

The structure of the endodermis during the development of wheat (*Triticum aestivum* L.) roots

GRAŻYNA GRYMASZEWSKA, WŁADYSŁAW GOLINOWSKI

Department of Botany, Institute of Plant Biology, Warsaw Agricultural University,
Rakowiecka 26/30, 02-528 Warsaw, Poland

(Received: December 31, 1985. Revision accepted: April 1, 1986)

Abstract

Four stages of development in the process of differentiation of the root endodermis of wheat (*Triticum aestivum* L. var. Grana) are described. The proendodermis cells have a meristematic nature. Their vacuoles accumulated an osmophilic material. In the next stage, Casparian strips arose in the walls of the endodermis. Dictyosomes and ER cisterns were numerous in the protoplasts of these cells. In the following stage, a suberin lamella was deposited over the entire internal surface of the primary cell wall. In the final stage, a secondary cell wall, thickened in the form of a letter U, was formed. In secondary wall simple pits arose. The endodermis of the wheat root developed asynchronously, more quickly over the phloem bundles.

Key words: root, wheat, differentiation, endodermis

INTRODUCTION

The endodermis, which is located on the border between the cortical parenchyma and the vascular tissues in root, serves several important functions (Van Fleet 1961, Clarkson and Robards 1975). Hence the reason why its ultrastructure, ontogeny (Bonnett 1968, Haas and Carothers 1975, Scott and Peterson 1979a), histochemical properties (Van Fleet 1961, Scott and Peterson 1979b) and interrelationships with other tissues and root functions (Clarkson et al. 1971, Harrison-Murray and Clarkson 1973, Karas and McCully 1973, Robards et al. 1973) have been under study for a long time. In spite of the wide interest in this tissue, many of the problems involved both in its structure as well as function have not yet been sufficiently resolved. This study presents the changes occurring in endodermis cells during its differentiation in wheat root.

MATERIAL AND METHODS

Wheat grains (*Triticum aestivum* var. Grana) were sprouted in the dark at 26°C on filter paper moistened with distilled water. Three-day-old seedlings were transferred for the next 7 days to pots filled with perlite and watered with Hoagland's medium. In addition, extra information was obtained from observations on 3-week-old plants. Roots which were to be examined in the light microscope, were fixed in FAA and embedded in paraffin. By using a rotary microtome, series of cross sections, 14 µm thick, and longitudinal sections, 8 µm thick, were obtained from entire roots. The slides were stained with safranin and fast green. Color reactions for localizing phenolic compounds were carried out on sections of fresh roots, made by cutting with an anatomic razor blade. Used in these reactions were: a solution of aniline with KJO_3 (Mace 1963), FeCl_3 , Mitchell's reagent and the nitroso-reaction (Turowska et al. 1970). The pieces of roots to be used for ultrastructural examination were fixed in a mixture of 3% glutaraldehyde and 4% paraformaldehyde in 0.1 M cacodylan buffer, pH 7.2 (Karnovsky 1965) at room temperature. The material was post-fixed in 1% OsO_4 at 4°C. The fixed material was dehydrated in a rising series of alcohol, acetone and propylene oxide concentrations. Next, they were embedded in Epon 812 (Luft 1961) and cut on an LKB microtome. The sections were stained with uranyl acetate and lead citrate and examined in an JEM-100C microscope. These studies were done exclusively on seminal roots.

RESULTS

The proendodermis differentiated very early in the wheat root, just above the initials. Its cells underwent radial and tangential divisions (Fig. 1) leading to increasing the number of layers of cortical parenchyma. The proendodermis cells were tangentially elongated (Fig. 1) and had delicate cellulose walls, through which plasmodesmata passed (Fig. 3). Numerous mitochondria, ribosomes, Golgi apparatuses, plastids and large lipid bodies were found in their dense cytoplasm (Figs. 2-5). The plastids had a poorly developed system of internal membranes. Osmophilic plastoglobules and starch grains were formed in them (Fig. 3). In a short time, the volume of the vacuoles increased, and on osmophilic, flocculent and granular material appeared inside of them (Figs. 4, 5). The color reactions done on fresh sections, intended to discover phenolic compounds in the proendodermis, did not give positive results.

In the next stage of ontogeny, Casparian strips were formed in the endodermis cell walls (Figs. 6-8). The strips were formed earlier in the

cells located across from the phloem bundles. They took up about 1/3 of the radial wall length and were shifted towards the border with the pericycle (Fig. 6).

The cell wall over the segment taken up by the strip was homogenous, had an increased electron density and smooth surface, to which the plasma membrane tightly adhered (Fig. 8). Such tight adhesion was not observed elsewhere over the circumference of the cell.

In the cytoplasm of the cells with strips (Figs. 6–8), ribosomes, Golgi apparatuses, mitochondria and rough endoplasmic reticulum arranged parallel to the walls, were found. The plastids were not numerous. Only a few of them contained starch grains. The lipid bodies observed in the pro-endodermis were rarely seen at this stage of development. The major part of the volume of the cells with Casparian strips was taken up by a large, centrally positioned vacuole. In the region of the strips (Figs. 8) in the cytoplasm or just next to the plasma membrane, numerous membrane-bound vesicles filled with a granular, flocculent or homogenous content, occurred. Between the plasma membrane and cell wall, on both sides of the strip or in the neighbouring cytoplasm, myelin configurations were also often found.

After the Casparian strips were formed in the endodermis, the suberin lamella and secondary wall were formed successively. The suberin lamella (Figs. 9–11) was deposited over the entire surface of the primary cell wall. It arose, similarly as the secondary cell wall, earlier in the cells neighbouring the phloem bundles. Initially, the suberin lamella was thin, homogenous. As it matured, small osmophilic plates, parallel to the surface, and membrane fragments (Fig. 11) appeared in it, and its thickness increased as the result of deposition of new strips of an electron-dense substance (Fig. 9). The lamella was usually a bit thicker near the Casparian strips, and had a convoluted surface. In mature cells, already having a secondary cell wall, two regions could be distinguished in the suberin lamella. The more external one was lighter, homogenous.

The secondary cell wall first arose as a thin layer over the entire cell, then thickened in the shape of the letter U. Initially, only rare aggregates of an osmophilic substance (Fig. 12) occurred in the secondary cell wall. Gradually, layers with differently oriented cellulose fibers became discernable, and the amount of osmophilic material increased, especially in the deeper regions (Fig. 14). Simple pits, through which plasmodesmata passed (Fig. 13), arose in the thickened secondary cell wall. In mature endodermis cells, this secondary cell wall stained pink with safranin.

In cells with a suberin lamella, and also later in those with a secondary cell wall (Fig. 12), ER cisterns arranged parallel to the walls and filled with an electron dense material, were mainly found, as were mitochondria and ribosomes. Less numerous Golgi apparatuses and plastids lacking

PLATE I

Fig. 1. A cross section through the meristematic region of a wheat root at 180 μm from the meristem apex. Proendodermis cells (pEn) undergo tangential divisions (arrow) which leads to an increase in the number of cortical parenchyma layers. 338 \times

Figs. 2–5. The ultrastructure of proendodermis cells. Fig. 2 — A diagram of the ultrastructure of proendodermis cells. The primary cell wall is marked by dots. Fig. 3 — The following can be seen in the cytoplasm of a proendodermis cell: cell nucleus (Nu), mitochondria (Mi), plastids (Pa), with starch grains, Golgi apparatuses (Ga), endoplasmic reticulum (ER) and ribosomes. Va — vacuole, Pd — plasmodesmata. 19000 \times . Fig. 4 — A fragment of a proendodermis protoplast. Lipid bodies (Li) are visible in the cytoplasm. Va — vacuole, 19000 \times . Fig. 5 — A fragment of a proendodermis protoplast. An osmophilic, granular material is visible in the vacuole (Va). 19000 \times

PLATE II

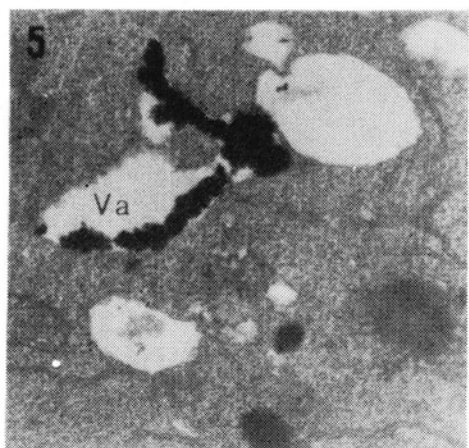
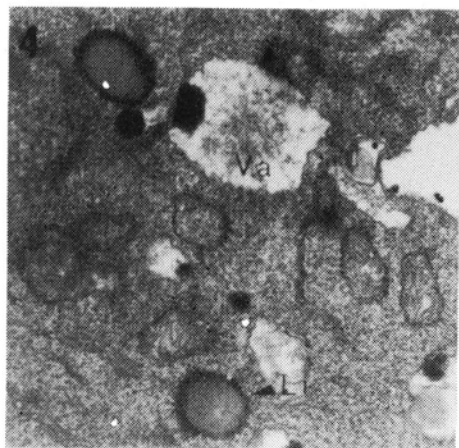
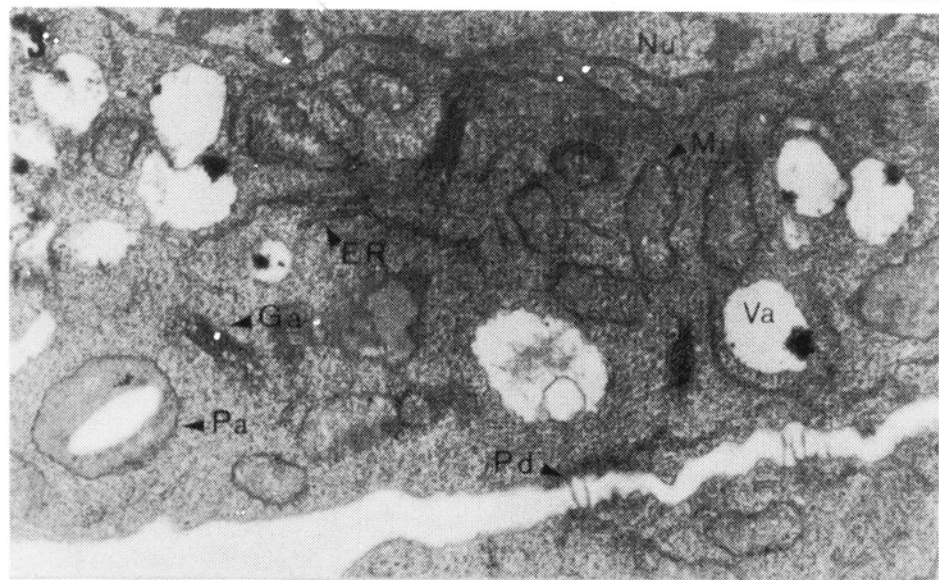
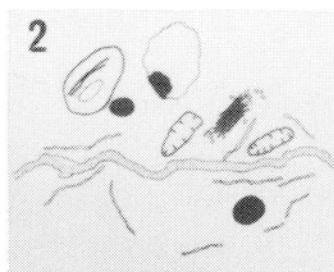
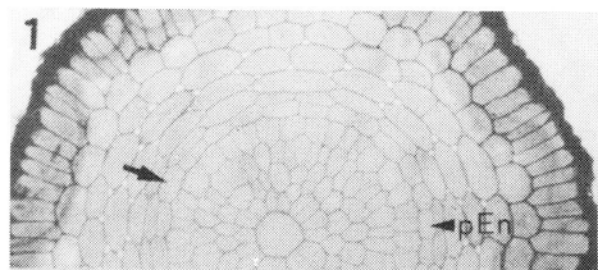
Figs. 6–8. The ultrastructure of endodermis cells with Casparian strips. Fig. 6 — A strip localized in the radial wall of an endodermis cell is marked with an asterisk. Pa — plastid, Ga — Golgi apparatus, Mi — mitochondrion, ER — endoplasmic reticulum. Va — vacuole. 9500 \times . Fig. 7 — A diagram of the structure of endodermis cells with Casparian strips. The primary cell wall containing the strip is marked by dots. Fig. 8 — An enlarged fragment from Fig. 6 of radial walls containing a Casparian strip. The area of the strip is marked by arrows. The plasma membrane adheres to the cell wall over the entire length of the strip. In the neighbouring cytoplasm, various vesicles (Ve) and myelin configurations (Mym) are numerous. 38000 \times

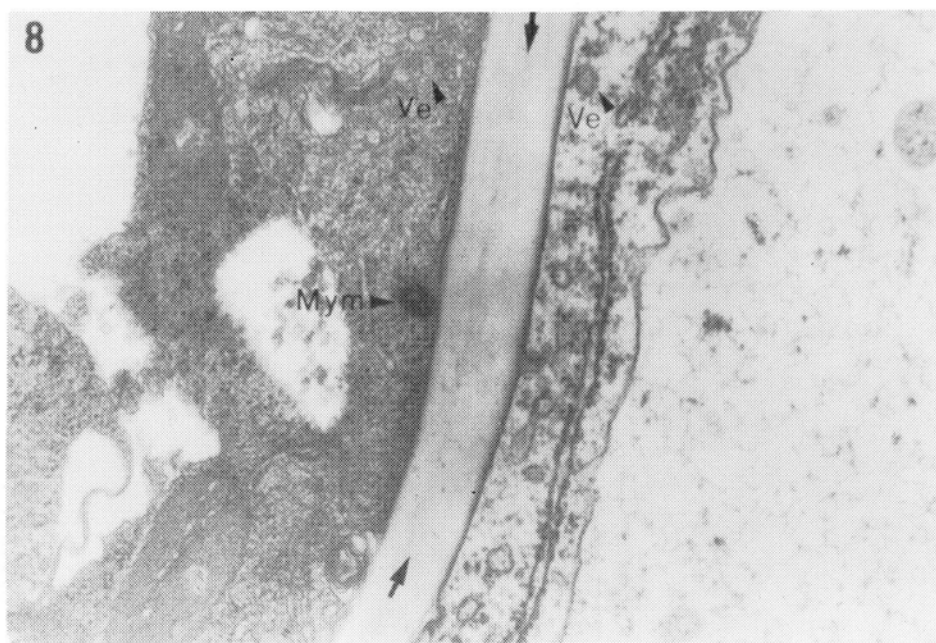
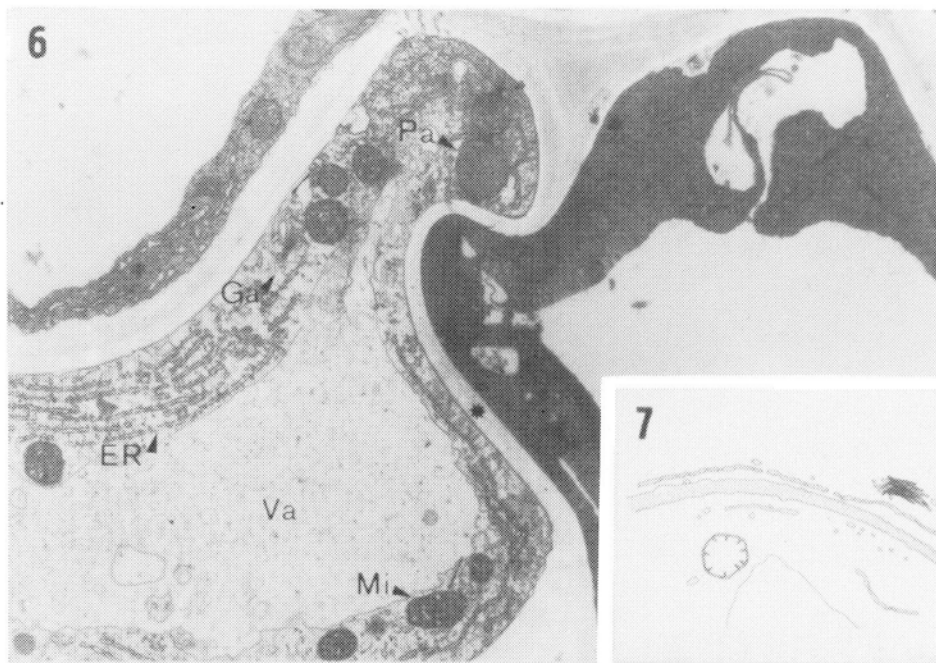
PLATE III

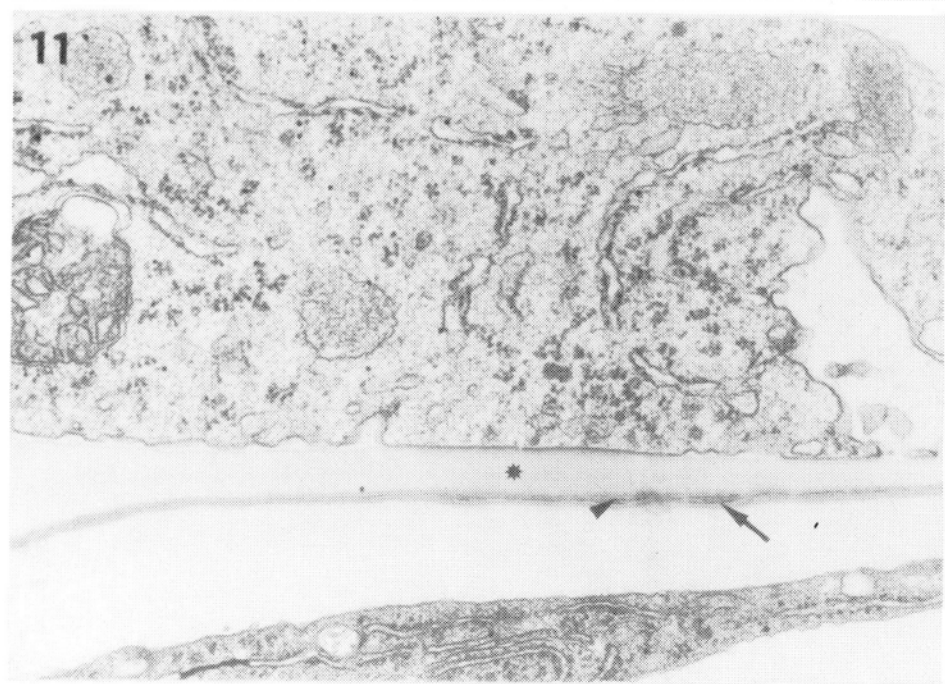
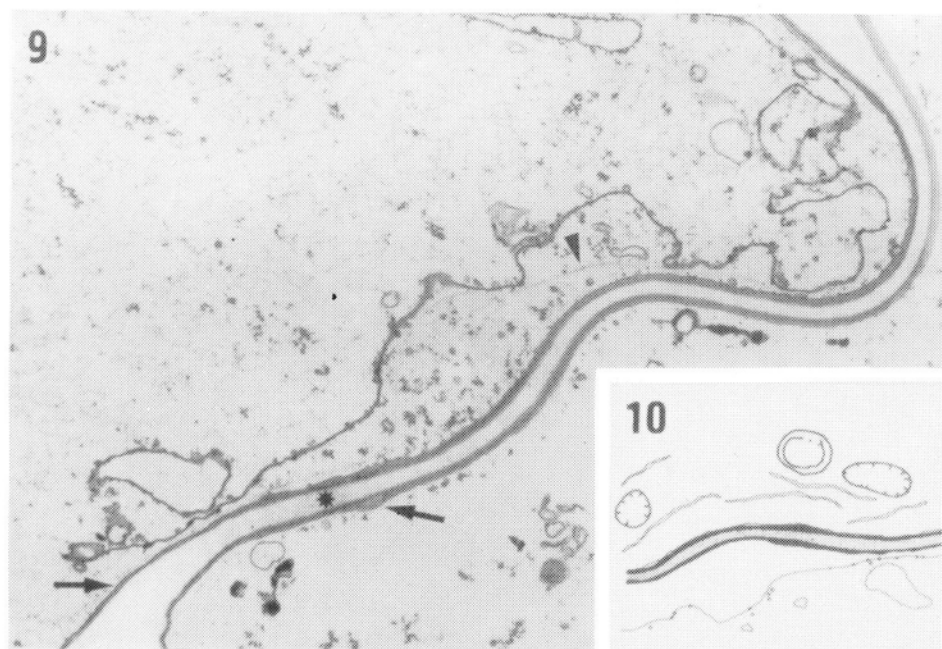
Figs. 9–11. The ultrastructure of endodermis cells with a suberin lamella. Fig. 9 — Radial walls of endodermis cells with a suberin lamella (arrows) on their surface. A strip of an electron-dense substance (arrow head) is visible between the plasma membrane and suberin lamella. The Casparian strip is marked by an asterisk. 19000 \times . Fig. 10 — A diagram of the ultrastructure of endodermis cells with a suberin lamella. The suberin lamella is marked by a thick black line. The primary cell wall is marked by dots. Fig. 11 — Parts of neighbouring endodermis cells during different stages of development. In the lower cell, on the surface of the primary cell wall, the suberin lamella and secondary cell wall are visible. Osmophilic platelets (arrow) and membrane fragments (arrow head) are embedded in the suberin lamella. The Casparian strip region is marked with an asterisk. 38000 \times

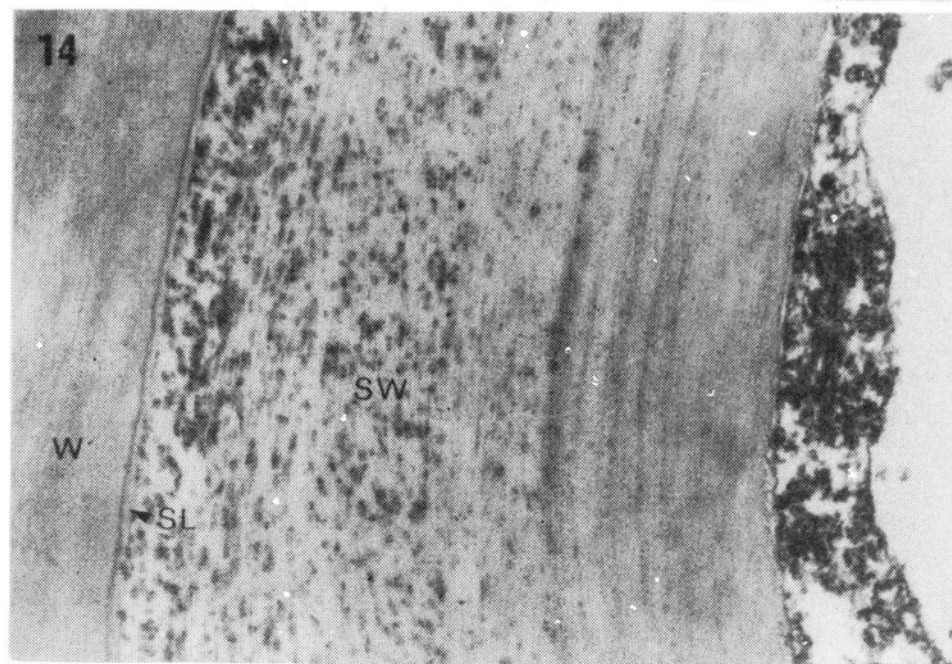
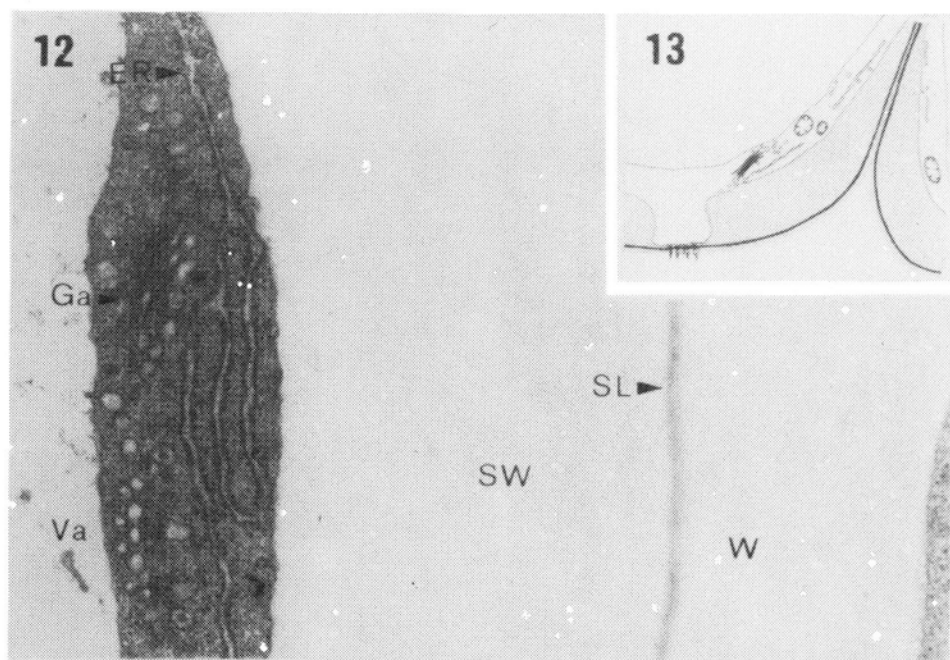
PLATE IV

Figs. 12–14. The ultrastructure of endodermis cells with a secondary cell wall. Fig. 12 — Visible in the cytoplasm of an endodermis cell with a thickened secondary cell wall (SW) are: rough endoplasmic reticulum (ER), Golgi apparatus (Ga) and vesicles with a varied content and size. Small vesicles are also found between the plasma membrane and cell wall. SL — suberin lamella, Va — vacuole, W — endodermis primary cell wall and wall of the neighbouring pericycle cell. 38000 \times . Fig. 13 — A diagram of the structure of an endodermis cells with a secondary cell wall. The primary and secondary cell walls are marked with dots. The suberin lamella between them is marked by a thick black line. Fig. 14 — A fragment of the internal tangential wall of a mature endodermis cell. The surface of the primary wall is covered by the suberin lamella (SL) and a thick secondary cell wall (SW), in which an osmophilic material is seen to be accumulating. The primary cell walls of the endodermis cell and of the neighbouring pericycle cell are jointly marked by the letter W. 38000 \times









starch grains were seen. In the neighbourhood of the ER and Golgi apparatuses, often next to the plasma membrane or between it and the cell wall, numerous, membrane-bound vesicles and, sometimes, multivesicular bodies were found. The sizes and contents of the vesicles were very diverse.

DISCUSSION

In wheat roots, as in many mono- and dicotyledons, the following stages could be discerned during the development of the endodermis: stage I, during which Casparian strips were formed in its walls, stage II, when the suberin lamella was formed on the internal surface of the primary cell wall, and stage III, when the secondary cell wall arose.

The proendodermis cells had an appearance typical for meristematic cells, and in reality, only their shape and localization similar to that in barley roots (Robards et al. 1973) allowed this tissue to be identified early. The osmophilic material found in the vacuoles had a similar appearance as the granular deposits in young endodermis cells from cotton roots, taken by Mueller and Beckman (1976) to be phenolic compounds. The presence of phenols is, according to Van Fleet (1961), what enables the endodermis to be identified by histochemical methods in the very early stages of ontogeny. In wheat, however, the color reactions done on fresh, unfixed material, did not confirm the presence of phenols in the proendodermis. Phenols also did not occur in the proendodermis of *Ranunculus acris* (Scott and Peterson 1979a).

The presence of Casparian strips in the radial walls of the endodermis was indicated by the smooth surface of the wall in this region, its increased electron density and tight contact with the plasma membrane. These traits are characteristic for the Casparian strip region in other plants also (Robards et al. 1973, Haas and Carothers 1975, Scott and Peterson 1979a). The characteristic adherence of the plasma membrane to the cell wall over the length taken up by the Casparian strip is interpreted by Bonnet (1968) to be the result of interactions between the hydrophobic, suberin-containing wall and the hydrophobic components of the plasma membrane. Similar views are shared by Haas and Carothers (1975) and Scott and Peterson (1979a).

Very little is known about the role played by organelles in the formation of Casparian strips. Bonnett (1968) and Haas and Carothers (1975) did not find any relationship between the strips and the organelles in the cytoplasm. According to Robards et al. (1973), the only indication that a Casparian strip is being formed in the cell wall is the presence of microtubuli. Scott and Peterson (1979a) suppose that the material needed

to expand the strips is supplied by paramural and multivesicular bodies which arise from Golgi apparatuses and lipid inclusions in the cytoplasm. In wheat, ER cisterns and Golgi apparatuses arranged mainly parallel to the cell wall were observed in the cytoplasm neighbouring the Casparian strips. Maybe they are the source of the membrane-bound vesicles with various contents, which are incorporated into the cell wall in the vicinity of the strips, or located in their immediate neighbourhood.

In fact, it is unknown which organelles participate in the formation of the suberin lamella. Haas and Carothers (1975) did not observe any particular spatial relationships between organelles and the suberin lamella. Scott and Peterson (1979a) suggest the participation of the ER in the transport of suberin precursors to endodermis walls. Our observations also indicate that the ER cisterns, often situated just next to the plasma membrane and surrounded by numerous vesicles, may play a role in the formation of this lamella.

The secondary cell wall formed in the endodermis of wheat roots, thickens in the shape of the letter U, similarly as in other grasses (Robards et al. 1973, Haas and Carothers 1975). According to Clarkson et al. (1971), in barley, the osmophilic material which accumulates in the secondary cell wall of the endodermis, is probably suberin or polyphenols. Aggregations of a similar, osmophilic material are also found in wheat. Color reactions did not, however, reveal that they were phenolic compounds. Only the reaction with safranine indicated that lignin is contained in the secondary cell wall. Lignin is also present in the endodermis secondary cell wall of *Ranunculus acris* roots (Scott and Peterson 1979b).

In wheat roots, similarly as in *Ranunculus acris* (Scott and Peterson 1979a), the endodermis cells did not mature uniformly. Those which were located across from the phloem bundles matured quicker. At the base of 10-day-old roots, cells with a thick secondary cell wall and ones with a wall just beginning to be formed, were found on one level. These cells touched cells having their primary cell wall covered only with a suberin lamella or having only a thin primary wall with Casparian strips. These latter cells, called passage cells, were found only across from protoxylem vessels. In time, these cells also, as in barley (Robards et al. 1973), attained full maturity. Passage cells have not been observed in maize also (Haas and Carothers 1975). According to Clarkson et al. (1971), in the older parts of barley roots, there is no more than one passage cell per thousand endodermis cells.

In the situation in which the modifications which the endodermis cell walls undergo during maturation cause this tissue to become a barrier to apoplastic transport, the authors of many studies combining ultra-structural observations with physiological studies (Clarkson et al. 1971,

structural observations with physiological studies (Clarkson et al. 1971, Harrison-Murray and Clarkson 1973, Robards et al. 1973, Clarkson and Robards 1975) indicate that the main connection between the cortex and stele, allowing symplastic transport to remain continuous, are the plasmodesmata.

REFERENCES

- Bonnett H. T., 1968. The root endodermis: fine structure and function. *J. Cell Biol.* 37: 199–205.
- Clarkson D. T., Robards A. W., Sanderson J., 1971. The tertiary endodermis in barley roots: fine structure in relation to radial transport of ions and water. *Planta* 96: 292–305.
- Clarkson D. T., Robards A. W., 1975. The endodermis, its structural development and physiological role. In: *The development and function of roots*. Torrey J. G., Clarkson D. T. (eds.), Academic Press, London-New York-San Francisco, pp. 415–436.
- Haas D. L., Carothers Z. B., 1975. Some ultrastructural observations on endodermal cell development in *Zea mays* roots. *Amer. J. Bot.* 62: 336–348.
- Harrison-Murray R. S., Clarkson D. T., 1973. Relationships between structural development and absorption of ions by the root system in *Cucurbita pepo*. *Planta* 114: 1–16.
- Karas I., Mc Cully M. E., 1973. Further studies of the histology of lateral root development in *Zea mays*. *Protoplasma* 77: 243–269.
- Karnovsky M. J., 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell Biol.* 25: 137A.
- Luft J. H., 1961. Improvements in epony resin embedding methods. *J. Biophys. Biochem. Cytol.* 9: 409.
- Mace M. E., 1963. Histochemical localization of phenols in healthy and diseased banana roots. *Physiol. Plant.* 16: 915–925.
- Mueller W. C., Beckman C. H., 1976. Ultrastructure and development of phenolic-storing cells in cotton roots. *Can. J. Bot.* 54: 2074–2082.
- Robards A. W., Jackson S. M., Clarkson D. T., Sanderson J., 1973. The structure of barley roots in relation to the transport of ions into the stele. *Protoplasma* 77: 291–311.
- Scott M. G., Peterson R. L., 1979a. The root endodermis in *Ranunculus acris*. I. Structure and ontogeny. *Can. J. Bot.* 57: 1040–1062.
- Scott M. G., Peterson R. L., 1979b. The root endodermis in *Ranunculus acris*. II. Histochemistry of the endodermis and the synthesis of phenolic compounds in roots. *Can. J. Bot.* 57: 1063–1077.
- Turowska I., Kohlmünzer S., Molik-Węgiel J., 1970. *Skorowidz fitohistochemiczny*. AM, Kraków.
- Van Fleet D. S., 1961. Histochemistry and function of the endodermis. *Bot. Rev.* 27: 165–220.

Struktura endodermy w rozwoju korzenia pszenicy
(*Triticum aestivum* L.)

Streszczenie

W procesie różnicowania endodermy w korzeniach *Triticum aestivum* L. var. Grana opisano cztery stadia rozwojowe. Komórki proendodermy mają charakter merystematyczny. W ich wakuolach gromadzi się materiał osmofilny. W następnym stadium, w ścianach radialnych endodermy powstają pasemka Caspary'ego. W protoplaście tych komórek licznie występują cysterny ER i diktiosomy. W dalszym stadium, na całej wewnętrznej powierzchni ściany komórek endodermy odkłada się blaszka suberynowa. W końcowym etapie tworzy się podkowiasto zgrubiała ściana wtórna. W ścianie wtórnej powstają jamki proste. Endoderma w korzeniu pszenicy rozwija się asynchronicznie, szybciej nad wiązkami łykowymi.