ULTRASTRUCTURE OF *GLADIOLUS X HYBRIDUS* ROOT ENDODERMIS

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ABSTRACT

Gladiolus x hybridus Van Houtte roots had primary, secondary and tertiary endodermis depending on the distance from the root apex. Free calcium ions were localized with the method of Slocum and Roux (1982). Electron microscopic observations revealed that electron dense calcium antimonite deposits were localized in some endodermis cells. Endodermis cells situated above xylem elements were rich in calcium antimonate deposits in contrary to those situated above the phloem elements and passage cells. The cells rich in calcium ions were present both in endodermis II and III stage of development.

Localization of calcium deposits was on plasma membrane and tonoplast. There were also numerous calcium antimonate deposits in vacuoles. Dictyosome's cisterns and Golgi derived vesicles show presence of calcium ions. Calcium rich endodermis cells had plastids with antimonate deposits present in their tylacoids lumen. Plasmodesmata looked the same in all endodermis cells, but those from calcium rich endodermis cells had calcium antimonate deposits in the lumen of plasmodesmata.

KEY WORDS: root endodermis, calcium uptake, electron microscopy.

INTRODUCTION

The root endodermis is a unicellular, continuous layer of cells separating the cortex from the stele (Esau 1977). Depending on species, the anatomical development of the root endodermis may pass through three stages: the primary endodermis (stage I) with Casparian bands in their radial cell walls; the secondary endodermis (stage II) with suberin lamella deposited on the internal surface of the primary cell wall; and the tertiary endodermis (stage III) with secondary lignified cell walls thickenings mainly on the radial and inner tangential surfaces. In the last stage the passage cells are present (Peterson and Enstone 1996). The primary and secondary endodermis is typical for dicotyledoneous species roots while tertiary endodermis is present much often in monocotyledonous species roots (Zeier and Schreiber 1998).

The endodermis plays an important physiological function as a barrier in the passive apoplastic diffusion of water and minerals across the root cortex (Nagahashi et al. 1974; Peterson et al. 1993), and also as a place of the active transport of water and ions to the stele (Kundt 1998). Clarkson (1984) proposed the model of calcium uptake, in which an important role is played by the endodermis with Casparian bands. The calcium channels and the energy-dependent calcium pumping units, situated on plasma membrane, were involved in pumping calcium ions

through endodermis cell protoplast, which was the way to transfer calcium to the stele omitting Casparian bands. The other types of endodermis have all cell walls impermeable for water and calcium and this is reason why Clarkson model cannot explain the transport by these types of endodermis. The examinations of the structure of endodermis and calcium ions distribution were helpful for understanding the processes of water and minerals uptake by roots, especially since calcium ions play an important role in water uptake (Quintero et al. 1999).

In the present study the calcium ions distribution in endodermis of *Gladiolus x hybridus* roots was examined in relation to the structure of secondary and tertiary endodermis.

MATERIAL AND METHODS

Roots of *Gladiolus x hybridus* Van Houtte corms were examined. There were two kinds of roots growing from the corms. The thick contradicle roots, which were not examined and ordinary ones. Among the latter there were the roots growing directly from the corm and lateral roots. Both the main roots and the lateral ones were taken. The root length was from 15 to 20 cm. The samples were taken at 1, 2, 3, 4, 5 and 15 cm from root apex.

Specimens for electronmicroscopical examination were fixed in a mixture of 3% glutaraldehyde and 4% paraformal-

dehydrate in cacodylate buffer (pH = 7.2), for 4 h. Postfixation in 1% osmium tetroxide for 2 h at 4°C was applied. The material was dehydrated in the concentration gradients of ethanol (10–100%), propylene oxide and embedded in Epon. The ultrathin sections were placed on copper grids, and stained with uranyl acetate and lead citrate (Reynolds 1963).

Subcellular calcium localization was analyzed according to the method of Slocum and Roux (1982) with minor modifications (Jian et al. 1997). Specimens for electron-microscopical calcium localization were fixed in a mixture of 2.5% (v/v) glutaraldehyde with 2% potassium antimonate in 0.1 M K-phosphate buffer (pH = 7.6), for 12 h in 4°C. Postfixation in 1% osmium tetroxide with 2% potassium antimonate in 0.1 M K-phosphate buffer (pH = 7.6) for 2 h at 4°C was applied. The same fragments of roots, fixed and postfixed in the same mixtures, but without potassium antimonate were used as a reaction control.

In order to confirm that the dark deposits present in the specimens contain Ca^{2+} , chelating of calcium ions with ethylene glycol-bis N, N, N', N'-tetraacetic acid (EGTA) was performed. The ultrathin sections, on copper grids, were immersed in 100 mM EGTA (pH 8.0), incubated for 1 h at 60°C and stained with uranyl acetate and examined.

Observations were performed with the aid of the JEM 1220 electron microscope (Jeol, Japan).

RESULTS

The main difference noticed between roots growing directly from the corm and the lateral ones was in the xylem

structure. The main roots had sclerenchyma cells in the middle of the stele, while the lateral ones had parenchyma cells with thin walls and big intercellular spaces in this location. Xylem vessels of the lateral roots had smaller lumen than vessels from the main roots (Fig. 1). Both types of the roots had primary, secondary (Fig. 2) and tertiary endodermis depending on the distance from the root apex. Also stages intermediate between secondary and tertiary endodermis were observed (Fig. 3). In hair zone there was tertiary endodermis.

In the secondary endodermis cells rich in calcium antimonate deposits were observed. They were situated exactly opposite to xylem vessels and for one xylem strand there was only a single such an endodermis cell. In stages intermediate between secondary and tertiary endodermis cell walls were thickened not evenly. In some cells this process began earlier than in others. Wall thickening of the endodermis cells rich in calcium antimonate deposits, began earlier than in other endodermis cells.

Tertiary endodermis was composed of typical cells and passage cells adjacent to xylem elements. While the typical cells had radial and outer tangential cell walls thickened, the passage cells had all their cell walls thin. The typical cells situated adjacent to xylem strands exhibited calcium antimonate deposits. On the contrary, endodermis cells adjacent to phloem had almost no deposits of calcium antimonate (Fig. 6, 20). In the root hair zone the number of cells rich in calcium antimonate deposits vary from one to three. The calcium-rich cells had a different shape (Fig. 1) than the other endodermis cells, although they also had thickened cell walls. In the older parts of the, root above the hair zone, most of endodermis cells were calcium positive.

Fig. 1. A cross section through the stele of gladiolus root at the level of tertiary endodermis. The endodermis cells rich in calcium deposits are marked by arrows. The number of cells rich in Ca^{2+} deposits situated adjacent to xylem (xylem vessels – X) varies from one to three per one xylem strand. Bar – 20 μm .

Fig. 2. Endodermis cells with suberin lamella (sl) on the internal surface of the primary cell wall (pw). Calcium deposits are present on the plasma membrane (arrowheads) and in plastids (P) envelope. Bar – 200 nm.

Fig. 3. Endodermis in the stage when secondary thickening (sw) of the wall has just begun. Calcium antimonate deposits are present on the plasma membrane, tonoplast and in the vacuole (V). Bar – 500 nm.

Fig. 4. The tertiary endodermis cell with secondary cell wall (SW). Calcium antimonate deposits present on plasma membrane, tonoplast and in vacuole (V). Bar – 500 nm.

Fig. 5. An endodermis cell with secondary cell walls (SW). Calcium antimonate deposits are present on the plasma membrane, tonoplast and in the vacuole (V). No deposits in mitochondria (M). Bar – 500 nm.

Ordinary tertiary endodermis cells stained with uranyl acetate and lead citrate looked the same. The central vacuole was situated in the middle of the cell and cytoplasm had small mitochondria, plastids and short endoplasmatic reticulum cisterns. The radial and inner tangential walls were thickened and had the suberin lamella. The calcium

antimonate deposits were present on plasma membrane and tonoplast (Fig. 4, 5, 11-14). Also vacuoles of the cells had a lot of deposits situated specially near the tonoplast. In protoplast of the cell plastids were strongly marked (Fig. 8-10). The space between outer and inner membrane of plastid envelope and tylacoids lumen were rich in calcium an-

Fig. 6. A plastid from the tertiary endodermis calcium poor cell. No deposits are visible. Bar – 200 nm.
Fig. 7. A plastid from secondary endodermis cell. The space between outer and inner membranes of plastid envelope and within the tylacoid lumen are filled with calcium deposits. Bar – 200 nm.
Fig. 8, 9. A plastids from the calcium rich tertiary endodermis cell. Calcium deposits are present in the space between outer and inner membranes of plastid envelope and in the tylacoid lumen. Bars – 200 nm.
Fig. 10. A part of the plastid from tertiary endodermis cell. Calcium antimonate deposits are present within tylacoid. Bar – 100 nm.

timonate deposits. Another part of protoplast exhibiting a positive reaction for Ca^{2+} was the maturing face cisterns of Golgi apparatus and vesicles derived from them (Fig. 11, 12). Antimonate staining was present in few places in cytoplasm, but neither in endoplasmatic reticulum nor in mitochondria. A similar location of calcium antimonate deposits was observed in secondary endodermis cells rich in

calcium deposits (Fig. 2). However, the plastids were smaller and had a less extended tylacoid system (Fig. 7).
Plasmodesmata joining endodermis cells and passage cells with cortex parenchyma cells, and endodermis with pericycle cells were observed. These plasmodesmata differ, because the structure of the cell walls is different. Plasmodesmata in the interface between passage cells and

Fig. 11, 12. Golgi apparatus from the tertiary endodermis cells. Calcium deposits in dictyosome cistern lumen and cistern derived vesicles (arrowheads). Bars – 200 nm.

Fig. 13, 14. Calcium deposits around plasma membrane of tertiary endodermis cell. Bars – 200 nm.

cortex parenchyma cells or pericycle, cross the thin and not modified walls and have thin and narrowed canals. The plasmodesmata in the interface between endodermis cells and pericycle cells were situated in very thick and modified cell walls of endodermis and in this region their canal was thicker and twisted. The suberin lamella was much thicker around the plasmodesmata and the plasmodesmata were thin and narrow in that region. Although plasmodesmata joining the endodermis cells with pericycle cells were all of the same structure in calcium antimonate deposits rich and poor cells, but the endodermis cells with calcium antimonate deposits in its protoplast had pla-

smodesmata strongly marked by calcium antimonate (Fig. 15-19). On the contrary, plasmodesmata from endodermis cells adjacent to phloem were not exhibiting calcium antimonate deposits (Fig. 20).

The passage cells showed little calcium antimonate deposits in their protoplasts. The deposits in endodermis cells situated adjacent to phloem and in passage cells were similar in size and shape.

The samples treated with EGTA showed no electron dense deposits, but transparent holes indicating where the calcium antimonate deposits were chelated by EGTA (Fig. 21, 22). There were dark deposits only in secondary cell

Fig. 15-19. Plasmodesmata from calcium rich tertiary endodermis cells. Dark deposits of calcium antimonate are present where they pass through the secondary cell wall. Bars – 200 nm.
Fig. 20. A plasmodesm from calcium poor endodermis cell. The lumen without calcium deposits. Bar – 200 nm.

wall, situated exclusively near the suberin lamella, and these were not chelated by EGTA (Fig. 13).

DISCUSSION

The investigations of *Gladiolus* root endodermis structure revealed that there were three distinct kinds of cells composing the endodermis: the passage cells and two kinds of endodermis cells with thickened cell walls. The difference between these two kinds of cells with thickened walls

consisted in the presence of calcium ions in their protoplast. Similar observations of differences in calcium level in endodermis cells were reported by Hodson and Sangster (1993). They showed that calcium is present at higher levels in *Sorghum bicolor* root endodermis than in surrounding tissues, and that some endodermis cells accumulate more Ca^{2+} ions than others.

The most intensive calcium uptake and transport take place in the apical part of roots (Haussling et al. 1988; Robards et al 1973). The endodermis of this region not only plays an important role as a barrier in apoplasmic transport

Fig. 21. Tertiary endodermis calcium rich cell. The sample treated with EGTA. Transparent holes indicate where the Ca^{2+} deposits were chelated by EGTA (star 1 on plasma membrane surface, star 2 – on tonoplast and star 3 – in vacuole). Bar – 500 nm.

Fig. 22. Plasmodesma from tertiary endodermis calcium rich cell. The sample treated with EGTA. Transparent holes in a plasmodesma (arrow heads) and along plasma membrane (star) indicating the places where calcium antimonate deposits were present. Bar – 200 nm.

of water and ions on the rim of cortex and stele, but also is a place where the pumping mechanism is located (Kundt 1998). The observed calcium-rich endodermis cells were situated adjacent to xylem elements, and thus they were supposed to be directly involved in transport of ions and water to the xylem.

Zeier and Schreiber (1998) analysed the chemical composition of tertiary endodermis walls isolated from the roots of five monocotyledonous species. They concluded that the barrier properties of the endodermis against the apoplastic transport of ions and water would increase from primary to tertiary endodermis, due to their walls chemical composition, so the transport in tertiary endodermis must be dependent on ability of symplasmic transport by plasmodesmata. The possible role of plasmodesmata in radial water and ions transport in plant roots was suggested by Kitorova and collaborators (1998). The inhibitor of plasmodesmata transport used by these authors decreased the exudation rate from pumpkin roots while the solute and water flows from wheat root to the shoot were completely blocked. The difference between those two plant roots is in the endodermis structure: wheat being a grass-family member has a typical tertiary endodermis. Also Ma and Peterson (2001) observed a constantly high plasmodesmata frequency in endodermis cells of onion roots, and they suggested that these plasmodesmata are involved in water and ions' transport to the stele.

The model of calcium uptake by roots proposed by Clarkson (1984) assumes an important role of endodermis with Casparian bands in these process. There, the presence of calcium channels arranged in outer tangential plasma membrane position and in the radial externally to Casparian bands, as well as the presence of energy dependent calcium transporters in the internal part of plasma membrane pumping calcium through endodermis, play the main role. The classic Clarkson's model of calcium transport through endodermis cells with Casparian bands does not explain the similar process in endodermis with thick lignin walls, as well as endodermis with suberin lamella, because these cell walls are situated on the way of calcium efflux to the stele and are a barrier in apoplastic transport. Only passage cells fulfil the function of calcium uptake, described by that model (Peterson and Enstone 1996). The presence of calcium-rich endodermis cells, situated directly opposite the xylem elements, suggests that they are involved in calcium transport to the stele, but this transport must go through plasmodesmata as the only possible passage.

However, a high level of cytosolic free ions of calcium is harmful and can result in a metabolic dysfunction. Thus, an active Ca^{2+} transport system on cellular membranes is necessary to obtain homeostasis. The presence of Ca^{2+} pumps in plasma membrane (Briskin 1990; Williams et al. 1990; Thomson et al. 1993; Rasi-Caldogno 1995) and tonoplast (Fukumoto and Venis 1986; Du Pont and Morris-

sey 1992; Pfeiffer and Hager 1993) was reported and a high level of calcium ions, both in plasma membrane and in tonoplast of endodermis cells, can be a sign of the active process to prevent the damages due to the high Ca^{2+} level.

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STRUKTURA KOMÓREK ENDODERMY KORZENIA *GLADIOLUS X HYBRIDUS* VAN HOUTTE

STRESZCZENIE

Endoderma korzeni *Gladiolus x hybridus* Van Houtte w strefie włośnikowej znajduje się w III stadium rozwoju. W partiach korzenia bliższych jego wierzchołkowi obserwowano wcześniejsze stadia rozwoju endodermis w tym również stadium II z lamellą suberynową. Przeprowadzono lokalizację Ca^{2+} za pomocą reakcji z antymonianem potasu. Komórki endodermis umieszczone na wysokości wiązek drewna charakteryzują się dużymi depozytami antymonianu wapnia w odróżnieniu od komórek endodermis występujących na wysokości wiązek łyka, jak i komórek przepustowych. Takie bogate w jony wapnia komórki znajdowano we wszystkich etapach rozwoju endodermis.

Komórki bogate w Ca^{2+} posiadają depozyty wapniowe zlokalizowane na plazmalemmie i tonoplaście komórek. Ponadto obserwowano rozległe depozyty Ca^{2+} w wakuoli. W protoplaście obserwuje się depozyty występujące w cysternach diktiosomów i pęcherzykach aparatu Golgi'ego. Plastydy tych komórek mają liczne depozyty antymonianu wapniowego w świetle tylakoidów oraz w przestrzeni między dwoma błonami otoczki plastydu. Chociaż plasmodesmy występujące w komórkach endodermis bogatych i ubogich w Ca^{2+} nie różnią się wyglądem, to te z komórek bogatych w depozyty wapniowe mają strąty Ca^{2+} w części plasmodesmy przebiegającej w zgrubiałej ścianie komórki endodermis. Funkcja opisanych komórek endodermis jest dyskutowana.

SŁOWA KLUCZOWE: endoderma korzenia, pobieranie wapnia, mikroskop elektronowy, lokalizacja wapnia.