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A Way to Interact with the World: Complex and Diverse Spatiotemporal Cell Wall Thickenings in Plant Roots

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Abstract

Plant cells are defined by their walls, which, in addition to providing structural support and shape, are an integral component of the nonliving extracellular space called the apoplast. Cell wall thickenings are present in many different root cell types. They come in a variety of simple and more complex structures with varying composition of lignin and suberin and can change in response to environmental stressors. The majority of these root cell wall thickenings and cell types that contain them are absent in the model plant *Arabidopsis thaliana* despite being present in most plant species. As a result, we know very little regarding their developmental control and function. Increasing evidence suggests that these structures are critical for responding to and facilitating adaptation to a wide array of stresses that a plant root experiences. These structures function in blocking apoplastic transport, oxygen, and water loss and enhancing root penetrative strength. In this review, we describe the most common types of cell wall thickenings in the outer cell types of plant roots—the velamen, exodermal thickenings, the sclerenchyma, and phi thickenings. Their cell-type dependency, morphology, composition, environmental responsiveness, and genetic control in vascular plants are discussed, as well as their potential to generate more stress-resilient roots in the face of a changing climate.

Contents

1. SIMON SAYS: MOVEMENT AND BLOCKAGE IN THE PLANT APOPLAST

Middle lamella: a structure in-between plant cells that is composed of pectic polysaccharides and is deposited after the cell plate is put down in cytokinesis

Apoplast: the extracellular plant cell space occupied by the plant cell wall

A fundamental feature of plant cells is the presence of a cell wall. The wall defines cellular form, provides structure and support, and serves additional diverse functions. A primary cell wall is deposited after cell division. First, a middle lamella, composed of pectic polysaccharides, is formed as the cell plate is put down during cytokinesis. Cell walls are then deposited on either side of the middle lamella. Primary walls are characterized by their flexibility, strength, and composition and contain cellulose, noncellulosic polysaccharides, pectin, and glycoproteins. As cells undergo elongation and differentiation along the path to acquire their final function, cell shape changes through localized loosening, breakdown, and deposition of the new (primary) cell wall. The area occupied by cell walls is referred to as the apoplast, and it serves underappreciated functions in interacting with the environment. The apoplastic extracellular space is an interconnected system of cell walls and their surrounding spaces. Water and mineral nutrients essential for plant growth are taken up by root systems belowground, and they passively diffuse through the apoplastic space until they are blocked. Apoplastic barriers prevent bulk flow from the outer environment directly into the vasculature. Selective water and nutrient uptake must then occur via either symplastic movement through the cytoplasm connected by plasmodesmata or transcellular transmembrane

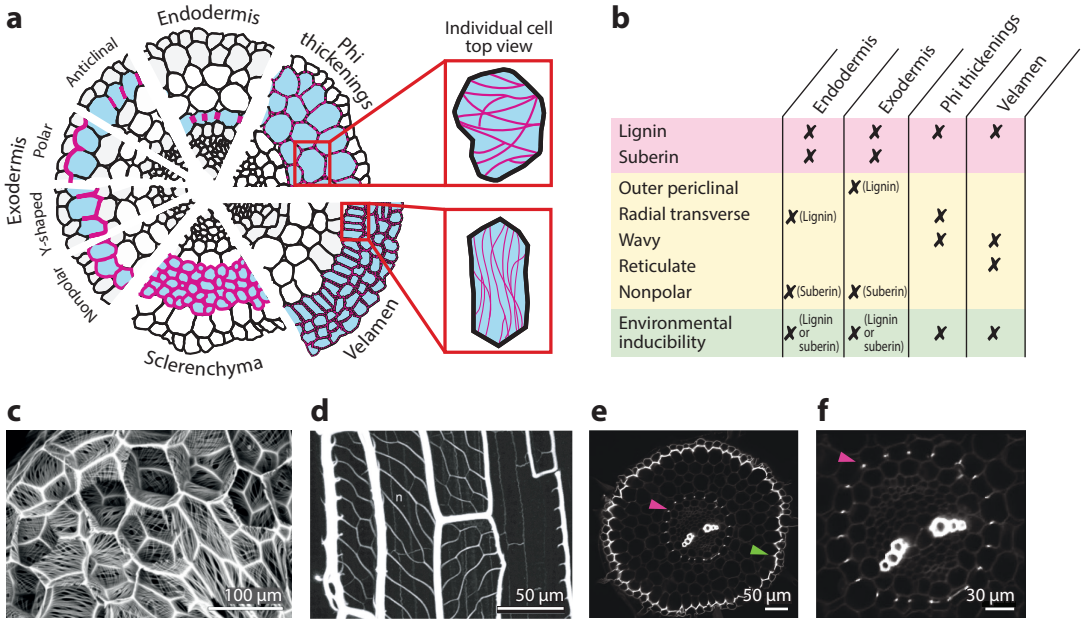

Figure 1

Overview of different cell wall thickenings. (*a*) Representation of the different lignified cell wall thickenings per cell type. Lignin is shown in magenta, and the cell types of interest are shown in blue. Exodermis: The four patterns of barrier deposition include nonpolar, in which the entire exodermal cell is surrounded by the barrier; Y-shaped, in which the barrier is deposited in a Y-shaped pattern on the anticlinal wall closer to the inner root; polar cap, in which the barrier is polarized to the periclinal cell wall facing the epidermis, forming a cap; and anticlinal, in which the barrier is deposited in the anticlinal walls. Endodermis: The lignified Casparian strip is deposited in the center of endodermal anticlinal walls. Cortical cells: Phi thickenings are found in the radial, transverse walls and can form a net-like set of thickenings. A detailed inset is represented to highlight this net-like structure from a top view perspective of a cell. The sclerenchyma cells (depicted here as is typical of a multiseriate cortical sclerenchyma) have thick lignin that uniformly coats all cell walls. Epidermis: In the velamen, a complex set of secondary cell-wall thickenings is generally oriented in the same direction in each cell. A detailed cell is represented to highlight this oriented cell wall pattern from a top-view perspective. (*b*) Table with different regulatory modules that control root cell wall thickenings, including the metabolic pathway (*pink*), cellular location (*yellow*), and environmental inducibility (*green*). (*c*) Velamen organization in *Miltoniopsis* sp. roots using Basic Fuchsin–stained lignin. Panel adapted from Reference 54 (CC BY 4.0). (*d*) Fine reticulate networks associated with phi thickenings in a *Brassica napus* root using berberine-stained lignin. Panel adapted from Reference 3 (CC BY 4.0). (*e*) Exodermis (*green arrow*) and endodermis (*pink arrow*) in a *Solanum lycopersicum* root cross section stained with Basic Fuchsin (lignin; *white*) and Calcofluor white (cellulose; *gray*). Panel adapted with permission from Reference 59 (CC BY-NC-ND 4.0). (*f*) Endodermal Casparian strip (*pink arrow*) in *Solanum lycopersicum* (enlarged section from panel *e*).

transport with specific membrane carriers. Apoplastic barrier composition changes depending on the root cell type in which it is present.

Roots have deceptively simple cell-type patterning, with a series of largely radially symmetric cell types (**Figure 1**). The epidermis is the outer cell layer, and internal to the epidermis is the cortex tissue. In species with multiple cortical layers, the outermost cortex is defined as the hypodermis or the exodermis. Inner cortical layers can include the sclerenchyma, aerenchyma, periendodermal layer, or other cortical layers that have not been given a specific name. The endodermis flanks the stele, which includes the pericycle and the vasculature including the xylem, phloem, and (pro)cambium. All these cell types are initiated and specified at the root tip, the location of the root stem cell niche. As the root tip grows in the direction of gravity (downward), cells progressively differentiate. First, they undergo rapid cell proliferation in a zone called

Symplastic: related to the symplast, which is a cytosolic interconnected continuum of plant cells, interconnected by plasmodesmal bridges

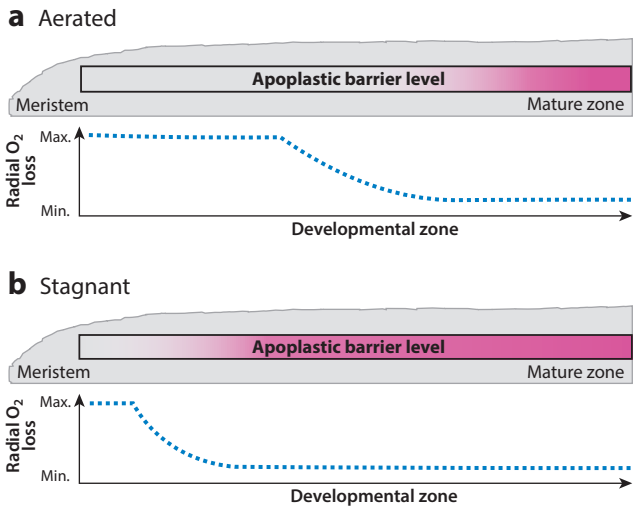

Figure 2

Exodermal lignosuberized barriers change in magnitude and developmental time in response to stagnant conditions. (*a*) The exodermal barrier in aerated conditions. (*b*) Root responses in stagnant conditions. (*a*,*b*, *top*) Root longitudinal section, with the meristem on the left and the maturation zone on the right. The lignosuberized barrier is represented by the rectangle. Increased magnitude of lignosuberized deposition is shown in pink. (*a*,*b*, *bottom*) Graph illustrating the levels of the radial oxygen loss. The y-axis represents the radial oxygen loss, and the x-axis represents the developmental gradient from the meristem to the maturation zone.

the meristem. Cells then rapidly elongate in the elongation zone and subsequently differentiate (**Figure 2**). This pattern is largely recapitulated in roots of different types, including lateral, nodal, and crown. Thus, there is a gradient of developmental age along the root's longitudinal axis, with the youngest cells at the root tip and the oldest cells near the root–hypocotyl junction.

Exodermis: the external cortical cell layer (under the epidermis) that can contain lignified and/or suberized cell wall thickenings

Sclerenchyma: a differentiated cortical cell, in single or multiple layers, that contains uniformly deposited lignin

Suberin lamellae: electron-dense and -light layers of suberin that are successively deposited

The best-characterized apoplastic barrier is the Casparian strip (CS), which resides in root endodermal cells, in large part because of the wealth of molecular, genetic, and genomic tools available in *Arabidopsis thaliana*. The CS is composed of lignin that seals the space between the plasma membranes of two neighboring endodermal cells in a precise band in the center of the transversal and anticlinal cell walls. The sealing of this space is characteristic of its presence and is revealed by band plasmolysis (13, 62; reviewed in 44). The CS is formed early in the root differentiation zone, and it inhibits apoplastic movement through the endodermis to vascular cells (87). After the CS has been laid down, and later in the endodermal differentiation trajectory, endodermal cell walls are coated with suberin lamellae. The endodermal CS and suberin lamellae have long been accepted as necessary to root hydraulic conductivity by generating sufficient resistance to facilitate the transpiration stream (17).

All angiosperm roots have a CS. However, the roots of most angiosperms contain a multitude of additional cell wall thickenings that are not present in *Arabidopsis*. Any modification to the cell wall that influences its diffusion, transport capacity, and mechanical properties in turn influences communication and exchange with the environment. Many of these cell wall thickenings have been described for centuries (21, 137) and in recent years have become of interest for their dynamic responses to the environment, including changes in water availability and interactions with microbes (7, 17, 37, 43, 51, 110). In this review, we explore the vast diversity of root cell wall thickenings and analyze them in the context of their cell type, developmental regulation, composition, function, and role in mediating environmental responses. We do not discuss root

peridermal wall thickenings that develop during secondary growth, as they are the subject of a recent review (119). We detail how common understanding of the function or composition of these cell wall thickenings from anatomical or metabolic profiling can be challenged using current and innovative experimental approaches. Finally, we discuss how advances in high-resolution cellular imaging, single-cell transcriptome sequencing, and gene editing and transformation allow us to revisit these beautiful developmental features and consider how their evolution contributed to plants' success in terrestrial colonization.

2. LIGNIN AND SUBERIN: PRIMARY COMPONENTS OF SECONDARY CELL WALLS AND APOPLASTIC BARRIERS

Cell walls are composed of a cellulosic matrix deposited by cellulose synthase complexes at the plasma membrane. Cell walls can be further supported with the deposition of a secondary or even a tertiary cell wall between the primary cell wall and the plasma membrane. Hemicellulosic molecules can be interwoven in this matrix, and additional matrix strengthening is provided by lignin and suberin. While pectin is considered to be underrepresented in secondary or tertiary cell walls, available evidence suggests that this is not necessarily the rule (58, 66). The main components of lignin are derived from the general phenylpropanoid pathway beginning with phenylalanine and the specific monolignol biosynthetic pathway (10, 104, 136), resulting in the production of hydroxycinnamyl alcohols (monolignols). These include *p*-hydroxyphenyl (H), guaiacyl (G-), and syringyl (S-)lignin units, which are coupled by peroxidases and laccases. The transcription of phenylpropanoid and monolignol biosynthetic enzymes is highly spatiotemporally regulated during development as well as in response to the environment (4, 14, 63, 82, 83, 132).

Suberin is an insoluble poly(acylglycerol) polyester composed of a polyaliphatic and a polyphenolic domain. The polyaliphatic domain consists of highly glycerol-esterified very long-chain fatty acid coenzyme A (CoA) derivatives; ω-hydroxyacids; and α,ω-dicarboxylic acids, while the polyphenolic domain consists of ferulic acid–esterified ω-hydroxyacids and primary fatty alcohols. These polyaliphatic and polyphenolic domains are deposited in layers called suberin lamellae. Diverse chemical and structural arrangements are present in many organisms and tissues, their biosynthetic enzymes have been generally elucidated, and their cell type and developmental stage deposition as well as their environmental responsiveness have been well-reviewed in Reference 117.

Bulk metabolite profiling has been instrumental in elucidating the varied biochemical composition of lignin and suberin found within root cell types (112). Since these cell wall thickenings strengthen the wall, they are also recalcitrant to treatments that degrade the cell wall, such as cellulases and pectinases. Therefore, treatment of the cell wall with these enzymes can separate the cell wall itself from the components that thicken and strengthen them. They can then be extracted with organic solvents and concentrated and can undergo several chemical degradation protocols to identify component composition (112). In the case of suberin, transesterification with BF_3 and methanol, derivatization, and analysis by gas chromatography can be used to identify ω-hydroxyacids, ω-diacids, primary carboxylic acids, primary alcohols, and 2-hydroxy fatty acids. After this transesterification, aromatics (coumaric acid and ferulic acid) are released. Thioacidolysis and gas chromatography–mass spectrometry (GC-MS) are used to identify lignin monomeric composition (112). These chemical profiling methods were profound in their determination of the complexity of secondary cell wall composition. However, they are also limited in that they detect only the subunits of the cell wall at bulk resolution and not their polymeric structure or the cell type in which they are present, much less where on the cell wall they are located. Histochemical stains and microscopy are tools to help determine the general biopolymer composition of cell wall thickenings at spatiotemporal resolution.

Stagnant: low- to no-oxygen (anoxic) conditions

Velamen: differentiated root epidermal cell, in single or multiple layers, that are composed of heavily lignocellulosic cell walls in elaborate structures that have undergone programmed cell death

Electron microscopy is perhaps the most precise method of lignin or suberin lamellae identification at cellular resolution. Transmission electron microscopy (TEM) coupled with potassium permanganate ($KMnO_4$) can detect lignin composed of S-lignin units. In the case of the CS, TEM is used to reveal the electron-dense structure that connects the two neighboring cell plasma membranes and blocks the apoplast. Further, it visualizes whether the CS structure is confined to the precise central position that is strictly aligned with the endodermal radial wall. Recently, confocal Raman spectroscopy was also used to identify lignin in the *Arabidopsis* endodermis at cellular resolution (103). Electron microscopy can further reveal the intermittent electron-dense and electron-light suberin lamellar structure (118) found in secondary or even tertiary cell walls. Lignin-specific histochemical stains include Phloroglucinol-HCl, which detects the cinnamaldehyde end groups of monolignols, preferentially recognizing G- and S-monolignols. The Maüle reaction primarily detects S-lignin. Basic Fuchsin stains lignin but is unable to discriminate between monolignol types (61). The current standard for suberin detection is Fluorol Yellow 088 (75), as well as other lipophilic stains, including Nile Red, Sudan III, Sudan Red, Sudan Red 7B, Sudan Black and Neutral Red. Classically, the endodermal CS was identified using autofluorescence. In subsequent studies it was detected with berberine hemisulfate, with Aniline Blue to counteract autofluorescence (15). However, these two approaches cannot distinguish between lignin and suberin. In this review, we will state the evidence for a wall thickening being lignified or suberized. If a berberine hemisulfate stain is included, the implicit caveat should be that additional experimentation is needed to conclusively determine the sole presence of lignin. Additionally, clearing of roots in conjunction with this staining has dramatically improved our ability to identify these cell wall structures in diverse plant species (135).

3. METHODS TO ELUCIDATE APOPLASTIC BARRIER FUNCTION

One of the ways in which barrier function is tested is by observing the diffusion of fluorescent molecules or dyes, known as apoplastic tracers, through the apoplast. When diffusion is blocked, the presence of an apoplastic barrier can be inferred. Many different molecules have been used to trace apoplastic movement in plant roots. These include uranyl (105), periodic acid and Schiff's reagent, Calcofluor or Tinopal (77, 95), berberine hemisulfate and potassium thiocyanate (38), tritiated water, ^{86}Rb (25), and propidium iodide (87, 109). While quite reliable, there are also limitations to each of these approaches, which are outlined in Reference 90.

Other approaches are also used to measure the ability of these cell wall structures to prevent loss of oxygen, water, H_2S as found in stagnant (low to no oxygen–containing, standing) water, or salt. These include the application of oxygen electrodes (34), pressure probes, hydrostatic pressure gradients (101), and the methylene blue stain (35, 122, 123, 126). One advantage of these approaches is that when interpreted relative to the position of the barrier along the root's longitudinal axis, we can infer whether barrier deposition is also regulated in developmental time.

4. DIVERSITY OF ROOT CELL WALL BARRIERS: FORM AND POSITION

Cell wall thickenings have been identified in many other root cell layers in addition to the endodermis. In this section, we revisit literature that describes these structures in specific cell types, as well as their cell wall composition as inferred using histochemistry and microscopy.

4.1. The Velamen

The velamen is a single or multiseriate epidermal cell type that undergoes programmed cell death after it has deposited a secondary cell wall (73) (**Figure 1**). Although generally associated with

epiphytic orchids, the velamen has also been documented to occur in terrestrial plants from almost 240 genera in 23 monocot families (150). Velamen secondary cell wall thickenings are composed of cellulose (54) and are heavily lignified (54, 58, 76, 97, 146). More recently, pectin has also been reported to be deposited in a nonpolar manner on all cell walls or even in specific walls within the velamen (58). Some lipidic substances are present in these walls, although their composition is unknown, and an absence of suberin has been reported in others (58, 76). The complexity and elegance of cell wall structures in the velamen are stunning (**Figure 1**). They vary across and even within species. There is no canonical structure, and the adjectives used to describe them are manifold: banded, mesh-like, crisscrossed, helical, wickerwork-like, thick and parallel, uniform, polarly localized at periclinal walls, wavy, and striated (8, 54, 58, 73, 76, 97, 146) (**Table 1**).

4.2. The Exodermis

Under the epidermis, uniseriate and multiseriate cortical layers can have thickened cell wall features. Perumalla & Peterson (92) defined an exodermis as a hypodermis that contains a lignosuberized CS. Since then, however, our understanding of the nature of the CS has changed (87). Our comprehensive review of exodermal wall thickenings in 47 species revealed only one case of a true exodermal CS (**Table 2**) in *Glyceria maxima*. Here, we define a CS based on its precise radially localized position and function as revealed by band plasmolysis. Although there are two presumed exodermal layers (biseriate) in this species, only the innermost exodermal layer undergoes band plasmolysis (127). Therefore, we have redefined the exodermis as any cell layer under the epidermis that contains thickened cell walls (either lignified or suberized) that may, but does not necessarily, act as an apoplastic barrier.

There are four lignin-containing wall morphologies in the exodermis (**Figure 1**): (*a*) complete or nonpolar lignification around all cell walls (22, 35, 36, 57, 59, 68, 77, 116, 120, 134, 143, 145), (*b*) a polar cap in the epidermal cell wall face and along the anticlinal walls (59, 77, 127), (*c*) anticlinal walls (64, 67, 71, 92, 95, 101, 114, 115, 143), and (*d*) an upside-down Y deposition toward the inner part of the root (79, 80). By contrast, there are three suberin-containing exodermal wall morphologies: (*a*) complete suberization of the cell (19, 22, 25, 30, 35, 36, 59, 68, 96, 114–116, 120, 133, 134), (*b*) polar localization facing the epidermal cell wall and along anticlinal walls (65), and (*c*) anticlinal wall deposition (30) (**Table 2**). Lignin and suberin morphologies are identical for 14 species (11, 22, 35, 36, 68, 76, 91, 114, 116, 120) and distinct for 10 (34, 59, 64, 79, 80, 115) (**Table 2**). We can speculate that in cases where the lignin and suberin cell wall thickening morphologies are the same in a single species/cultivar, the wall is collectively lignosuberized, while in the cases of different morphologies, they are under distinct developmental regulation. Accessions or cultivars of the same species that have the same lignin and suberin morphologies suggest species-level genetic control in control conditions (35, 36, 64, 91, 101, 122, 123).

Exodermal cell wall structures are highly responsive to a variety of environmental stressors, with an increase in the magnitude of lignin (36, 64, 68, 91, 101, 120), suberin (19, 30, 65, 68, 120), or inducibility (122, 123). Such stressors include excess salt (120), hypoxia (35, 36, 57, 127), H_2S, and water limitation (19). There is also genetic variation in the direction of inducibility of cell wall thickenings within species. Suberin levels increased in magnitude for *Hordeum marinum* (sea barley) (var. H21) and decreased in magnitude in *H. marinum* (var. H90) in stagnant (anoxic) conditions, and lignin and suberin levels increased in response to salt in only two of three almond varieties (65, 120). Potentially relevant to its function as an apoplastic barrier, exodermal lignin or suberin can be induced earlier in response to many stresses (closer to the root tip), including salt, hypoxia, stagnant conditions, and water deficit (19, 64, 68).

Table 1 Cell wall thickenings in the velamen

Species	Number of velamen layers	Cell wall morphology and composition	Reference
Bifrenaria harrisoniae	NA	Unknown lignin[a] (PG); cell junctions (pectin-RR)	58
Caularthron bilamellatum	1	Unknown lignin (PG)	58
Cleisostoma yersinii	3	Nonpolar in second and third layer (UV-AF)	97
Cymbidium sinense	~7	NA	72
Cymbidium tracyanum	~15	Banded/criss-cross (LM)	72
Dendrobium	3–5	Helical lignin (F)	54
Dendrobium fimbriatum	2–3	Nonpolar (pectin-RR); unknown lignin (PG)	58
Dendrobium nobile	NA	Anticlinal (pectin-JIM5)	58
Dimerandra emarginata	3–4	Cell corners (pectin-RR)	58
Doritis pulcherrima	1–2	Unknown lignin (PG); Nonpolar (pectin-RR)	58
Encyclia ghillanyi	NA	Periclinal (DAPI-AF); unknown lignin (PG)	58
Epidendrum ciliare	NA	Unknown lignin (PG)	58
Epidendrum nocturnum	NA	Unknown lignin (PG); periclinal and nonpolar; (pectin-JIM5; JIM7)	58
Gongora unicolor	NA	Unknown lignin (PG)	58
Laelia anceps	3+	Banded/wave-like lignin (F)	54
Miltonia bluntii	5	Cell–cell junctions (pectin-RR; DAPI-AF); unknown (PG)	58
Miltoniopsis	6–8	Mesh-like/Crisscrossed/irregular striations/cell wall ridges (F)	54
Oncidium sp.	NA	Unknown lignin (PG); Cell–cell junctions (pectin-JIM5;JIM7)	58
Phalaenopsis	2–3	Crossed/wickerwork-like (F)	54
Phalaenopsis cornu-cervi	2	Periclinal wall (pectin-RR); autofluorescence only in second layer (DAPI-AF); unknown lignin (PG)	58
Phalaenopsis hybrid	NA	Unknown lignin (PG); nonpolar (JIM5)	58
Sobralia macrantha	NA	Thick and parallel; (EM; LM+TBO)	8
Stemona japonica	4	Striations (lignin-PG)	146
Stemona sessilifolia	3	Wavy (lignin-PG)	146
Stemona tuberosa	3	Nonpolar (lignin-PG)	146
Trichoglottis bipunctata	NA	Unknown lignin (PG)	58
Vanda tessellata (Roxb.) Hook. ex G. Don	3	Wavy (S)	76

[a]Unknown lignin indicates that morphology is not provided.

Abbreviations: DAPI-AF, DAPI autofluorescence; EM, electron microscopy; F, Basic Fuchsin; JIM5 and JIM7, pectin antibodies; NA, not available; PG, Phloroglucinol-HCL; RR, Ruthenium Red; S, Safranin; TBO, Toluidine Blue; UV-AF, UV autofluoresence.

4.3. Phi Thickenings

Phi thickenings were originally named by Van Tieghem in 1871 (139) for their visual similarity to the Greek letter phi (ϕ). Their classical morphology comprises two half circles on either of the anticlinal walls of a given root layer. Thus, in radial cross sections, this appears as a circular cell wall formation with an anticlinal cell wall through its middle. In three dimensions, the phi thickening would form a rectangular, centralized structure around the radial cell wall (**Figure 1**). Phi thickenings were originally categorized as three types, depending on which cortical layer they

Table 2 Exodermal lignin and suberin cell wall thickenings

Species	Number of exodermal layers[a]	Lignin morphology	Suberin morphology	Stress test?	Lignin stress	Suberin stress	Function: barrier?	Function: other method	Reference(s)
Aegiceras corniculatum	1	Everywhere (PG)	NA	NA	NA	NA	NA	NA	22
Allium cepa	1	Anticlinal (BNaOH)	Everywhere (TEM)	NA	Increased (F)	NA	Epi/Exo (Uvitex)	NA	92, 96
Almond (*Empyrean Prunus persica* × *Prunus davidiana*)	1	Everywhere (F)	Everywhere (NR)	Salt	Increased (F)	Increased (NR)	NA	NA	120
Almond Controller-5 (*Prunus salicina* × *Prunus persica*)	1	Everywhere (F)	Everywhere (NR)	Salt	Increased (F)	Increased (NR)	NA	NA	120
Almond Krymsk-86 (*Prunus cerasifera* × *Prunus persica*)	1	Everywhere (F)	Everywhere (NR)	Salt	NA	Increased (NR)	NA	NA	120
Avicennia officinalis	2	Everywhere (BAB)	NA	Salt	Increased (BAB)	Increased (FY)	Reduction in apoplastic byflow	NA	68
Bruguiera cylindrica	2	Everywhere (BAB)	Everywhere (FY)	NA	NA	NA	NA	NA	68
Caltha palustris	1	Anticlinal (BAB)	Everywhere (FY)	NA	NA	NA	NA	NA	115
Capsicum annuum	1	Polar Lignin Cap (F)	NA	NA	NA	NA	NA	NA	77
Caulartbron bilamellatum	1	NA	Thick at outer face, thin at inner face (SR)	NA	NA	NA	NA	NA	58
Chlorophytum comosum	1	Anticlinal (BAB)	NA	NA	NA	NA	NA	NA	143
Echinochloa crus-galli var. praticola	1	Everywhere (F, PG) Anticlinal wall (BAB)	Everywhere (FY)	Stagnant	Increased (F)	Same (FY)	Epi/Exo (PA)	Oxygen electrode/ Methylene Blue	35
Echinochloa crus-galli var. crus-galli	1	Everywhere (F, PG) Anticlinal wall (BAB)	Everywhere (FY)	Stagnant	Same (PG, BAB)	Same (FY)	Epi/Exo (PA)	Oxygen electrode/ Methylene Blue	35
Echinochloa colona	1	Everywhere (F, PG) Anticlinal wall (BAB)	Everywhere (FY)	Stagnant	Same (PG, BAB)	Same (FY)	Epi/Exo (PA)	Oxygen electrode/ Methylene Blue	35

(*Continued*)

Table 2 (*Continued*)

Species	Number of exodermal layers[a]	Lignin morphology	Suberin morphology	Stress test?	Lignin stress	Suberin stress	Function: barrier?	Function: other method	Reference(s)
Echinochloa oryzicola	1	Everywhere (F, PG) Anticlinal wall (BAB)	Everywhere (FY)	Stagnant	Same (PG, BAB)	Same (FY)	Epi/Exo (PA)	Oxygen electrode/ Methylene Blue	35
Pontederia crassipes	1	Everywhere (BAB)	NA	NA	NA	NA	NA	NA	143
Encyclia stellata	1	Everywhere (S)	NA	NA	NA	NA	NA	NA	33
Glyceria maxima	3	NA	NA	Stagnant	Everywhere (PG); Casparian strip[b] (second layer)	Everywhere (SR7B)	Epi/first Exo; Epi/second Exo (PA)	Not a radial oxygen loss barrier (oxygen electrode)	127
Glycine max (L.) Merr.	1	NA	Everywhere (FY)	NA	NA	NA	NA	NA	133
Hordeum marinum Huds H21	1	NA	Polar cap (FY)	Stagnant	NA	Increased (RY)	NA	Oxygen electrode	65
Hordeum marinum Huds H90	1	NA	Polar cap (FY)	Stagnant	NA	Decreased (FY)	NA	Not a radial oxygen loss barrier (oxygen electrode)	65
Hordeum vulgare	1	Anticlinal (BAB)	NA	NA	NA	NA	NA	NA	71
Iris germanica	1–4	Upside-down Y (BAB)	Everywhere (SR, FY)	NA	NA	NA	NA	NA	79, 80
Iris pumila	Multiple	Unclear (BNaOH)	NA	NA	NA	NA	NA	NA	93
Laetia corymbulosa	1	NA	Everywhere (NR)	Stagnant	NA	Same (NR)	NA	Not a radial oxygen loss barrier (oxygen electrode)	30
Nicotiana benthamiana	1	Polar lignin cap (F)	NA	NA	NA	NA	NA	NA	77
Nymphaea odorata	1	Anticlinal (BTB)	Everywhere (SR7b)	NA	NA	NA	NA	NA	115
Oryza glumaepatula W2165	1	NA	Everywhere (FY)	Stagnant	Everywhere (F)	Increased (FY)	NA	Oxygen electrode	34
Oryza sativa cv. Azucena	1	Anticlinal (BAB; BTB)	Everywhere (FY)	Stagnant	Same (BAB)	Increased (FY)	NA	Oxygen electrode; pressure probe	64, 101

(*Continued*)

Table 2 (*Continued*)

Species	Number of exodermal layers[a]	Lignin morphology	Suberin morphology	Stress test?	Lignin stress	Suberin stress	Function: barrier?	Function: other method	Reference(s)
Oryza sativa cv. IR42	1	Anticlinal (BAB)	Everywhere (FY)	Stagnant; H_2S	Everywhere (PG)	Same (FY)	Epi/Exo (PA)	Oxygen electrode/ Methylene Blue	126
Oryza sativa cv. Nipponbare	1	NA	NA	Stagnant	Everywhere (F)	Everywhere (FY)	Exo/Scl early; Epi/Exo late (PA)	Oxygen electrode	34, 122, 123
Phoenix dactylifera	3–5	Everywhere (F/S)	Everywhere (FY)	Stagnant	NA	NA	NA	NA	11
Phragmites australis	2–3	Polar lignin cap (F, S)	Everywhere (SR)	NA	Same (PG)	NA	Epi/Exo (PA)	NA	127
Pontederia cordata	2	Everywhere (BAB)	Everywhere (FY)	NA	NA	NA	NA	NA	116
Quercus robur L.	1	NA	Everywhere (SR7B)	NA	NA	NA	NA	NA	128
Rhizophora stylosa	~4	Everywhere (PG)	Everywhere (FY)	NA	NA	NA	NA	NA	22
S. lycopersicum cv. M82	1	Polar lignin cap (F)	Everywhere (FY)	Drought	NA	Increased (FY)	NA	NA	77
Saccharum officinarum	1	Anticlinal (BNaOH)	NA	NA	NA	NA	NA	NA	95
Salix martiana	1	NA	Anticlinal (NR)	NA	NA	NA	NA	Oxygen electrode	30
Sobralia macrantha	1	Polar lignin cap (TBO)	NA	NA	NA	NA	NA	NA	8
Solanum tuberosum	1	Polar lignin cap (F)	NA	NA	NA	NA	NA	NA	77
Tabernaemontana juruana	1	NA	Everywhere (NR)	Stagnant	NA	Increased (NR)	NA	Oxygen electrode	30
Trillium grandiflorum	1	Anticlinal (BNaOH)	NA	NA	NA	NA	NA	NA	95
Triticum aestivum L. cv. Huamai 8	1	Everywhere (PG)	NA	NA	NA	NA	NA	NA	145

(*Continued*)

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Table 2 (*Continued*)

Species	Number of exodermal layers[a]	Lignin morphology	Suberin morphology	Stress test?	Lignin stress	Suberin stress	Function: barrier?	Function: other method	Reference(s)
Typha × *glauca* Godr.	4–6	Everywhere (BTB)	Everywhere (FY, SR7B)	NA	NA	NA	NA	NA	114
Typha angustifolia	4–6	Everywhere (BTB)	Everywhere (FY, SR7B)	NA	NA	NA	NA	NA	114
Urochloa humidicola	1	Inward polar lignin cap (PG)	Everywhere (FY)	Stagnant	Same (PG)	Everywhere (FY)	NA	Oxygen electrode	57
Vanda tessellata	1	Everywhere (S)	Everywhere (SB)	NA	NA	NA	NA	NA	76
Vinca minor	1	Polar lignin cap (BNaOH)	NA	NA	NA	NA	NA	NA	95
Zea mays cv. Cefran	1	Anticlinal (BAB)	Everywhere (SR)	NA	NA	NA	NA	NA	134
Zea mays cv. LG11	1	NA	Everywhere (TEM)	NA	NA	NA	NA	NA	25
Zea mays inbred line Mi29	1	NA	NA	Stagnant	NA	Everywhere (FY)	NA	Not a radial oxygen loss barrier (oxygen electrode); positive barrier (Methylene Blue)	141
Zea nicaraguensis	1	NA	NA	Stagnant	NA	Everywhere (FY)	NA	Radial oxygen loss barrier (oxygen electrode); negative barrier (Methylene Blue)	141
Zea nicaraguensis introgression line 468 (in Mi29)	1	NA	NA	Stagnant	NA	Everywhere (FY)	NA	Radial oxygen loss barrier (oxygen electrode); negative barrier (Methylene Blue)	141

[a]We define an exodermis as any layer (one or more) that is under the epidermis with ligno- or suberized-cell wall thickenings.

[b]Note that we only name a Casparian strip if there is functional evidence and precise positioning.

Abbreviations: BAB, Berberine-Aniline Blue; BNaOH, Berbernine-NaOH; BTB, Berberine-Toluidine Blue; F, Fuchsin; FY, Fluorol Yellow; NA, not available; NR, Nile Red; PA, Periodic Acid; PG, Phloroglucinol-HCl; SR7B, Sudan Red 7B; TBO, Toluidine Blue; TEM, Transmission Electron Microscopy.

were found in. Type I thickenings are found in the innermost cortical layer, sometimes termed the peri-endodermal layer (138). Type II are found in the outermost cortical layer, and type III in the middle cortical layer (138). There are also reports of phi thickenings in the hypodermis (78, 94) and the epidermis (28). Aleamotu'a et al. (2, 3) refined the Van Tieghem definition to include any secondary cell wall outgrowth that forms only in localized bands around cells of the root cortex rather than a uniform secondary wall thickening across the entire wall. However, this definition does not include variations of the classical phi thickening morphology, which indeed are much more complex and pervasive, including reticulate, branched, net-like, crescent, and even pitted structures (**Figure 1**; **Table 3**) that have been found in 22 species (3, 12, 16, 45, 55, 89, 98, 125). Aleamotu'a's definition also excludes hypo/exodermal and epidermal thickenings with phi morphology.

Phi thickening: a lignified anticlinal band, primarily found in cortical cell walls but occasionally present in epidermal cells, with partial or full radial shape that can elaborate into reticulated structures

Histochemical analyses support phi thickenings as lignified wall structures (12, 41, 45–47, 55, 66, 125, 128, 129, 147) (**Table 3**), and a lack of signal when stained with Sudan Red 7B and Sudan III clearly demonstrates that the phi thickening structure is not suberized (55, 66, 125, 128, 144, 147). Antibodies and other stains suggest a dynamic trajectory of phi thickening with varying cell wall composition. Callose marked the position of a newly forming phi thickening in the orchid *Miltoniopsis*, and cellulose appears to form the scaffold for the phi thickening prior to it being encrusted with lignin in *Brassica oleracea* cv. Marathon (1, 55). This cellulose is subsequently likely impregnated with pectin and/or hemicellulose (55, 66).

Phi thickenings are found only in cortical cell types; thus, there must be developmental control of their production. Given their general presence in cortical cell types, phi thickenings are likely under developmental control. Interestingly, phi thickenings are not necessarily radially continuous, and their presence along the root's longitudinal axis (i.e., developmental time) is variable (1, 16, 55, 74, 89). Phi thickenings are highly responsive to the environment, with reports of induction in response to salt, sucrose, diverse growth media, osmotic stress, soil compaction, and drought (3, 41, 56, 74, 89, 125, 128). In some species, phi thickenings are induced upon waterlogging or in aquatic environments (49, 147), while, in another, phi thickenings are decreased upon waterlogging (46).

4.4. Sclerenchyma

The sclerenchyma are uniformly lignified cells within the cortical layer, located inside of the exodermis, and are found in many cereals, some wetland species, and *Phoenix dactylifera* (date palm) (11, 27, 34, 42, 64, 91, 101, 111, 122, 127) (**Figure 1**; **Table 4**). Presently, there is no molecular marker for the sclerenchyma; therefore, this cell layer is only defined based on the presence of uniformly deposited lignin. In addition to lignin-detecting stains and chemical profiling, laser tomography provides an alternative higher-throughput mode to identify sclerenchyma based on its lignified thickenings (111). While in *Oryza sativa* and *G. maxima* the sclerenchyma is uniseriate, multiseriate cortical sclerenchyma has been reported in nodal roots of some modern maize (111).

4.5. Additional Uncharacterized Cell Wall Thickenings

Although not given a specific name, lignin and suberin have additionally been identified in the epidermis of several species (for lignin/likely lignin, see 22, 77, 80, 93, 134, 143; for suberin, see 79, 102, 114, 133, 134). The function and pervasiveness of these structures are unknown.

5. DOES A WALL ALWAYS HAVE TO SERVE A PURPOSE? FORM AND FUNCTION

Cell wall composition is dynamic throughout plant growth, from new synthesis after cell division to loosening during expansion and increasing rigidity in response to the environment. The

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Table 3 Phi thickenings in diverse plant species

Species	Morphology	Epidermal	Outer cortex (type II)	Middle cortex (type III)	Inner Cortex/ peri-endodermal (type I)	Stress inducible?	Reference(s)
Brassica napus Hyda 971 CL	(*a*) Radial, transverse and thick (*b*) Polar endodermal facing in peri-endodermal layer, ladder-like thickenings, and reticulate	NA	NA	Yes (PG)	Yes (PG, B)	NaCl	3
Brassica oleracea cv. Marathon	(*a*) Radial, transverse and thick (*b*) Polar endodermal facing in peri-endodermal layer, ladder-like thickenings, and reticulate	NA	NA	Yes (EM, TBO)	Yes (EM, PG, F, B)	NaCl, sucrose	1, 3
Caesalpinia peltophoriodes	Radial, transverse	NA	NA	NA	Yes (S)	Flooding	49
Cardamine hupingshanensis	NA	NA	NA	NA	Yes (B)	NA	144
Catasetum barbatum	Reticulate	NA	Not provided (EM)	Not provided (EM)	Not provided (EM)	NA	16
Catasetum laminatum	Ledge-like	NA	Not provided (S/AB)	Not provided (S/AB)	Not provided (S/AB)	NA	16
Catasetum oerstedii	Wavy	NA	Not provided (S/AB)	Not provided (S/AB)	Not provided (S/AB)	NA	16
Clowesia russelliana	Pitted	NA	Not provided (S/AB)	Not provided (S/AB)	Not provided (S/AB)	NA	16
Clowesia thylaciochila	Thickened, reticulate	NA	Not provided (S/AB)	Not provided (S/AB)	Not provided (S/AB)	NA	16

(*Continued*)

Table 3 (*Continued*)

Species	Morphology	Epidermal	Outer cortex (type II)	Middle cortex (type III)	Inner Cortex/ peri-endodermal (type I)	Stress inducible?	Reference(s)
Clowesia warscewiczii	Thickened, reticulate	NA	Not provided (S/AB)	Not provided (S/AB)	Not provided (S/AB)	NA	16
Cordaites rootlet (Grand'Croix)	Radial, transverse	NA	NA	NA	Yes (fossil section)	NA	130
Cordaites rootlet (Cuzieu)	Radial, transverse	NA	NA	NA	Yes (fossil section)	NA	130
Cryptomeria japonica	(*a*) Radial transverse for type I (*b*) Net-like for type III	NA	NA	Yes (PG)	Yes (PG)	NA	45
Cunninghamia lanceolata	Radial, transverse	NA	NA	NA	Yes (PG)	NA	45
Cymbidium	Reticulate, pitted	NA	Not provided (EM)	Not provided (EM)	Not provided (EM)	NA	98
Dressleria dilecta	Rod-like	NA	Not provided (S/AB)	Not provided (S/AB)	Not provided (S/AB)	NA	16
Eriobotrya japonica Lindl. "Mogi" loquat	Radial, transverse, complex	NA	NA	Yes (AF)	NA	Drought	89
Gingko biloba	Radial, transverse, net-like	NA	NA	NA	Yes (B, PG)	NA	12
Grammangis ellisii	Helical-like, scattered	NA	Yes (S/AB)	Yes (S/AB)	Yes (S/AB)	NA	16
Juniperus virginiana	(*a*) Radial, transverse for type I (*b*) Net-like for type II	NA	NA	Yes (PG)	Yes (PG)	NA	45
Laelia anceps	Radial, transverse	NA	Yes (F)	Yes (F)	Yes (F)	NA	54
Metasequoia glyptostroboides	Radial, transverse	NA		Yes (B/PG)	Yes (B/PG)	Water inducible	147
Miltoniopsis (*orchid*)	Radial, transverse, occasional branching and interconnection	NA	Yes (B)	Yes (B/PG)	Yes (B/PG)	NA	55

(*Continued*)

Table 3 (*Continued*)

Species	Morphology	Epidermal	Outer cortex (type II)	Middle cortex (type III)	Inner Cortex/ peri-endodermal (type I)	Stress inducible?	Reference(s)
Myrica rubra (Sieb. et Zucc.	U-shaped	NA	Yes (PG)	Yes (PG)	Yes (PG)	Dry conditions	125
Noccaea caerulescens	Radial, transverse	NA	NA	NA	Yes (EM, PG)	NA	66
Pelargonium	(*a*) Radial, transverse in type II (*b*) Scattered in types I and III	NA	Yes (PG)	Yes (PG)	Yes (PG)	NA	47
Pelargonium hortorum	Radial, transverse	NA	Yes (B/AB)	NA	NA	NA	78, 94
Prunus avium L.	Radial, transverse	NA	NA	Yes (PG)	Yes (PG)	NA	128
Rhizophora mangle from Santa Cruz site	Radial, transverse	NA	Yes (PG)	Yes (PG)	Yes (PG)	NA	129
Sequoiadendron gigantea	(*a*) Radial, transverse in type I (*b*) Net-like in type II	NA	NA	Yes (PG)	Yes (PG)	NA	45
Sinapis alba	Radial, transverse; net-like structure at periclinal endodermal face	NA	NA	NA	Yes (B)	Sucrose	3
Thlaspi caulescens	Radial, transverse; pitted, crescent thickenings at periclinal endodermal face	NA	NA	NA	Yes (B)	Sucrose	3
Taxus cuspidata	Radial, transverse; crescent at periclinal endodermal face	NA	NA	NA	Yes (PG)	NA	45
Taxus media	Radial, transverse	NA	NA	NA	Yes (fossil section)	NA	81
Thuja occidentalis	Net-like	NA	Yes (B/AB, F)	Yes (B/AB, F)	Yes (B/AB, F)	NA	45
Z. mays cv. Garant FAO 240	NA	Yes (B)	NA	NA	NA	Slag	28

Abbreviations: AF, autofluorescence; B, berberine; B/AB, Berberine/Aniline Blue; EM, electron microscopy; F, basic fuchsin; NA, not available; PG, phloroglucinol; S/AB, Safranin/Astral Blue.

Table 4 Sclerenchyma identified in multiple plant species

Species	Number of sclerenchyma layers	Lignin identification	Reference(s)
Aegilops speltoides tausch 79TKO21-131	Unknown	No (LT)	111
Aegilops tauschii TA2570	Unknown	No (LT)	111
Glyceria maxima	1	Yes (PG)	127
Hordeum vulgare (Arena)	~6–7	Yes (LT)	111
Hordeum vulgare (Golf)	~4–5	Yes (LT)	111
Hordeum vulgare (Barke)	Unknown	No (LT)	111
Hordeum vulgare (BCC776)	Unknown	No (LT)	111
Hordeum vulgare (HOR4727)	Unknown	No (LT)	111
Hordeum vulgare (MorexIPK)	Unknown	No (LT)	111
Hordeum vulgare (Nurenburg)	Unknown	No (LT)	111
Hordeum vulgare (Tkn24b)	Unknown	No (LT)	111
Oryza glumaepatula W2165	1	Yes (PG, chemical)	34
Oryza sativa cv. IR42	1	Yes (PG)	65
Oryza sativa cv. Azucena	1	Yes (B, PG, Maule)	64, 101
Oryza sativa cv. Dawn	2–3	Yes (PG)	27
Oryza sativa cv. Nipponbare	1	Yes (PG)	34, 122
Oryza sativa cv. Pamp	1	Yes (PG)	27
Oryza sativa cv. Selenio	1	Yes (B)	27
Phoenix dactylifera	~2–3	Yes (PG)	11
Triticum aestivum (Einkorn)	Unknown	No (PG)	111
Triticum aestivum (Hope)	~3	Yes (LT)	111
Triticum aestivum (Marfed)	~3	Yes (LT)	111
Triticum aestivum (Paragon)	~2	Yes (LT)	111
Triticum aestivum (Seri82)	~3–5	Yes (PG, LT)	111
Triticum aestivum (Vandal)	~4	Yes (LT)	111
Triticum aestivum (Chinese spring)	Unknown	No (LT)	111
Triticum aestivum (Currawa)	Unknown	Yes (LT)	111
Triticum aestivum (Era)	Unknown	No (LT)	111
Triticum aestivum (Marquis)	Unknown	No (LT)	111
Triticum aestivum (Sonora)	Unknown	No (LT)	111
Triticum aestivum (Weebill 1)	Unknown	No (LT)	111
Triticum turgidum durum Mahmoudi 552	Unknown	No (LT)	111
Triticum urartu G1812	Unknown	No (LT)	111
Triticum timopheevii (Zhuk) 357	Unknown	No (LT)	111
Triticum turgidum dicoccoides G3211	Unknown	No (LT)	111
Triticum turgidum dicoccoides PI233288	Unknown	No (LT)	111
Triticum turgidum dicoccon Khapli	Unknown	No (LT)	111
Triticum turgidum durum Malvi Ekdania 69	Unknown	No (LT)	111
Triticum zhukovskyi 69Z5.71	Unknown	No (LT)	111
Zea mays ssp. mexicana (Ames 21808)	Unknown	No (LT)	111
Zea mays ssp. mexicana (Ames 21857)	Unknown	No (LT)	111
Zea mays ssp. mexicana (Ames 8083)	Unknown	No (LT)	111
Zea mays ssp. mexicana (PI 566674)	Unknown	No (LT)	111

(*Continued*)

Table 4 (*Continued*)

Species	Number of sclerenchyma layers	Lignin identification	Reference(s)
Zea mays ssp. parviglumis (PI 384063)	Unknown	No (LT)	111
Zea mays ssp. parviglumis (Ames 21861)	Unknown	No (LT)	111
Zea mays ssp. parviglumis(Ames 21803)	Unknown	No (LT)	111
Zea mays ssp. parviglumis (Ames 21814)	Unknown	No (LT)	111
Zea mays ssp. parviglumis (Ames 21830)	Unknown	No (LT)	111
Zea mays ssp. parviglumis (Ames 21802)	Unknown	No (LT)	111
Zea mays IBM14	~7	Yes (LT)	111
Zea mays IBM146	Unknown	Yes (LT)	111
Zea mays IBM178	Unknown	No (LT)	111
Zea mays IBM284	Unknown	No (LT)	111
Zea mays IBM86	Unknown	Yes (LT)	111
Zea mays OHW128	Unknown	No (LT)	111

Abbreviations: LT, laser tomography that can identify multiseriate cortical sclerenchyma; PG, phloroglucinol.

vast range of types of cell wall thickenings across plants lends support to their importance as evolutionary adaptations facilitating growth and reproductive success under varying environmental conditions. However, determining their exact function is not a straightforward endeavor. In *Arabidopsis*, genetic tools facilitate probing of one cell wall type and its composition relative to its function. Exemplary experiments on the *Arabidopsis* CS showed, by apoplastic tracer assays coupled with perturbation of either lignin or suberin biosynthesis, that the lignified CS is the primary apoplastic barrier in root development (87). Additional experiments used the vast array of CS and suberin mutants to specify the roles of each in plant physiology. While suberin directly affects root hydraulic conductivity (131), the CS directly blocks mineral solute entry into the xylem stream (17). Endodermal suberin levels are also modulated by altered nutrient status (deficiencies or increased abundance), and depletion of suberin in the endodermis perturbs elemental composition in the shoot and blocks the coupled transcellular pathway (5). Suberin plasticity is genetically controlled by nutrient availability (5), as well as hormone signaling, which is elegantly demonstrated by the use of cell type–driven mutant signaling factors (5, 124).

Unfortunately, these tools are not present in the majority of species described in this review, and the function of additional cell wall thickenings is largely inferred through correlative evidence. In this section, we summarize these data and provide a perspective on available tools that could be used to conclusively determine their function.

5.1. Velamen Wall Thickenings Enable Growth in Unusual Environments

Coupled transcellular pathway: transport between plant cells that occurs through polarized influx and efflux transporters

The velamen has best been described in epiphytic orchids. These possess spongy roots that consist of a multiseriate velamen with hollow cells encased by elaborate cell wall structures. Zotz & Winkler (151) found that rapid water uptake (~15 s) and slow water loss were positively correlated with velamen thickness in multiple orchid species. This relationship was corroborated when comparing the epiphytic orchid *Cymbidium tracyanum* with the terrestrial orchid *Cymbidium sinense*, where the drought-tolerant epiphytic orchid had a higher ratio of velamen thickness relative to root diameter (72). Velamen thickness was also positively correlated with leaf area and dry mass among 21 *Dendrobium* species (99). In addition to functional traits associated with water relations, the velamen can take up P_i and Rb in multiple orchid species and is responsible for ~80% of uptake relative to the root tip (151). Finally, flavonoid molecules located within the velamen of

photosynthetic roots act as ultraviolet (UV) protectants (23). In the future, establishment of tools to genetically probe and identify ideal velamen cell patterning and secondary cell wall architecture for specific stressors could help to enable environmental resilience.

5.2. The Exodermis as Goldilocks: Facilitating Responses to Too Little or Too Much Water

In conditions of flooding or in stagnant water, there is little oxygen availability to the root, resulting in hypoxia or anoxia. Plant roots have evolved two mechanisms to facilitate survival in these environments. First, exodermal cells are lignified or suberized to provide a radial oxygen loss barrier. Second, aerenchyma—air spaces resulting from programmed cell death in cortical cell files—can retain oxygen. These developmental features do not act in isolation. In this section, we review evidence for exodermal cell wall thickenings acting as a radial oxygen loss barrier, with the caveat that aerenchyma additionally contribute to this phenomenon. The barrier function of the wall is tested using apoplastic tracers, while its ability to prevent radial oxygen loss is assessed via oxygen-sensing electrodes or Methylene Blue (35, 64, 123, 127) (**Figure 2**; **Table 2**). A review of the literature where paired measurements were made in control and oxygen-deprived conditions, usually in adventitious roots, suggests that lignified or suberized exodermal barriers are present in both control and stress conditions with no change in magnitude, present in both conditions but with increased magnitude in the stress condition, or absent in a nonstress condition and present in a stress condition (30, 34, 35, 57, 64, 65, 101, 127). In cases where cell wall thickenings increase in intensity in response to stagnant conditions, there is often greater deposition closer to the tip (**Figure 2**). Both of these result in less oxygen loss along the root's longitudinal axis (64, 65, 101) (**Figure 2**). Exodermal barriers not only function to regulate oxygen loss in excess water but also control salt and water uptake in excess or limiting conditions, respectively (19, 68, 120).

Which constituent of the exodermal wall is responsible for barrier function in stagnant conditions—lignin or suberin? The history of research on the CS tells us that we should not necessarily accept the dogma that suberin is the critical component. Based on the available literature (**Table 2**), both lignin and suberin are present in the exodermis of multiple plant species when there is a functional barrier (35, 36, 68, 127). Genetic analysis aimed to untangle this conundrum in rice. Shiono et al. (121) tested the requirement for exodermal suberization via a mutation in the ABCG transporter (RCN). ABCG transporters of this type have been linked to suberin monomer transport (148). The exodermal barrier is inducible in *O. sativa* cv. Nipponbare (**Figure 1*b***); the presumable CS (recognized by berberine staining), lignin, and suberin (Phloroglucinol and Fluorol Yellow) are present only in stagnant conditions. In the *rcn* transporter mutant, these features are not induced, although there is increased lignin in the sclerenchyma (121). Chemical profiling of suberin in the outer part of the root (epidermis, exodermis, sclerenchyma, and other inner cortex cells) indicated half of the amount of aliphatic suberin in the mutant, in stagnant conditions, and approximately double the amount of aromatic suberin. No quantification of total lignin was performed. Apoplastic tracers linked the defect in exodermal cell wall composition to a compromised apoplastic barrier function. In wild type, blockage occurred at the junction between the epidermis and exodermis in stagnant conditions, while there was no blockage in the mutant (121). This suggests that suberin composition is definitely important to barrier function but does not exclude the role of lignin.

The hormone abscisic acid (ABA) underlies exodermal suberin accumulation in excess water and in water deficit. Perturbation of ABA biosynthesis in rice using fluridone reduces both aliphatic and aromatic suberin in the outer portions of the root in stagnant conditions; while application of 10 μM ABA was sufficient to induce aliphatic and aromatic suberin in aerated conditions only

(123). The loss and gain of suberin were reflected accordingly in measurements of radial oxygen loss (higher and lower, respectively). In the tomato root, suberin levels increase when exogenous ABA is applied (19). Reduced exodermal suberin by mutation of the tomato biosynthetic enzyme ASFT, and of the transcription factor MYB92, with no corresponding change in lignin levels, resulted in the plants' increased sensitivity to reduced water availability (19). Again, these results suggest that exodermal suberin is a barrier component that controls plant water relations.

The polar lignin cap in the tomato root exodermis is also found in other solanaceous species (77). Its function as an apoplastic barrier was determined in tomato by the addition of an apoplastic tracer to the wild type in control conditions, in the presence of a lignin biosynthetic inhibitor, and upon rescue with an exogenous application of monolignols (77). Further, this lignified barrier acts to regulate ion uptake, with changes in aboveground mineral ion composition (77). Based on this evidence, it is possible that lignin and suberin in the exodermis can have distinct functions in interacting with the environment. Mutants that can truly uncouple exodermal lignin and suberin are needed to support this conclusion.

5.3. The Unknown Function of Phi Thickenings

Although phi thickenings are highly responsive to the environment, there is only one report stating that they can act as an apoplastic barrier (14). Use of apoplastic tracers in *Zea mays* cv. Garant FAO240 (corn), *Miltoniopsis* (orchid), *Malus domestica* (apple), and *Pelargonium hortorum* (geranium) showed movement throughout the apoplast despite the presence of phi thickenings. Movement was only blocked at the endodermal apoplastic barrier (28, 56, 94). By contrast, when propidium iodide was used as a tracer in *Noccaea caerulescens*, apoplastic blockage was observed in the peri-endodermal layer with phi thickenings (66). Thus, the majority of the evidence rejects phi thickening function as an apoplastic barrier. The apoplastic tracer experiment with *N. caerulescens* should be reperformed with localized blockage of localized lignin biosynthesis to determine if this conclusion stands. Alternative functions for phi thickenings could include providing mechanical strength, such as in the case of sclerenchyma, or contributing to perception and signaling with biotic organisms (104). Given these collective data, a refined definition of a phi thickening is that it is a lignified band present nearly exclusively in cortical cell walls, with partial or full radial shapes—which can elaborate into reticulated structures with varying densities, including at periclinal walls—that likely does not serve as an apoplastic barrier.

5.4. Strong Sclerenchyma

The exact functional importance (with genetic evidence) of the sclerenchyma's secondary cell wall is unknown, although many lines of correlative evidence do exist. Increased lignin levels in *O. sativa* cv. Dawn are correlated with resistance to the pest rice water weevil (*Oryzophagus oryzae*) (27). In response to silica addition, lignin levels increase in rice (*O. sativa* cv. Selenio) sclerenchyma as determined by berberine staining (42). A potential role for sclerenchyma as a radial oxygen loss barrier is indicated by apoplastic tracer assays in *Oryza glumaepatula* (W2165) and *O. sativa* cv. Nipponbare. In aerated conditions, there was an increase in lignin within the sclerenchyma lignin in the middle of the root, and an apoplastic tracer was blocked between the exodermis and the sclerenchyma (34). Perhaps the most extensive data collected with respect to the sclerenchyma are an assessment of multiseriate cortical sclerenchyma (lignified sclerenchyma) presence relative to a variety of functional traits in modern cultivars, land races, and wild accessions of maize, barley, and wheat (111). Among these grasses, root tensile strength and penetration ability in compacted soils were positively correlated with the presence of multiseriate cortical sclerenchyma. This type of sclerenchyma is absent in land races and wild accessions, suggesting that it may have been

selected for during domestication. One can also hypothesize that lignin within the sclerenchyma wall could be associated with root hydraulic conductivity and ion uptake (particularly given the silica responsiveness). However, the influence of the exodermal and endodermal barriers would need to be experimentally and genetically uncoupled from the sclerenchyma in order to unequivocally determine such a function.

6. REGULATORY PATHWAYS

6.1. Evolutionary Novelty, Co-Option, and Rewiring

Numerous types of cell wall thickenings are present in plant species. Such thickenings vary in morphology, chemical composition, the cell type in which they are present, and their environmental responsiveness. How have their regulatory pathways arisen and changed over evolutionary time? While there is extensive molecular data describing the mechanisms by which the CS and suberization occur in the *Arabidopsis* endodermis, only a paucity of this type of data exists for these two endodermal cell wall structures in other plant species except for rice, rendering the question of their evolutionary origins difficult to answer. In the case of the exodermis, such molecular data have only just been revealed in tomato (19, 77). No direct evidence exists for genes responsible for the velamen, sclerenchyma, or phi thickenings.

Given this conundrum, we propose four scenarios for the regulation of the cell wall thickenings described in this review. We further speculate about which model may be more likely given existing data for exodermal and endodermal lignification and suberization.

6.1.1. A cell wall network was originally present in multiple cell types and was lost in one or more of these cell types in a given species. Here, the same regulators and enzymes (orthologs) would control deposition of a cell wall component in multiple cell types of multiple species. Loss of a pathway in one cell type in one or more species could occur by loss/perturbation of cell type–specific regulatory elements in these pathway genes or by loss of a cell type regulator. Indeed, suberin is present in the exodermis and endodermis of many species (**Table 2**). *Solanum lycopersicum* and its wild relative *Solanum pennellii* both have only exodermal and not endodermal suberin (19). Characterization of exodermal and endodermal suberization in other *Solanum* species could be used to test this hypothesis. Indeed, potato (*Solanum tuberosum*, which is related to tomato) has both a suberized exodermis and endodermis (32). If the same genes regulate exodermal and endodermal suberization in multiple *Solanum* species, and tomato and *S. pennellii* are outliers, then this could provide evidence that the endodermal suberin pathway was lost in tomato and this wild relative.

6.1.2. A whole network was completely co-opted to control cell wall thickenings in a given cell type. This is an alternative to the model described in Section 6.1.1. There is no endodermal suberization in the tomato or *S. pennellii* primary root under the conditions examined, and the core steps of suberin biosynthesis and its regulation are localized to the exodermis and have the same environmental responsiveness in tomato (19, 142). In this scenario, related species would have either exodermal or endodermal suberization. The presence of the suberin module in one cell type would be sufficient for function. The same genes would be used, but in a different place, and would be inherently flexible in their function irrespective of cell type. Study of other tomato relatives and their more recent common ancestors can provide additional data to see if such flexibility does occur.

6.1.3. Regulators and enzymes are duplicated and sub-functionalized to drive these processes in other cell types. Gene duplications give rise to multiple family members. Members

sub-functionalize such that each member functions in a different cell type. There is evidence for this model in rice where both the exodermis and endodermis are suberized. Here, a mutation in the presumed suberin monomer transporter *RCN1/OsABCG5* dramatically reduces exodermal but not endodermal aliphatic suberin (121), suggesting that other related suberin transporters act within the rice endodermis. We propose that this scenario is the most likely when there is deposition of a cell wall modification of a similar morphology in multiple cell types. Thirteen species have anticlinal deposition of exodermal lignin (**Table 2**). It is reasonable to hypothesize that the genes responsible for the central positioning of the endodermal CS within the anticlinal cell walls may have been duplicated and sub-functionalized to control this process in their exodermis. Deposition of lignin in the velamen and phi thickenings is helical or pitted, similar to that observed in xylem cells (69) (**Figure 1**; **Tables 3** and **4**), and could be controlled by paralogs of the VND6/VND7 transcription factors that arise by local or genome duplications. Single-cell transcriptome profiling in these species could identify candidate genes that operate under this scenario. Subsequent mutation of each member of the duplicate pair could test whether this hypothesis is true.

6.1.4. Uncharacterized novel regulatory genes and pathways. Exodermal lignification in tomato appears to represent one of these cases. Reverse genetic experiments demonstrated that the tomato exodermal polar lignin cap does not share known regulators with the lignified CS, and, to date, no activators of the tomato polar lignin cap have been identified (77). Additionally, repressors of exodermal polar lignin cap deposition were identified in inner cell types (77), suggesting that regulation of this pathway is distinct from any previously described endodermal regulation. There are reports of less common morphologies of lignin deposition in the exodermis, such as an inward-facing polar lignin cap and an upside-down Y (57, 79, 80) that could represent such novelty.

These four hypothetical scenarios are not necessarily mutually exclusive and all-encompassing. Distinct elements of these complex regulatory networks might each fall into a different category. Additionally, signaling pathways that perceive environmental changes and elicit or enhance thickening for adaptive purposes must exist, increasing the complexity of the regulation of cell wall thickenings. Environmentally adaptive thickening must integrate three likely discrete modules (**Figure 1*b***): cell type developmental programs, metabolic regulation, and factors that coordinate exactly where in a cell the thickening is deposited. Cell wall thickenings also respond to environmental signals by turning on earlier or later in developmental time. Thus, these cell wall thickenings must also integrate temporal developmental cues (**Figures 1*b*** and **2**). These stages in regulation of thickening are outlined below, as well as illustrative examples from *Arabidopsis* that provide a framework for our current understanding of these processes.

6.2. Cell Type Dependency

One defining factor for these different categories of cell wall thickenings is their presence in a specific cell type. Such spatial restriction suggests that their synthesis and deposition are dependent on the specification of a given cell type identity and its respective differentiation program. Potential paradigms can be found in the endodermis and xylem differentiation programs, where both cell-autonomous and non-cell-autonomous cues are responsible for the cell type–dependent deposition of the CS and the xylem secondary cell wall. In the case of the CS, this includes the nonautonomous transcription factor SHORTROOT (SHR) that activates an autonomous transcriptional network to specify endodermal identity and differentiation (resulting in the CS) (24, 26, 48, 84). In the xylem, non-cell-autonomous hormone signals and mobile microRNAs specify xylem cell identity (9, 20), with subsequent activation of an autonomous transcriptional network that controls xylem differentiation (the secondary cell wall) (69). Chromatin-mediated repression keeps the xylem differentiation program off in nonvascular cells (29, 132), and, in tomato, the

SlSCZ and *SlEXO1* transcription factors repress formation of the polar lignin cap in inner cortical cells (77).

6.3. Metabolism

From the metabolic perspective, many of the families of enzymes and transporters that control lignin and suberin biosynthesis and deposition have been identified. However, these gene families have undergone expansion and loss within angiosperms (117, 136). Thus, we lack knowledge of which specific genes are responsible for synthesizing and depositing lignin and suberin in the diverse examples of cell wall thickenings. Chemical profiling methods can provide us with monomeric composition at the whole-root resolution or from bulk measurements of the outer sleeve [epidermis + exodermis + inner cortical layer(s)] relative to the endodermis and vascular tissue. What we know less of is the extent of variability in biopolymeric composition at cell type resolution—both between cell types in a single species and within the same cell type in different species. This is not yet experimentally tractable with modern chemical profiling approaches. Plant cell wall glycan-directed monoclonal antibodies, as well as those that can recognize G- and S-containing lignin within the cell wall (50), and fluorescently labeled monolignols (113) can provide clues to this complexity. Unfortunately, these cannot be used to detect changes in suberin monomer composition. Nonetheless, linking the different enzymes and regulators to specific cell wall composition in a given cell type and to their function (i.e., strengthening, penetration, water uptake or retention, or apoplastic blockage) is critical to assessing the diverse ways in which evolution has facilitated the coordination of metabolism to produce these elegant and functional structures.

6.4. Cellular Location

Considering the number of adjectives used to describe cell wall thickening morphologies, one cannot help but assume that their position within the context of the cell's three-dimensional shape must be not only precisely regulated but also functionally advantageous. Again, we turn to *Arabidopsis* as our cornerstone for putative models. Cell wall components are trafficked to polar nano- or microdomains within the plasma membrane. Possible factors that could be linked to the asymmetric (polar) deposition observed for the polar lignin or polar suberin cap in the plant species described in **Table 2** include *INFLORESCENCE AND ROOT APICES RECEPTOR KINASE* (*IRK*) and *KINASE ON THE INSIDE* (*KOIN*), which have polar (either facing to the outside or the inside of a root, in a cell type–dependent manner) periclinal accumulation (18, 106). Their polar localization is dependent on endomembrane trafficking and targeted secretion to the plasma membrane (18, 106). One could hypothesize that signaling proteins of this type could positionally direct cell wall components (either monolignols or suberin monomers) to this location. Further local cues are likely, and presumably non-cell-autonomous, to indicate if the polarity domain is located on the outward-facing or inward-facing periclinal cell wall. In the case of precise radial positioning of the CS, the CASPARIAN STRIP MEMBRANE DOMAIN PROTEINs (CASPs) assisted by the exocyst complex are the first to mark CS formation (60, 108). They establish a protein-exclusion plasma membrane domain and conduct enzymatic activities essential for the proper sealing of the CS (6). A dirigent domain-containing protein complex that includes ENHANCED SUBERIN1 is recruited to the CASP location in the plasma membrane, where it is required for lignin polymerization and assembly in a CS (53). In rice, the OsCASP1 protein similarly defines the CS domain, and mutation of *OsCASP1* results in a broader accumulation of lignin along the endodermal radial walls, which could be interpreted as conservation in the formation of the CS (140, 149). Given this, it is reasonable to speculate that other CASPs or CASP-like

proteins may be similarly responsible for the anticlinal deposition of lignin in the exodermis in other plant species or even the radial, transverse structure of phi thickenings in cortical cells.

Apoplastic pathway: passive diffusion through the plant cell wall extracellular space (apoplast)

Symplastic pathway: transport between plant cells that occurs through plasmodesmata

Monolignol and suberin monomer (ABC) transporters act downstream of positional (polar or nonpolar) cues to secrete these compounds outside of the plasma membrane (113, 121; reviewed in 117). In the case of xylem secondary cell wall synthesis, ABC transporters are located in discrete plasma membrane domains that form the base of helical secondary cell wall deposition. Oxidative enzymes such as Respiratory Burst Oxidase H (RBOH), laccases, and peroxidases can all polymerize lignin but in different contexts depending on the type of cell wall thickening (107, 113). In xylem, laccases also are located in discrete domains where the helical secondary cell wall is laid down. Given the similarity in secondary cell wall thickenings of the velamen and phi thickenings, it is plausible to consider that ABC transporters and laccases would be localized in a similar manner in these cell types. In the case of the phi thickening, however, these elaborated shapes seem to be anchored in the rectangular phi thickening structure; thus, this structure may serve as a first enucleating point.

7. DEVELOPMENT AND PHYSIOLOGY

In this review, we present a compendium encompassing the spectrum of known root cell wall thickenings and their astounding diversity in roots and across different species. But amid this diversity, some questions emerge: What threads of commonality bind these thickenings together, and where do their differences lie? Can these cell wall thickenings functionally compensate for one another? When considering these questions, we begin first with nutrient transport, which occurs via three different pathways: apoplastic, symplastic, and coupled transcellular. These pathways have been defined based on studies in *Arabidopsis* roots using tracer assays (dyes or fluorescent molecules that visualize symplastic or apoplastic transport) as well as functional characterization of polarized nutrient transporters (5, 100, 124). The apoplastic pathway constitutes transport between cells through the apoplast, which is then barred by the endodermal CS. Intercellular transport in the symplastic pathway occurs via plasmodesmata. In the coupled transcellular pathway, transport occurs via polarized carriers and is then barred by endodermal suberin (**Figure 3**). How then does transport via these pathways occur when there are lignified or suberized cell wall thickenings in cell types other than the endodermis?

In tomato, two of these pathways are distinct from what has been described in *Arabidopsis*. Apoplastic tracer assays in the presence and absence of lignin biosynthetic inhibitors and exogenous monolignol application demonstrated that the tomato polar lignin cap in the exodermis blocks apoplastic transport (77). Therefore, relative to *Arabidopsis*, the tomato apoplastic pathway is constricted and blocked at the exodermis. In the coupled transcellular pathway in *Arabidopsis*, nutrients move via selective polarized transporters and are then predicted to be barred by endodermal suberin (5). In tomato, suberin is present only in the exodermis. It is unknown whether exodermal suberin can block nutrient movement via the polarized transporters. If it does, the coupled transcellular pathway will also be significantly restricted in the tomato root relative to *Arabidopsis* (**Figure 3**). Potato and rice both have exodermal and endodermal suberin. Does suberization in the exodermis bar nutrient movement via polarized transporters, also shortening the path length of the coupled transcellular pathway? Alternatively, passage cells (i.e., cells lacking suberin) could also facilitate continued transcellular transport (52). A final overarching question remains: How would these differences in the apoplastic and coupled transcellular pathways influence shoot physiology and nutrient relations?

Functional compensation in the case of barrier loss is illustrated in *Arabidopsis*, where a surveillance system stands vigilant in the endodermis, detecting and addressing defects in the CS. The

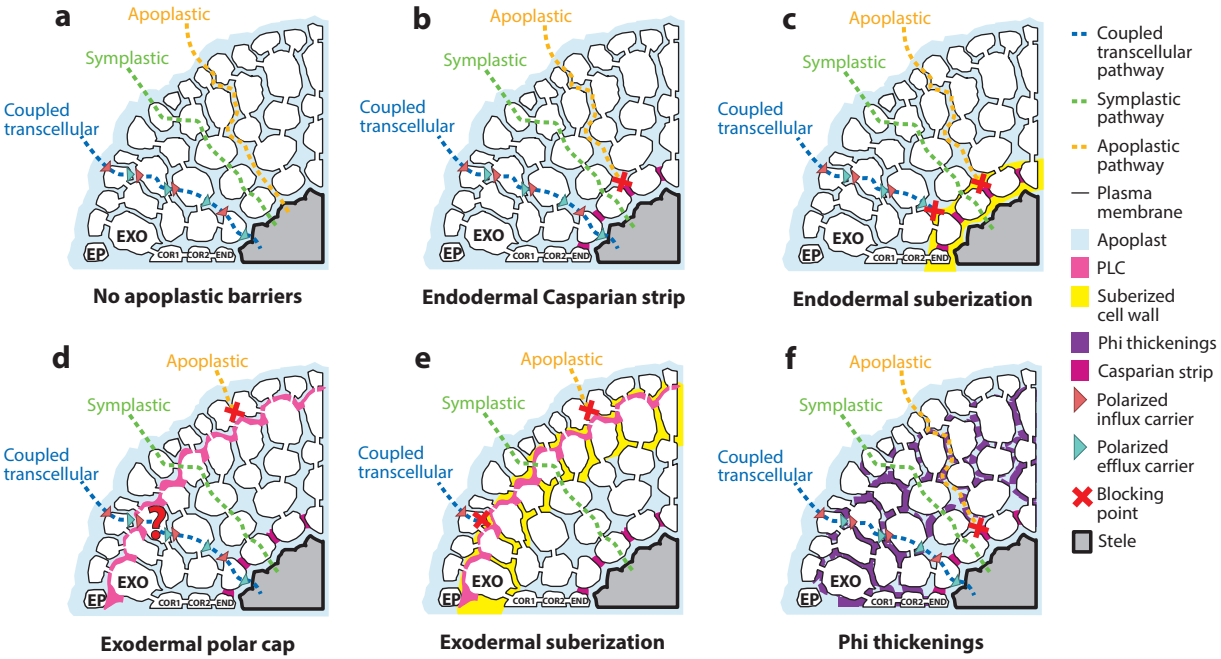

Figure 3

Pathways for radial transport of molecules into the vasculature. (*a*) Root with no apoplastic barriers. The apoplastic, symplastic, and coupled transcellular pathways are active in all cell types. (*b*) Root with a Casparian strip in the END. The apoplastic pathway is blocked at the endodermal Casparian strip (END), while the symplastic and coupled transcellular pathways are active. (*c*) A root with an endodermal Casparian strip and suberin lamellae. The apoplastic pathway is blocked by the Casparian strip, while the coupled transcellular pathway is blocked by the endodermal suberin lamellae (END). (*d*) A root with an exodermal PLC and an endodermal Casparian strip. At the EXO, the apoplastic pathway is blocked at the PLC, the symplastic pathway is probably active, and it is unknown whether the coupled transcellular pathway is active. At the END, the symplastic pathway continues to be active, and if the coupled transcellular pathway is active in the EXO, then it will continue to be active in the END. (*e*) A root with an exodermal PLC, exodermal suberin lamellae, and the endodermal Casparian strip. At the EXO, the apoplastic pathway is blocked by the PLC, the coupled transcellular pathway is blocked by the suberin lamellae, and the symplastic pathway is active. The symplastic pathway continues to be active at the END. (*f*) A root with phi thickenings. The apoplastic, symplastic, and coupled transcellular pathways are active in all cell types. Abbreviations: COR1, inner cortical layer 1; COR2, inner cortical layer 2 or peri-endodermal layer; END, endodermis; EP, epidermis; EXO, exodermis, PLC, polar lignin cap.

stelar CASPARIAN STRIP INTEGRITY FACTOR1 (AtCIF1) and AtCIF2 peptides diffuse and bind to the AtSGN3 receptor, activating a compensatory pathway resulting in corner lignification and early endodermal suberization when the CS is perturbed (31, 85). There is evidence for evolutionary conservation of this surveillance system in the tomato and rice endodermis (77, 149). It is less clear whether other surveillance mechanisms exist and activate when cell wall thickenings in the exodermis, cortical cells, or sclerenchyma are disturbed. To explore potential compensation mechanisms among these barriers in these species, it is essential to generate mutants lacking lignin within the exodermis, sclerenchyma, and endodermal CS or suberin to meticulously compare the effects of alterations of each of these wall structures. Lignin in root cell wall thickenings is associated with multiple functions—as a barrier in the exodermis and endodermis but also as a determinant of tensile strength and penetration ability (111). It is possible that lignin in another cell type, such as the sclerenchyma or even in phi thickenings, could compensate for a perturbation in exodermal lignin or exodermal suberin. The REDUCED CULM NUMBER1 (RCN1)

ATP-binding cassette transporter in *O. sativa* cv. Nipponbare is a likely exodermal suberin transporter (121). Decreased exodermal suberin is present within the *rcn1* mutant, with a concomitant increase in sclerenchyma lignin, in stagnant conditions (121). This increased sclerenchyma lignin supports the hypothesis that lignification or suberization of other cell types may serve as functional compensation for the lack of a cell wall thickening.

Stress-induced changes in cell wall thickening types, positions, and morphologies may facilitate plasticity—that is, tailored solutions to meet heterogenous environmental cues (39). This plasticity could be linked to gradients of nutrient availability within the soil matrix, with more of a need for selectivity in upper parts of the soil, which is supported by asymmetric exodermal barrier deposition in split root systems with different nutrient levels (86). Sections 4 and 5 of this review describe numerous examples of the stress responsiveness of cell wall thickenings. In the simple case of the *Arabidopsis* root, suberin in the endodermis is deposited closer to the root tip in response to salt stress (5). Can distinct stresses be recognized by the root such that responses to multiple stresses are partitioned across multiple cell wall thickenings? This could include differential cell wall composition, morphologies, and deposition in different cell types [e.g., in a single layer of a multiseriate exodermis or a phi thickening in only a subset of cortical cells (10, 40, 70)].

8. A FRAMEWORK FOR THE FUTURE

A common thread throughout this review is the importance of *Arabidopsis* in the provision of a conceptual framework to interrogate cell wall thickenings, from their form to function. It is now time to move past our reliance on this useful plant species, to take advantage of newly developed analytical and functional tools, and to explore new model systems. These efforts should be guided by fundamental questions and similar tools developed to enable cross-comparisons in each respective species. In order to determine the dependence of cell wall thickenings on a given cell type, our efforts should (*a*) define the programs responsible for epidermal and cortex cell specification in representative plant species, (*b*) determine if the disruption of these cell identities results in loss of the cell wall thickening, and (*c*) determine whether the particular cell wall thickenings are able to be (re)produced in other cell types. Forward mutant screens should still be a fundamental assay to identify genes responsible for cell type specification and differentiation. The variety of histochemical dyes presented in this review coupled with microscopy can provide a rapid view of cell wall composition and/or differentiation status. A caveat with such screens is that any phenotype requiring the sectioning of material can be laborious. Ideally, methods such as two- or multiphoton microscopy can be used to image cell layers deep within root tissue but need to be optimized with reporters of cell wall thickening composition. Cell wall–directed antibodies can assist in the discrimination of cell type identity in both uniseriate and multiseriate cell layers (as in 58) and can be used to determine a more specific composition of a given thickening, for instance, prevalence of S- versus G-lignin monomers or specific hemicellulose molecules.

Reverse genetic approaches can be used to test whether orthologous genes that control, for instance, the polar lignin cap in the tomato exodermis also control it in the distantly related *Phragmites australis* (**Table 2**). Single-cell transcriptome profiling can provide additional reverse genetic candidates for early cell-autonomous regulators (19). In both of these cases, though, transformation capacity precludes our ability to use reverse genetics to test their function. Thus, when choosing representative species to study a given cell wall thickening, transformation capacity should be one of the most important criteria. Identification of non-cell–autonomous cues could include the blunt force tool of external application of plant hormones, the more elegant use of tissue-specific drivers, the optogenetic control of mutants in hormone signaling pathway genes, or even translation of the inducible callose synthase system to block plasmodesmal transport in specific tissues.

An additional theme with respect to these cell wall thickenings is the incredible diversity in their morphology within a given cell. In order to identify the genes that control these diverse patterns, fluorescent and other reporters must be generated to determine the influence of transporters, peroxidases, laccases, CASP-like, and other as-yet-unidentified proteins in setting up their respective subcellular locations on the plasma membrane. Again, this will require the application of cutting-edge microscopy techniques such as light sheet and super-resolution microscopy (88).

Determining the function of these cell wall thickenings is equally important in future studies. We must distinguish between lignin and suberin in their contribution to apoplastic barrier function. This will require cell type–specific disruption of lignin and suberin biosynthesis, either genetically or chemically, coupled with apoplastic tracers and other functional assays. However, not all cell wall thickenings function as apoplastic barriers. The use of genetically diverse germplasm and high-throughput techniques to visualize plant anatomy linked multiseriate cortical sclerenchyma to two distinct root traits (111). Employment of such methods is complementary to previously described molecular approaches. Further, they leverage the extensive histories of plant domestication and breeding germplasm. Once these additional functions are determined, they still must be linked to a given morphology by targeted disruption of lignin or suberin biosynthesis, cell type specificity, and subcellular location.

The link between these cell wall thickenings and environmental responses seems clear, but the exact ways that this link occurs are less so. Can specific stresses elicit changes in cell wall morphology or chemical variation in the cell wall of a single cell type, directly enabling an adaptive response to the given stress? If so, how does this effect change when this stress is part of an onslaught of multiple, simultaneous environmental changes? If we study species that are tolerant to specific abiotic or biotic perturbations, do they have a preferred way of producing a cell wall thickening that enables this tolerance? If yes, can this mode be introduced into another sensitive species with the same positive consequence? Setting up these tools will not be straightforward, but their potential is immense. It is awe inspiring to review the literature over the last century and more, where careful observations of plant cells and their modifications led to profound conclusions regarding the way that plants have evolved to interact with a harsh environment. Future research on root cell wall thickenings must continue to learn from the past, while embracing novel technologies applied to diverse species, to understand how to harness these intricate structures to build a more resilient future.

SUMMARY POINTS

1. Multiple types of cell wall thickenings, composed of lignin, suberin, or both, exist in different root cell types, depending on the plant species.
2. These cell wall thickenings have diverse functions: The velamen can rapidly transport water and phosphate, exodermal wall thickenings and the endodermal Casparian strip and suberin act as apoplastic barriers, and lignin with the sclerenchyma functions in mechanical strength, while the function of phi thickenings is unknown.
3. These cell wall thickenings are environmentally inducible and in many cases facilitate their responses to harsh and dynamic environmental stimuli.
4. The molecular underpinnings of all but the endodermal Casparian strip and suberin are largely unknown, as are their evolutionary origin.

DISCLOSURE STATEMENT

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