nodules that were developmentally inhibited by neighboring nodules. Some nodules 25 DAI and 60 DAI were dissected, and fragments containing nodule vascular traces or nodule vascular bundles were sampled separately. Undamaged nodules were sampled for paraffin embedding.

FURTHER PROCEDURES

All the material was fixed identically in a mixture of 5% glutaraldehyde and 4% paraformaldehyde in 0.1 M sodium cacodylate buffer, pH 7.2–7.3, at room temperature and air pressure -0.3 hPa. Rinsing with the same buffer followed fixation. Next, the samples intended for resin sections were postfixed with 2.5% osmium tetroxide in 0.1 M cacodylate buffer for 2 h at 4°C and rinsed again. The probes sampled 10 DAI were examined under a dissecting microscope, and those that after osmification revealed nodule primordia, as well as samples of 25 DAI and 60 DAI nodules, were dehydrated in ethanol and acetone and embedded in Glycid Ether 100 epoxy resin. The samples were positioned in embedding molds to ensure sectioning in transversal, radial or tangential planes versus the root axis. Semithin serial sections 3 μm thick were cut with a Jung–2065-Supercut microtome (Leica/Reichert-Jung) and stained at 70°C with 1% aqueous azure A and 2% methyl blue on 2% borax. For fine structure observations, ultrathin serial sections were cut with an Ultracut E microtome (Reichert), collected on formvar-coated slot-grids and contrasted with lead citrate and uranyl acetate.

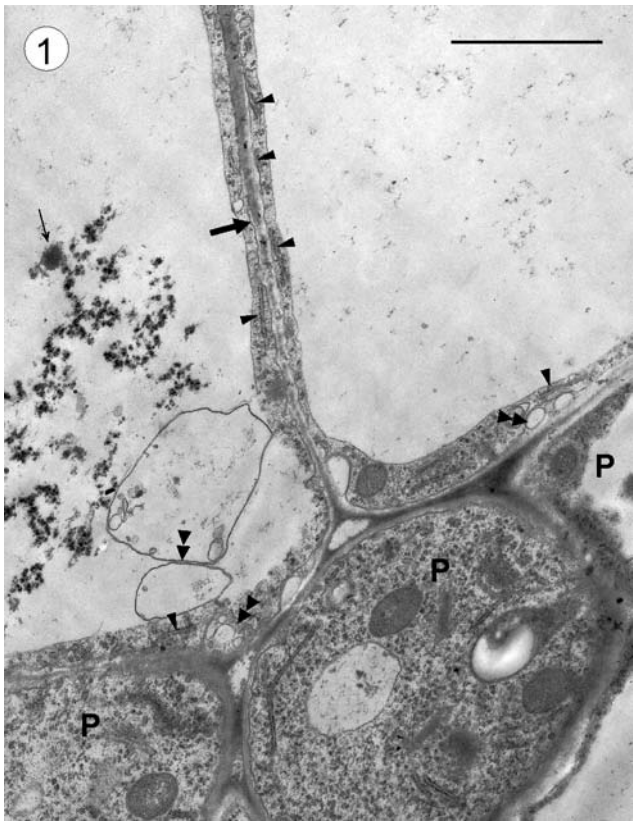

Fig. 1. Pro-endodermis close to the meristematic apex of the nodule vascular bundle (images are of *Lupinus luteus* root nodule fixed 60 DAI). In anticlinal cell wall (arrow) no Casparian band is visible yet. P – pericycle of nodule vascular bundle; thin arrow – electron-dense deposits within endodermal vacuole; arrowheads – RER; double arrowheads – membranous structures. Bar = 2.38 μm.

The samples intended for paraffin sections were dehydrated in ethanol and xylene, embedded in paraffin wax, serial-sectioned transversely, radially or tangentially at 8–20 μm thickness depending on nodule size, and stained with safranin/fast green (Broda, 1971).

Observations and microphotography employed an Axioskop light microscope (Zeiss-Opton) equipped with a Contax 167MT camera, or a JEM 100C or JEM 1220 transmission electron microscope (JEOL). Negatives were scanned into electron-

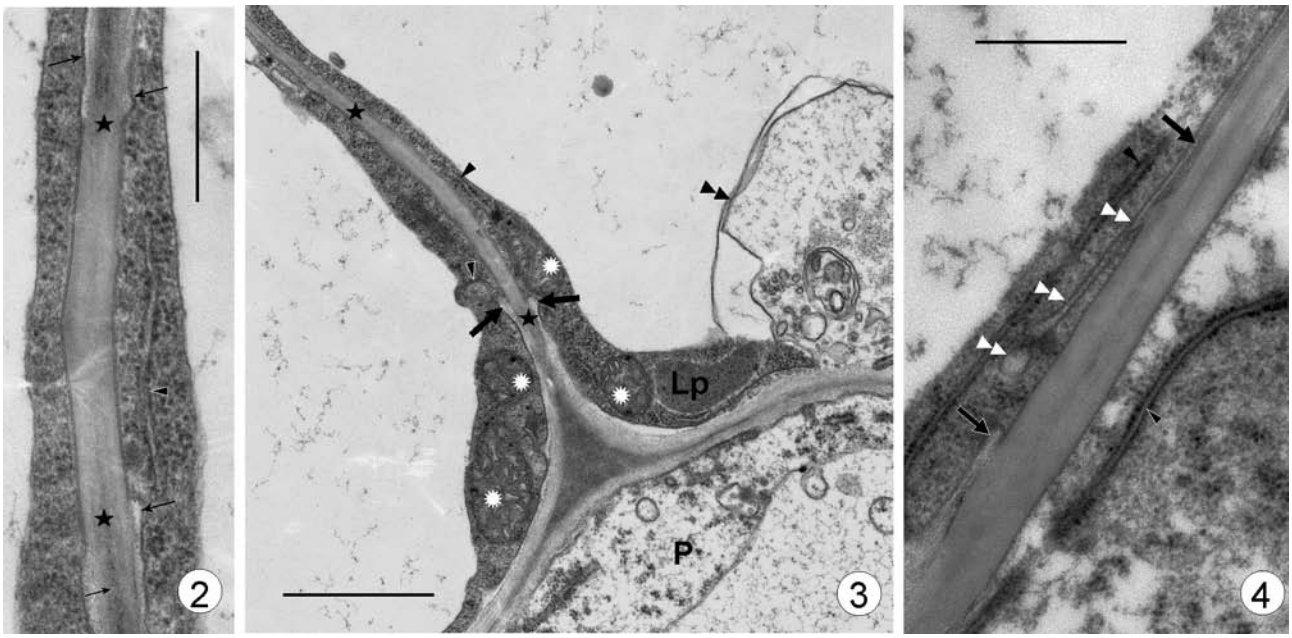

Fig. 2. Endodermis in first developmental stage, nodule vascular bundle. Asterisks – region of Casparian band in anticlinal cell wall of endodermal cell; arrows – plasma membrane beyond Casparian band loosely associated with the cell wall; arrowhead – RER. Bar = 0.53 μm. **Fig. 3**. In the endodermis of the nodule vascular bundle, deposition of the secondary cell wall (arrows) is initiated asymmetrically starting at the site of the bundle pericycle (P). Lp – leucoplast; asterisks – Casparian band; arrowheads – RER; double arrowheads – membranous structures; rosettes – mitochondria. Bar = 1.23 μm. **Fig. 4**. Deep pockets of plasma membrane (double arrowheads) folded at the site of secondary cell wall deposition (arrows) at the Casparian band. Arrowheads – RER. Bar = 0.80 μm.

ic files of 1600 dpi resolution using a CanoScan 9900F flatbed scanner. The gamma level and contrast of the resulting grey-tone images were adjusted using Adobe Photoshop 7.0 software.

RESULTS

In 10 day-old root nodules, the nodule vascular system consisted of the nodule vascular trace (NVT) only. In its distal part, the location of the first nodule bundles, still at the stage of meristematic strands, was discernible. At this time the NVT was ensheathed by pro-endodermis (not shown), which was ultrastructurally similar to the cells of meristematic apices of vascular bundles.

In nodules fixed 25 DAI, the vascular endodermis ultrastructure was similar to that of nodules fixed 60 DAI. However, since more developmental stages of vascular endodermis differentiation were observed in the latter, the description of endodermis in 25 DAI nodules is omitted as redundant.

In elongating apices of the nodule vascular bundles (NVBs) located close to the meristems of 60 DAI nodules, pro-endodermis cells were observed (Fig. 1). Their cell walls did not differ from the primary cell walls of adjoining cells in differentiating bundle pericycle. The pro-endodermis cells were already vacuolated, and the vacuoles accumulated electron-dense deposits. In the cytoplasm, numerous short cisternae of rough endoplasmic reticulum (RER) were present, and plasmodesmata connected the protoplasts of NVB pericycle cells, pro-endodermal cells and nodule cortex cells. Differentiation of the Casparian band co-occurred with the maturation of conductive elements, but preceded the formation of the wall labyrinth in bundle transfer cells. Differentiation of the band was not uniform in the bundle cross section, but there was no correlation between the differenriation stage attained by the particular endodermal cell and the location of bundle phloem or xylem. The Casparian band usually was positioned within the anticlinal wall closer to the NVB pericycle, The cell wall within the Casparian band stained red in safranin/fast green paraffin sections, indicating lignification (not shown). In TEM, the band exhibited more homogenous ultrastructure, in contrast to the other parts of the cell wall, but had similar electron density, and the plasma membrane adhered tightly to it (Fig. 2). Plasmo-desmata connected the protoplasts of the inner cortex, vascular endodermis and NVB pericycle. Gradually a new layer of cell wall (secondary cell wall) was deposited. The process occurred in the vicinity of the bacteroid tissue cells, which exhibited no symptoms of degradation yet. Within an endodermal cell

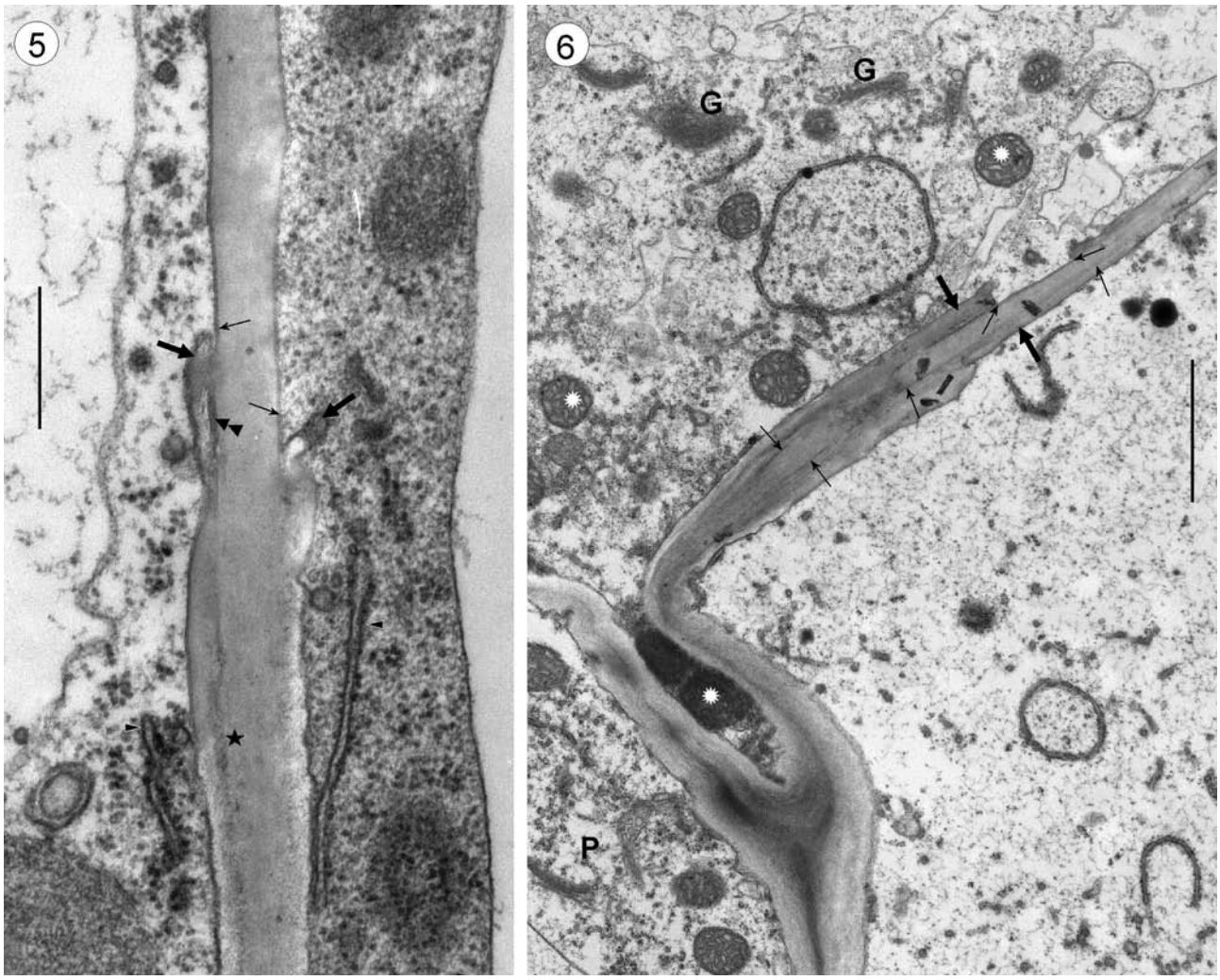

Fig. 5. Deposition of the secondary cell wall (arrows) at the Casparian band, nodule vascular bundle. No electron-dense suberin layer is discernible. Asterisk – possible position of inner edge of Casparian band; double arrowhead – Casparian band-adhering fragment of plasma membrane, which might be embedded within the secondary cell wall; thin arrows – parts of Casparian band not covered with secondary cell wall yet; arrowheads – RER. Bar = 0.45 μm. **Fig. 6**. The most advanced stage of differentiation of the endodermis ensheathing the nodule vascular bundles. Note the absence of tonoplast, and "diluted" cytoplasm in the lower cell. P – bundle pericycle cell; G – Golgi bodies; arrows – secondary cell wall; thin arrows – position of Casparian band; in the upper cell the band is only partially covered by secondary cell wall; rosettes – mitochondria. Bar = 1.54 μm.

the secondary wall deposition was often initiated unevenly (Fig. 3), starting from the wall adjoining the NVB pericycle or the nodule inner cortex. The secondary wall had fine-fibrillar ultrastructure similar to the primary cell wall. At the edge of the Casparian band, at the site of secondary wall deposition, the plasma membrane usually formed deep pockets (Figs. 4, 5). Characteristically, RER cisternae were always observed in the adjoining cytoplasm, and often the sections (as in Fig. 5) suggested fusion of RER-derived vesicles with the plasma membrane pocket. In NVBs of second and higher orders, the Casparian bands were never completely covered with the secondary cell wall layer, even in NVBs fixed 60 DAI (Fig. 6). In older parts of the NVB system, some cells of the vascular endodermis facing the nodule outer cortex developed a thick three-layered cell wall similar to that described below for the nodule vascular trace. Such cells were often flattened but remained alive.

The nodule vascular trace (NVT) connected the system of NVBs with the root vascular tissues. In the samples fixed 25 DAI, endodermis in the first developmental stage, that is, with the Casparian band formed, surrounded the NVT (not shown). In the nodules dissected and fixed 60 DAI, the oldest NVBs (i.e., first-order ramifications of the NVT), as well as the NVT itself were ensheathed with vascular endo-

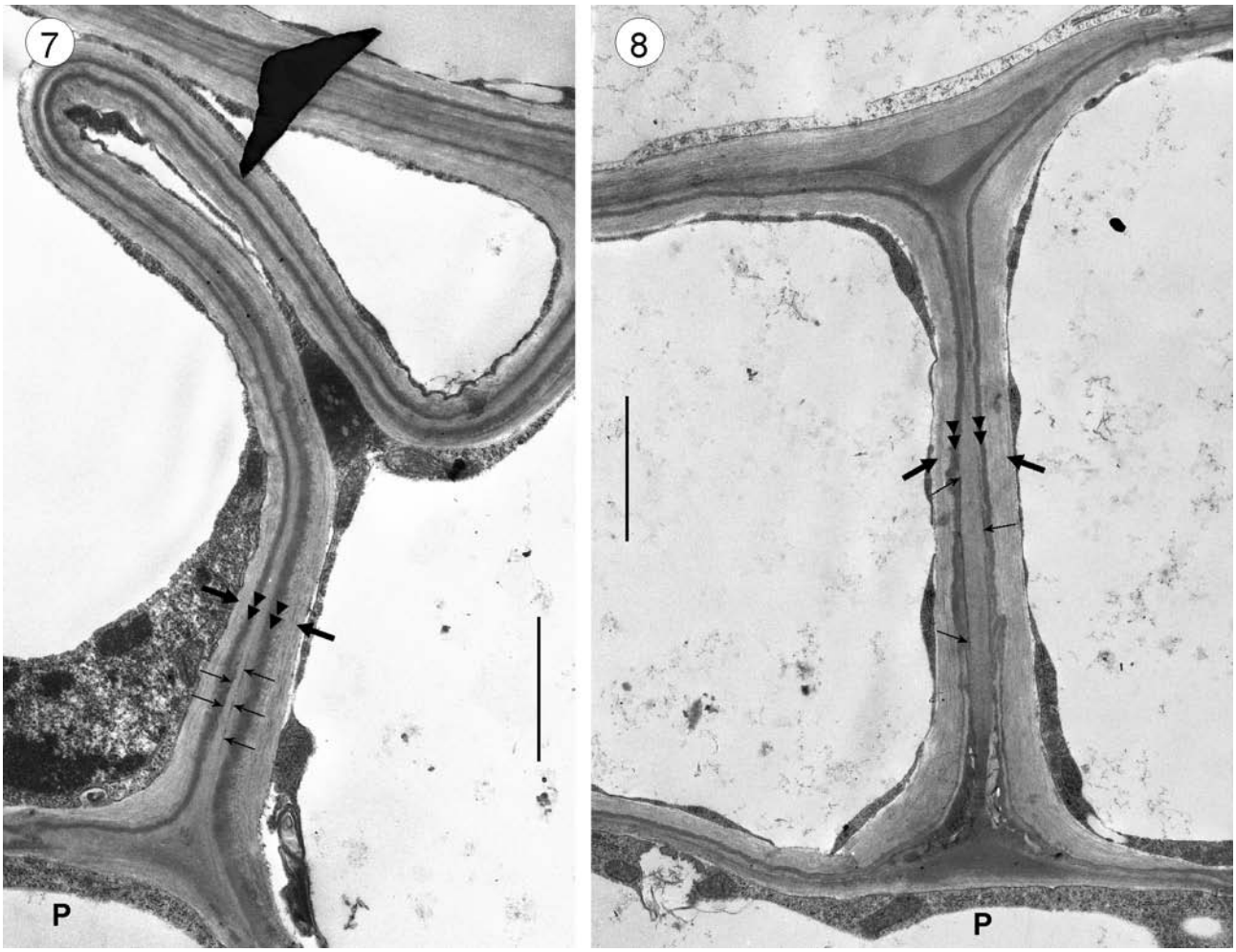

Fig. 7, 8. The most advanced stage of differentiation of the endodermis ensheathing the nodule vascular trace. Note three-layered structure of the thick cell wall. P – cells of nodule vascular trace pericycle; thin arrows – position of Casparian band remnants in anticlinal wall of endodermal cell; double arrowheads – electron-dense suberin layer; arrows – secondary cell wall. Bars = 1.80 μm in Fig. 7, 2.21 μm in Fig. 8.

dermis with a thick three-layered cell wall (Fig. 7). The outermost (oldest) layer corresponded to the primary cell wall, and remnants of the Casparian band were discernible in this layer (Fig. 8). The middle layer (suberin lamella) was more electron-dense and homogenous than the innermost (youngest) layer of secondary cell wall (Fig. 9), which resembled the primary cell wall ultrastructurally. In some places the layers of the primary cell wall and suberin lamella were discontinuous. In nodules fixed 60 DAI, the NVT endodermal cells still contained protoplasts, which were strongly vacuolated (Fig. 7), with electron-dense deposits in vacuoles and numerous RER cisternae and mitochondria in the cytoplasm. Discontinuity of the tonoplast was observed in some older endodermal cells, giving the cytoplasm a diluted appearance (Fig. 6).

DISCUSSION

In lupine, the first-stage root endodermis existing at the time of, and in the root zone of, nodule initiation undergoes dedifferentiation, and its derivative cells take part in the formation of the nodule primordium (Łotocka et al., 2000). Between the subrhizodermal initials of bacteroid tissue and the primary xylem strand, the nodule vascular trace forms. It is ensheathed with vascular endodermis, which differentiates in continuity with the root endodermis. The continuity of the nodule vascular endodermis with the root endodermis is a uniform feature of legume root nodules (Hirsch, 1992). In indeterminate root nodules of *L. luteus* all the developmental stages of endodermis differentiation can be found from the meristematic apices of nodule vascular bundles

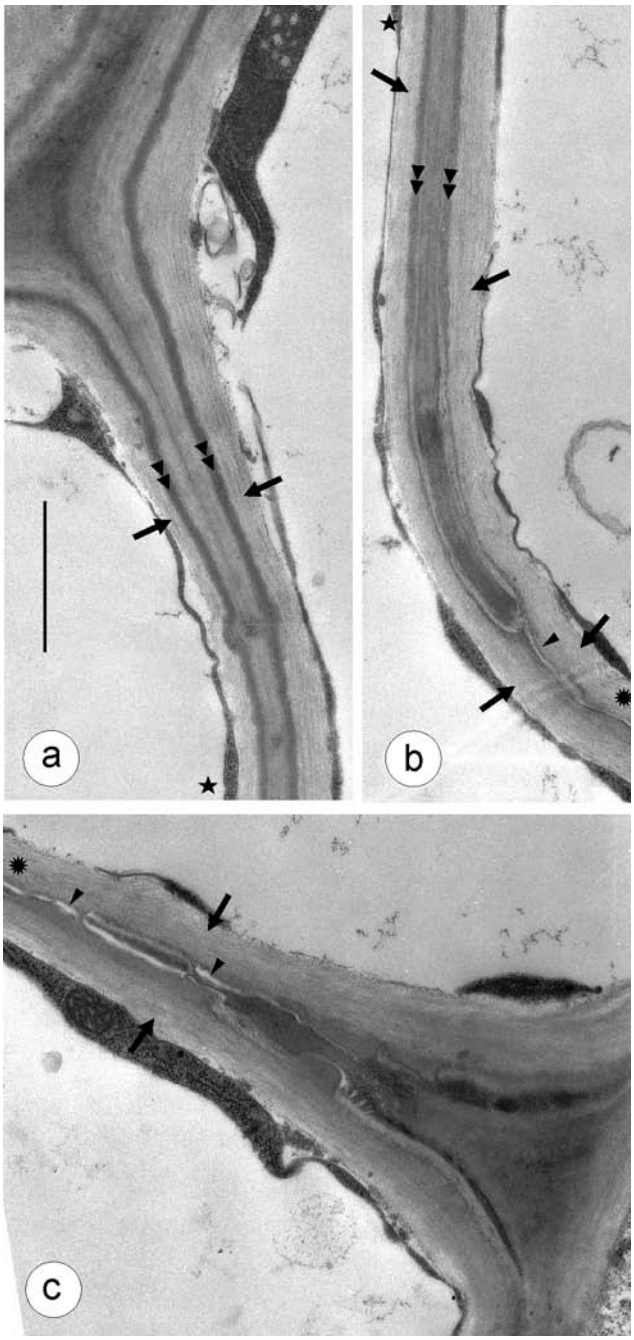

Fig. 9. Anticlinal wall of endodermis ensheathing the nodule vascular trace. The whole wall was cut in three fragments, and asterisks and rosettes indicate the matching points of the wall. Note discontinuities (arrowheads) in the ultrastructure of electron-dense suberin layers (double arrowheads) and the primary cell wall located between them; remnants of Casparian band are not recognizable. Arrows – secondary cell wall. Bar = 1.61 μm.

towards their older parts and then through the nodule vascular trace. Such an observation was not reported earlier for nodule vascular endodermis of the other legumes. In the nodule vascular endodermis of *Trifolium repens* and *Vicia faba*, only the second developmental stage, the formation of the suberin layer, was reported in the oldest part of the vascular system (Łotocka et al., 1997; Abd-Alla et al., 2000).

The developmental stages of the nodule vascular endodermis in *L. luteus* are ultrastructurally identical to the development of the root endodermis in this species (author's unpublished observations). Nor does first-stage ultrastructure differ from earlier data on root nodule vascular endodermis in other legume species (Pate et al., 1969) or on the root endodermis (Clarkson and Robards, 1975; Kopcińska and Golinowski, 1987).

After differentiation of the Casparian band in the vascular endodermis of lupine NVBs, a new layer was deposited. It did not resemble the published pictures of the formation of the suberin layer, which are electron-dense and homogenous. On the contrary, in lupine the new layer was fibrillar and similar to the primary cell wall. Such ultrastructure does not suggest it to be an efficient barrier to apoplast solute movement. However, in the NVT of nodules fixed 60 DAI, the thick cell wall of the vascular endodermis contained an electron-dense and homogenous suberin layer internal to the fibrillar secondary cell wall. No plasmodesmata were found in these cell walls. Therefore, in the lupine nodules older than 25 DAI, both symplastic and apoplastic continuity between the cells of the NVT and adjoining parenchymatous nodule cortex probably were broken.

A common feature of root nodule structure is the boundary layer, consisting of bacteroid tissue-adjoining parenchymatous cells with very few, small intercellular spaces and distorted radial walls (Parsons and Day, 1990; Brown and Walsh, 1994). The distortion results from the lack of turgor due to fragmentation of the tonoplast. In consequence, the cytoplasm of boundary layer cells has a diluted appearance. The boundary layer functions as part of the nodule's oxygen diffusion barrier. Interestingly, in lupine's nodule vascular endodermis, which often was in contact with the boundary layer, numerous cells of the same protoplast ultrastructure were observed in this work. It cannot be excluded that the discontinuity of the tonoplast resulted from poor fixation of endodermal cells beyond the first developmental stage, as problems with fixative penetration in this tissue have been noted elsewhere (Pate et al., 1969).

ACKNOWLEDGEMENTS

The author thanks Ewa Znojek and Alicja Żuchowska for expert technical assistance.

REFERENCES

ABD-ALLA MH, KOYRO H-W, YAN F, SCHUBERT S, and PEITER E. 2000. Functional structure of the indeterminate *Vicia faba* L. root nodule: implications for metabolite transport. *Journal of Plant Physiology* 157: 335–343.

BONNETT HT. 1968. The root endodermis: fine structure and function. *Journal of Cell Biology* 37: 199–205.

BRENCHLEY WW, and THORNTON HG. 1925. The relation between the development, structure and functioning of the nodules of *Vicia faba* as influenced by the presence or absence of boron in the nutrient medium. *Proceedings of the Royal Society of London. Series B, Biological Sciences* 98: 373–398.

BRODA B. 1971. *Metody histochemii roślinnej*. PZWiL, Warsaw.

BROWN SM, and WALSH KB. 1994. Anatomy of the legume nodule cortex with respect to nodule permeability. *Australian Journal of Plant Physiology* 21: 49–68.

CLARKSON DT. 1993. Roots and the delivery of solutes to the xylem. *Philosophical Transactions of the Royal Society of London, Series B* 341: 5–17.

CLARKSON DT, and ROBARDS AW. 1975. The endodermis, its structural development and physiological role. In: Torrey JG and Clarkson DT [ed.], *The development and function of roots*, 415–436. Academic Press, London, New York, San Francisco.

DAKORA FD, and ATKINS CA. 1989. Diffusion of oxygen in relation to structure and function in legume root nodules. *Australian Journal of Plant Physiology* 16: 131–140.

FRASER HL. 1942. The occurrence of endodermis on leguminous root nodules and its effect upon nodule function. *Proceedings of the Royal Society of Edinburgh, B* 61: 328–343.

GRYMASZEWSKA G, and GOLINOWSKI W. 1987. The structure of the endodermis during the development of wheat (*Triticum aestivum* L.) roots. *Acta Societatis Botanicorum Poloniae* 56: 3–10.

HAAS DL, and CAROTHERS ZB. 1975. Some ultrastructural observations on endodermal cell development in *Zea mays* roots. *American Journal of Botany* 62: 336–348.

HARTMANN K, PEITER E, KOCH K, SCHUBERT S, and SCHREIBER L. 2002. Chemical composition and ultrastructure of broad bean (*Vicia faba* L.) nodule endodermis in comparison to the root endodermis. *Planta* 215: 14–25.

HELDER RJ, and BOERMA J. 1969. An electron-microscopical study of the plasmodesmata in the roots of young barley seedlings. *Acta Botanica Neerlandica* 18: 99–107.

HIRSCH AM. 1992. Developmental biology of legume nodulation. *New Phytologist* 122: 211–237.

KARAHARA I, and SHIBAOKA H. 1992. Isolation of Casparian strips from pea roots. *Plant and Cell Physiology* 33: 555–561.

KOPCIŃSKA J, and GOLINOWSKI W. 1987. The structure of the endodermis during the development of pea (*Pisum sativum* L.) roots. *Acta Societatis Botanicorum Poloniae* 56: 11–18.

ŁOTOCKA B, ARCISZEWSKA-KOZUBOWSKA B, DĄBROWSKA K, and GOLINOWSKI W. 1995. Growth analysis of root nodules in yellow lupin. *Annals of Warsaw Agricultural University – SGGW, Agriculture* 29: 3–12.

ŁOTOCKA B, KOPCIŃSKA J, and GOLINOWSKI W. 1997. Morphogenesis of root nodules in white clover. I. Effective root nodules induced by the wild type of *Rhizobium leguminosarum* biovar. *trifolii*. *Acta Societatis Botanicorum Poloniae* 66: 273–292.

ŁOTOCKA B, KOPCIŃSKA J, GÓRECKA M, and GOLINOWSKI W. 2000. Formation and abortion of root nodule primordia in *Lupinus luteus* L. *Acta Biologica Cracoviensia Series Botanica* 42: 87–102.

MUELLER WC, and BECKMAN CH. 1976. Ultrastructure and development of phenolic-storing cells in cotton roots. *Canadian Journal of Botany* 54: 2074–2082.

PARSONS R, and DAY DA. 1990. Mechanism of soybean nodule adaptation to different oxygen pressures. *Plant Cell and Environment* 13: 501–512.

PATE JS, GUNNING BES, and BRIARTY LG. 1969. Ultrastructure and functioning of the transport system of the leguminous root nodule. *Planta* 85: 11–34.

ROBARDS AW, JACKSON SM, CLARKSON DT, and SANDERSON J. 1973. The structure of barley roots in relation to the transport of ions into the stele. *Protoplasma* 77: 291–311.

SANDERSON J. 1983. Water uptake by different regions of the barley root. Pathways of radial flow in relation to development of the endodermis. *Journal of Experimental Botany* 34: 240–253.

SCOTT MG, and PETERSON RL. 1979a. The root endodermis in *Ranunculus acris*. I. Structure and ontogeny. *Canadian Journal of Botany* 57: 1040–1062.

SCOTT MG, and PETERSON RL. 1979b. The root endodermis in *Ranunculus acris*. II. Histochemistry of the endodermis and the synthesis of phenolic compounds in roots. *Canadian Journal of Botany* 57: 1063–1077.

THORNTON HG, and RUDORF JE. 1936. The abnormal structure induced in nodules on lucerne (*Medicago sativa* L.) by the supply of sodium nitrate to the host plant. *Proceedings of the Royal Society of London. Series B, Biological Sciences* 120: 240–252.

TJEPKEMA JD, and YOCUM CS. 1974. Measurement of oxygen partial pressure within soybean nodules by oxygen microelectrodes. *Planta* 119: 351–360.

VAN FLEET DS. 1961. Histochemistry and function of the endodermis. *The Botanical Review* 27: 165–220.

WARMBRODT RD. 1986. Structural aspects of the primary tissues of the *Cucurbita pepo* L. root with special reference to the phloem. *New Phytologist* 102: 175–192.

WITTY JF, SKOT L, and REVSBECH NP. 1987. Direct evidence for changes in the resistance of legume root nodules to O_2 diffusion. *Journal of Experimental Botany* 38: 1129–1140.

ZEIER J, GOLL A, YOKOYAMA M, KARAHARA I, and SCHREIBER L. 1999. Structure and chemical composition of endodermal and rhizodermal/hypodermal walls of several species. *Plant, Cell and Environment* 22: 271–279.