Formation of Endodermis-like Cells with Casparian Strip and Thick Wall Cells Derived from Pericycle in the Roots of Feijoa sellowiana (Myrtaceae)

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Two anatomical features of cells derived from the pericycle of the roots of Feijoa sellowiana are reported: (1) appearance of endodermis-like cell layer with a Casparian strip, which was confirmed by histological examination; (2) occurrence of a thick wall cell between endodermis-like cells. These features were considered to develop as follows: two tangential cell walls appeared successively in the pericycle cells, resulting in the formation of three cell layers. The walls of the cells of the outer layer, adjacent to the endodermis, became thicker on the interior side of the cell and heavily encrusted with lignin. The walls of the cells in the middle layer derived from the pericycle became suberized and developed into Casparian strips. These cells subsequently developed into endodermis-like cells. The innermost cell layer derived from the pericycle retained their original morphology for a while, but again divided tangentially and developed as mentioned above. Consequently, in aged roots, there were several layers, in which endodermis-like cells with Casparian strips alternated with cells with thick inner walls. The tangential width of endodermis-like cells reduced in sequence from the outer to inner layer. It is quite important to know how the anatomical features of feijoa trees appear under different soil conditions.

Key Words: anatomy, endodermis, lignin, root, suberin.

Introduction

Feijoa trees, which belong to Myrtaceae, are distributed in warm temperate and tropical regions. Although their fruits are edible, these trees are mainly used for ornamental purposes in Japan. In contrast to other plant structures (Canhoto et al., 1996; Kitayama et al., 1997; O’Brien, 1994; O’Brien et al., 1996), there are few if any reports on the root structure of members of the Myrtaceae. The development of suberin lamellae and Casparian strips in the endodermis, root lignification, and thickening of the cortical cell wall associated with root maturation are particularly important in perennial tree roots. Suberin lamella deposition and Casparian strip formation in the endodermis and exodermis (a layer of hypodermal cells adjacent to the epidermis) have been studied extensively in the roots of many plant species, particularly fruit trees, such as pear (Esau, 1943), apple (Mackenzie, 1979; Riedhart and Guard, 1957), loquat (Nii et al., 2004; Pan et al., 2006), grapevine (Song et al., 2011a), and red bayberry (Song et al., 2011b). The Casparian strip has been shown to block the free apoplast movement of water, ions, heavy metals, and fluorescent dyes (Bücking et al., 2002; Nagahashi et al., 1974; Peterson et al., 1981; Robards and Robb, 1972).

Nii et al. (2004) and Pan et al. (2006) reappraised cell wall growth (phi thickening) in the cortical cells of loquat (Rosaceae) tree roots. These authors found that phi thickening occurred before suberin lamella formation in the Casparian strip of loquat roots. In loquat trees, when roots were sampled from trees planted under drought stress conditions, phi thickening was observed to have developed markedly compared to normal conditions. The development of phi thickenings of the cortex in loquat roots under drought conditions may be regarded as a defense mechanism against water stress. Phi thickening is primarily considered to function as mechanical strength in young roots with poorly developed Casparian strips. We recently reported the occurrence of crescent-shaped thickening in the cortex of red bayberry tree roots, particularly under conditions of drought stress (Song et al., 2011b).

When we examined the anatomical features of feijoa roots, the formation of endodermis and endodermis-like...
cells with a Casparian strip was observed, and the endodermis-like cells increased with root aging. In addition, the cell wall between the endodermis and the next endodermis-like cell layers and between the endodermis-like cell layers thickened with lignin accumulation. We confirmed that these cells derived from the pericycle. So it is quite important in feijoa tree cultivation to know how these anatomical features appear in the roots.

In the present examination, we describe the detailed anatomical characteristics involved in the formation of endodermis-like cells with a Casparian strip and thick wall cell layers in cells derived from the pericycle in feijoa roots. Further, the development of these endodermis-like cells layers from the pericycle is schematized.

Materials and Methods

Plant materials

Three-year-old seedlings of feijoa (Feijoa sellowiana Berg.) were used in the present investigation. All of the trees were grown in baskets (diameter and depth: 300 mm each, stitch size: 30 mm) filled with sandy soil and covered with non-woven fabric in a greenhouse. When we collected roots, the plants were removed from the pots and the existing new white roots were cut from the middle part of root zone.

Root anatomy

The roots of the trees were collected for anatomical examination during the summer of 2010. Roots were sectioned by hand after collection using a new stainless steel razor blade. Serial transverse sections, between 5–120 mm from the root tip, were cut at 10 mm intervals to examine the structural development of the root tissues in relation to root age. Although more than twenty roots were sectioned and observed, a representative root was selected as the anatomical features were basically similar among root samples (Fig. 1). The root color was white until 20 mm from the root tip. Lateral roots continued from 20 mm behind the root tip to the basal portion of the root. Tissues were examined by fluorescent microscopy (excitation wavelength: 365 nm) without staining or after staining with several different staining solutions, including berberine hemisulfate-aniline blue for suberin lamellae of Casparian strips (Brundrett et al., 1988), safranin O or phloroglucinol-HCl for lignin (Jensen, 1962), and Sudan red 7B for lipids (Brundrett et al., 1991). Recently, Lux et al. (2005) reported a novel method for simultaneously clearing and staining tissue in which the fluorochrome 0.1% berberine hemisulfate is dissolved in a clearing mixture consisting of pure lactic acid saturated with chloral hydrate. We used this method and incubated the root sections in 0.1% berberine hemisulfate solution at 40°C for 30 min. The sections were then counterstained with 0.5% aniline blue (dissolved in H2O) at room temperature for 30 min, followed by safranin O for 1 min. All specimens in a drop of H2O on the slide glass were observed under ultraviolet light using fluorescent microscopy.

To examine the anatomical features of the cells, the remaining root tissues were fixed in 3% glutaraldehyde (0.1 M cacodylate buffer, pH 7.2) and stored at 4°C after sectioning by hand. The samples were then dehydrated through a graded ethanol series and embedded in Technovit 7100 resin (Heracus Kulzer GmbH, Wehrheim, Germany). Semi-ultra thin sections (1.5 µm) were cut using a glass knife before staining with methylene blue for histological examination under a BX60 optical microscope (Olympus, Tokyo, Japan).

Results and Discussion

During the young age of the root in feijoa trees, a Casparian strip developed in one layer of the endodermis (Figs. 2A and 3A). In the next stage, during root aging, one layer of an endodermis-like cell with a Casparian strip appeared inside the original endodermis (Figs. 2B and 3B). Cell layers with Casparian strips adjacent to the endodermis were referred to as an endodermis-like cell layer in the present study. In this way, multiple endodermis-like cell layers were observed with aging: two layers of endodermis-like cells (Fig. 2C) and three layers of endodermis-like cells (Fig. 2D). When the endodermis-like cell layer increased, cells with a thicker cell wall appeared between the endodermis and the next endodermis-like cell layers (Figs. 2B and 3B) and between the endodermis-like cell layers (Fig. 2C, D).

The process of the increase in endodermis-like cells was observed by fluorescent microscopy without staining (Fig. 3). The root section in Figure 3B is a more basal part from the root tip than that in Figure 3A. Detailed observation of serial transverse sections of the root was carried out in Figure 3A and 3B. Before the formation of the new root structure, the endodermis and pericycle were both arranged normally around the vascular bundle (Fig. 4A). The initial developmental stage of the novel structure in the pericycle involved two tangential divisions of each cell in the pericycle to produce three cells (Fig. 4B, C, D). After one cell wall appeared in the

![Fig. 1. Representative appearance illustrating new root development in feijoa plants. A–D indicate the positions corresponding to the cross-sections in Figure 2A–D, respectively.](image-url)
pericycle (Fig. 4B), an additional cell wall appeared and developed in the same pericycle cells (Fig. 4C). Consequently, three cells were formed from one pericycle cell (Fig. 4D, E, F).

The cell walls of the cells derived from the pericycle and located adjacent to the endodermis toward the pericycle side became thicker (Figs. 3B and 4F). The thickened cell wall became pink when stained with phloroglucinol and observed under an optical microscope, and red when stained with berberine hemisulfate-aniline blue-safranin O and observed under a fluorescence microscope (Fig. 2B). Based on the observed staining characteristics, the thickened walls were lignified strongly.

Fluorescence of the cells in the middle layer of three cells derived from the pericycle revealed that suberin accumulated in the cell walls (Figs. 2B and 3B). These characteristics resembled those of the Casparian strip in the endodermis (Fig. 2A). The suberin lamellae of the new endodermis-like layers were m-shaped and "opened" centripetally (Fig. 2B, C, D).

Finally, the innermost cellular layer of the pericycle-derived cells retained their original pericycle characteristics and did not develop a Casparian strip (Figs. 2B and 3B). Interestingly, this new pericycle could not be stained with any staining solution.

The anatomical features mentioned above explained the increase from one layer to two layers in cells derived from the pericycle. Next, in the case of three layers to four layers, the new pericycle again divided tangentially and developed in the same way (unpublished data). Thus, in the mature roots, two or three layers of endodermis-like cells were observed (Fig. 2C, D), i.e. these endodermis-like layers contained a Casparian strip. We also confirmed similar features in other relative species: wax apple (Syzygium samarangense), guava (Psidium guajava), and surinam cherry (Eugenia micheli) (unpublished data). In addition, in the mature roots 110 mm from the root tip in wax apple (Syzygium samarangense), 5 layers of endodermis-like cells and endodermis were confirmed (unpublished data). The tangential width of the cells with Casparian strips decreased progressively from the endodermis to the endodermis-like cells toward the interior of the root (Fig. 2). The width of the endodermis, first layer of endodermis-like cells, second layer of endodermis-like cells, and third layer of endodermis-like cells was 46.1 ± 2.4 µm (mean ± SD, n = 14), 38.5 ± 1.8 (n = 24), 28.9 ± 1.1 (n = 33) and 18.3 ± 7.3 (n = 40), respectively.

The development of these endodermis-like cell layers from the pericycle is schematized in Figure 5. In stage 1, a tangential cell wall appears in the cells of the pericycle (Fig. 5A, B), but before cell division is completed, a second cell wall appears in the inner part of the same cell in Figure 4C (Fig. 5C), and thus three cells are formed from a single pericycle cell (Fig. 5D). In stage 2, the walls of the outermost cells adjacent to the endodermis become thick (Fig. 5E, F). Suberin is deposited on the cells of the middle layer, resulting in the formation of a Casparian strip (Fig. 5F). The innermost cell layer of the pericycle-derived cells retained the original characteristics of a pericycle.

![Fig. 2. Development of suberin lamellae with Casparian strips and thick wall cells between endodermis or endodermis-like cells derived from pericycle in relation to root age in feijoa roots illustrated in Figure 1. Panel A: one layer endodermis 20 mm from the root tip; B: two layers of endodermis (endodermis and endodermis-like cell layer each) and one layer of thick wall cells 80 mm from the root tip; C: three layers of endodermis (one endodermis and two endodermis-like cell layers) and two layers of thick wall cells 90 mm from the root tip; D: four layers of endodermis (one endodermis and three endodermis-like cell layers) and three layers of thick wall cells 120 mm from the root tip. Arrows, Casparian strip; arrowhead, thick wall cell; en, endodermis; nen, new endodermis (endodermis-like cell layers derived from pericycle). Roots were sectioned by hand and tissues were examined by fluorescent microscopy after staining with berberine hemisulfate-aniline blue. Scale bars, 50 µm.](image-url)
Although one layer of endodermis generally surrounded the central stele, several layers of endodermis-like cells appeared inside the endodermis in mature feijoa roots. The increase of these endodermis-like cell layers resulted from the pericycle and the innermost cell layer of the pericycle-derived three cells retaining the original characteristics of a pericycle. Before the cell division of the pericycle, the endodermis and pericycle were arranged (Fig. 4A) and the pericycle did not generate fluorescence without staining or staining solution (Figs. 2 and 3). When new cell layers from the pericycle increased, the pericycle again divided tangentially and developed as mentioned above. The innermost cell layer in cells derived from the pericycle did not generate fluorescence, similar to the original pericycle. Consequently, we concluded that the innermost cell layer in cells derived from the pericycle preserved its original morphology.

The increase of cell layers derived from the pericycle has been described for the formation of the periderm (Esau, 1977). It was also described that a special type of protective tissue called polyderrm occurred in roots and underground stems of Hypericaceae, Myrtaceae, Onagraceae, and Rosaceae. Further, Fahn (1982) reviewed in detail the polyderrm in the same families as follows. A special phellogen is formed in the pericycle. This phellogen produces centrifugally a few layers of thin-walled non-suberized cells which alternate with a layer of endodermal-like cells. At the start of the differentiation of the latter into cork cells, Casparian strips appear on the cells which, with further development, become entirely lined by a suberin layer. Nelson and Wilhelm (1957) reported previously in the strawberry that the polyderrm continued to expand by the addition of new cell layers, probably a thickness of 20 or more cell layers. From these expressions, the formation of cell layers in the pericycle and the appearance of a suberin layer with a Casparian strip in feijoa roots were similar to polyderrm; however, the occurrence of a thick wall cell between endodermis-like cells is a newly discovered structure.

In the present study, thick wall cells occurred in a different area from phi thickening in Rosaceae trees (Nii et al., 2004; Pan et al., 2006) and crescent thickening in red bayberry (Song et al., 2011b). The appearance of the thick wall cells observed in this study was similar to that of the crescent-shaped thickening reported in red bayberry roots (Song et al., 2011b). We therefore proposed that the thick wall cells in the feijoa roots in this study was analogous to the crescent-shape thickening observed in red bayberry roots (Song et al., 2011b), even though the families are different. The thickening of cortical cell walls occurred erratically in the cortex of red bayberry (Song et al., 2011b), but in the present study it always occurred in cells derived from the pericycle. Unlike the thickening of the cortical cell walls, thickening in walls of the cells between the endodermis and the pericycle occurred sequentially. Song et al. (2011b) reported that the crescent-shaped thickening observed in red bayberry was promoted by drought conditions. In addition, Pan et al. (2006) described differences in the extent and distribution of phi thickening in loquat roots under different water stress conditions. They indicated that phi thickening in loquat roots and crescent thickening in the cortex of red bayberry roots play an important role in the adaptive response to soil drought and provide structural support for the root.

Peterson et al. (1981) reported that phi thickening did not function as a barrier against the apoplastic transport of relatively small molecules. They also reported the difficulty in determining whether phi thickening played an active role in apple (Weerdenburg and Peterson, 1983). Mackenzie (1979) suggested that phi thickening in the apple had a primarily structural function and that detailed study was required of the physiology of uptake and transport through apple roots in relation to structure. Further studies are required to understand the correlation...
of the formation of endodermis-like structures and thick wall cells in cells derived from the pericycle with unfavorable soil water conditions.

To conclude, we propose that a novel structure consisting of endodermis-like cells with a Casparian strip and thick wall cells originated from cells derived from the pericycle in members of the Myrtaceae. This new type of thick wall cell in feijoa roots was different from the \textit{phi} thickening observed in Rosaceae fruit trees. These

Fig. 4. Serial trans-sections of feijoa roots showing the arrangement of endodermis and pericycle between 30 to 40 mm from the root tip at a similar position to Figure 3. Panel A: before cell division of pericycle; B: appearance of one cell-wall in pericycle cells; C: appearance of two cell-walls in pericycle cells; D, E: formation of three cells after cell division of pericycle cells; F: three cells derived from pericycle developed differently. en, endodermis; pe, pericycle; ca, cambium; cw, cell wall formed in a pericycle cell; nen, new endodermis (endodermis-like cell layer derived from pericycle); twc, thick wall cell; npe, new pericycle derived from pericycle. Tissues were embedded in Technovit and sections were stained with methylene blue. Scale bars, 20 \mu m.

Fig. 5. Scheme of appearance of endodermis-like cells with a Casparian strip and thick wall cells from pericycle in feijoa roots. A: parallel arrangement of endodermis and pericycle; B: appearance of cell wall in pericycle cell; C: appearance of second cell wall in pericycle cell immediately after appearance of one cell wall; D: three cell layers formed after cell division of pericycle cell; E: some radial cell divisions occurred in innermost cell layers derived from pericycle; F: three cells derived from pericycle developed into completely different cells. Cells adjacent to the endodermis became thicker, cells in the middle layer changed to endodermis-like cells with a Casparian strip, and innermost cells retained their original pericycle characteristics. The formation of a Casparian strip in endodermis-like cells was confirmed by histological examination. EN, endodermis; PE, pericycle.
cells was analogous to the crescent thickening in the cortex of red bayberry roots. It is possible that these endodermis-like cells and thick wall cells have an adaptive function to protect the plant against drought stress. Future studies under a variety of soil conditions are required to elucidate the mechanism underlying root development and the plasticity of thick wall cells in members of the Myrtaceae.

**Literature Cited**


