Laying it on thick: a study in secondary growth

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Abstract

The development of secondary vascular tissue enhances the transport capacity and mechanical strength of plant bodies, while contributing a huge proportion of the world’s biomass in the form of wood. Cell divisions in the cambium, which constitutes the vascular meristem, provide progenitors from which conductive xylem and phloem are derived. The cambium is a somewhat unusual stem cell population in two respects, making it an interesting subject for developmental research. Firstly, it arises post-germination, and thus represents a model for understanding stem cell initiation beyond embryogenesis. Secondly, xylem and phloem differentiate on opposing sides of cambial stem cells, making them bifacial in nature. Recent discoveries in Arabidopsis thaliana have provided insight into the molecular mechanisms that regulate the initiation, patterning, and maintenance of the cambium. In this review, the roles of intercellular signalling via mobile transcription factors, peptide–receptor modules, and phytohormones are described. Crosstalk between these regulatory pathways is becoming increasingly apparent, yet the underlying mechanisms are not fully understood. Future study of the interaction between multiple independently identified regulators, as well as the functions of their orthologues in trees, will deepen our understanding of radial growth in plants.

Keywords: Arabidopsis, auxin, cambium, cytokinin, phloem, procambium, stem cells, signalling, transcription factors, xylem.

Introduction

Plants exhibit an extraordinary capacity for growth and developmental plasticity, owing to their ability to maintain populations of continuously dividing stem cells in tissues called meristems. Two indeterminate meristems are established during embryogenesis. These are the root apical meristem (RAM), which gives rise to the subterranean root system, and the shoot apical meristem (SAM), from which all aerial tissues are derived. The elongation of plants along the apical–basal axis that results from RAM and SAM activity is termed primary growth. In addition, plants develop a variety of post-embryonic meristems, from which axillary branches, floral organs, and lateral roots are derived (Taiz and Zeiger, 2002). As the architectures of land plants (Embryophyta) became larger and more complex during the Devonian period ~400 million years ago, the development of specialized tissues for mechanical support and efficient long-distance transport of fluids became increasingly advantageous (Tonn and Greb, 2017). As a result, the ability to expand roots and stems along the radial axis (termed secondary growth) has evolved multiple times in the Embryophyte lineage, and can be observed in extant gymnosperms and the majority of dicotyledonous angiosperms (Spicer and Groover, 2010; Nieminen et al., 2015).
Secondary growth arises from tightly controlled cell divisions in post-embryonic meristems known as the vascular and cork cambia. The vascular cambium, which is the focus of this review, gives rise to a network of interconnected transport cells and their supporting tissues, which span the entire primary plant body and its lateral organs. Uniquely among plant meristems, the vascular cambium harbours a single, bifacial stem cell in each radial file, which divides periclinal (parallel to the surface of the organ) to drive the development of distinct specialized tissues on opposing sides (Bossinger and Spokevicius, 2018; Shi et al., 2019; Smetana et al., 2019). Stem cells in the vascular cambium give rise to xylem centripetally and phloem centrifugally. Together, these dividing cells and their undifferentiated daughters (xylem and phloem progenitors) form a ‘cambial zone’, which is especially visible in transverse sections of mature Arabidopsis hypocotyls (Fig. 1A).

Xylem tissue is composed of conductive vessel elements that become hollow and vertically connected following programmed cell death (reviewed in Bollhöner et al., 2012). The resulting channel facilitates the transport of a continuous acropetal stream of water and dissolved minerals. Interspersed with vessel elements are dead xylem fibres and living parenchyma cells, the latter of which store starches, oils, and tanniniferous compounds (Esau, 1977). Vessel elements and fibres possess thick secondary cell walls of cellulose and hemicellulose, reinforced with lignin biopolymers to confer a high tensile strength. This allows xylem to mechanically support plant tissues as they elongate and withstand the negative pressures required for water transport (Taiz and Zeiger, 2002). On the opposing side of the vascular cambium, the phloem supports the bidirectional transport of sugars, proteins, amino acids, and other metabolites. Like xylem, phloem is composed of multiple specialized cell types, including conductive sieve elements (Fig. 1A). As phloem progenitors exit the cambial zone, organelles including the nucleus, vacuole, and cytoskeleton are degraded, and perforated sieve plates form to establish continuous connections between vertically adjoining cells (Furuta et al., 2014). Sieve elements have highly restricted metabolic activities and are thus supported by companion cells, to which they are connected via plasmodesmata. Other supporting cells include the phloem parenchyma and fibres, which fulfil storage roles and provide mechanical support, respectively (Esau, 1977). In addition to their roles in transporting photoassimilates and other nutrients, the importance of phloem highways for carrying long-distance RNA, phytohormone, and electrical signals is becoming increasingly apparent (Hilleary and Gilroy, 2018; Johns et al., 2021). This gives phloem tissue a central role in both plant growth and stress responses.

While the vascular cambium produces xylem and phloem, the outer cork cambium (also known as the phellogen) contributes to radial growth by producing protective tissue (Fig. 1A, D, E). In a layer known as the periderm, the cork cambium divides periclinaly to produce phelloderm centripetally and phellem centrifugally. The dead cork cells that comprise the phellem have walls layered with suberin and lignin, making them difficult for insects and phytopathogens to penetrate (Wunderling et al., 2018; Campilho et al., 2020). Therefore, replacement of the epidermis with phellem in woody stems and roots protects the internal transport tissues from environmental stress by reducing water loss and susceptibility to biotic threats.

**Arabidopsis thaliana as a secondary growth model**

While secondary growth is evidently important for plant development, the position of cambial meristems deep within internal tissues has hampered efforts to study their activity.

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**Fig. 1.** Secondary growth in Arabidopsis root and hypocotyl. (A) Cell types and their distribution in the Arabidopsis hypocotyl. (B–E) Transverse sections of Arabidopsis roots at different stages of secondary development. Red arrowheads mark cambial cell divisions. (B) Primary diarch pattern, in 7-day-old root, characterized by a central row of xylem with two adjacent phloem poles. (C) Formation of xylem vessels adjacent to the central xylem axis and initial cambial cell divisions (14-day-old root). Differentiating secondary xylem (marked yellow) acts as a cambium organizer, promoting adjacent cells to divide, thus initiating secondary growth. (D) In 18-day-old root, cambial divisions surround the xylem. Divisions in the cork cambium are apparent (black arrows). Secondary growth pushes the vascular cylinder through the cortex and epidermis. The radial pattern is complete in a 30-day-old root (E). (F) Transverse section through a 36-day-old hypocotyl showing xylem formed during the proportional growth phase (i) and xylem expansion growth phase (ii). Scales are 20 µm in (B–D); 50 µm in (E) and (F). X, xylem; ph, phloem.
Nevertheless, advances in plant genetics, tissue processing, and microscopy have recently accelerated vascular development research (reviewed in Nieminen et al., 2015; Lehmann and Hardtke, 2016; Fischer et al., 2019; Wang, 2020). Despite its herbaceous nature (i.e., lack of a persistent woody stem), Arabidopsis exhibits secondary thickening of its roots, hypocotyls, and inflorescence stems. Comparative anatomical studies have revealed striking similarity between the concentric patterns of xylem—cambium—phloem in the hypocotyls of Arabidopsis, stems of angiosperm trees, and storage roots of numerous crop species (Chaffey et al., 2002; Campilho et al., 2020; Hoang et al., 2020b). However, notable differences include the inability of Arabidopsis to form annual growth rings and its lack of ‘ray’ systems, which facilitate the radial transport of resins and gums in mature trees (Esau, 1977; Chaffey and its lack of ‘ray’ systems, which facilitate the radial transport of resins and gums in mature trees (Esau, 1977; Chaffey, 2020; Hoang et al., 2020). Even so, key genetic regulators of vascular development are conserved between Arabidopsis and its distant tree and root crop relatives (Wunderling et al., 2018; Hoang et al., 2020a, b), as discussed later in this review.

A robust primary pattern sets the stage

Before secondary growth commences in any plant, a precise primary vascular pattern is established. In Arabidopsis, the initiation of vascular stem cells and the specification of xylem and phloem in the embryo have been well characterized (reviewed in Miyashima et al., 2013; De Rybel et al., 2016). At the late globular stage of embryogenesis, vascular stem cells and surrounding pericycle tissue arise from the periclinal divisions of four procambial initials. Further division and differentiation of these cells during the subsequent heart stage give rise to a stereotypical radial pattern of cell types, which is mimicked in the post-embryonic root. This arrangement includes five or six xylem vessels in a single file, spanning the diameter of the stele (Baum et al., 2002). The central metaxylem has highly lignified, pitted cell walls, while their smaller protoxylem neighbours have spiral wall thickenings. Either side of these central xylem cells is a pool of procambium cells and a phloem pole containing sieve element precursors (Rodriguez-Villalon et al., 2014). Having two strands of protoxylem, this primary pattern is referred to as a diarch (Fig. 1B).

The formation of a diarch vascular cylinder is heavily dependent on intercellular signalling (Fig. 2A). Being one of the most important and extensively studied growth substances in plant development (Leyser, 2018), it is unsurprising that the phytohormone, auxin, has been implicated in this process. AUXIN RESPONSE FACTOR 5 (ARF5), also known as MONOPTEROS (MP), is released from its Aux/indole-3-acetic acid (IAA) inhibitor, BODENLOS (BDL), when cotyledon-derived auxin arrives at the vascular initials during embryogenesis (Weijers et al., 2006). MP is essential for early procambium formation, given that mp loss-of-function mutants were deficient in embryonic provascular cell divisions (Hardtke and Berleth, 1998). In addition, expression of a mutated, stabilized version of BDL conferred a rootless phenotype (Weijers et al., 2006), highlighting the importance of auxin signalling and MP function for root vascular development.

The cell to cell movement of transcription factors and miRNAs is known to play crucial roles in the establishment of root vascular patterns (De Rybel et al., 2016). The GRAS transcription factor SHORT ROOT (SHR) is expressed in the stele of developing roots and moves outwards to the endodermis, where it induces the expression of another GRAS protein, SCARECROW (SCR) (Nakajima et al., 2001). By binding SCR and the zinc finger BIRD transcription factors, JACKDAW (JKD) and BALDIBIS (BIB), SHR is sequestered in the nucleus of endodermal cells (Koizumi et al., 2012; Long et al., 2015, 2017). Here, SHR binds the promoters of MIR165 and MIR166, thereby inducing the expression of cell to cell diffusible miRNA, miR165/6 (Carlsbecker et al., 2010). The five vascular-expressed class III HOMEODOMAIN LEUCINE ZIPPER (HD-ZIP III) transcription factors, REVOLUTA (REV), PHABULOSA (PHB), PHAVOLUTA (PHV), ARABIDOPSIS THALIANA HOMEBOX 8 (ATHB8), and CORONA (CNA/ATHB15), are known targets of miRNA165/6-mediated post-transcriptional silencing (Mallory et al., 2004; Smetana et al., 2019). Accordingly, HD-ZIP III protein concentrations across the stele exhibit a gradient opposing that of their miRNA inhibitors, with abundance peaking in the stele centre and decreasing towards the pericycle (Carlsbecker et al., 2010; Fig. 2A). phb-1d gain-of-function roots demonstrated ectopic development of metaxylem in place of protoxylem, while simultaneous knockout of four HD-ZIP III members triggered ectopic protoxylem marker gene expression in the centre of the xylem axis (Carlsbecker et al., 2010). Thus, in a wild-type root, it is predicted that high HD-ZIP III levels promote metaxylem differentiation, whereas low levels promote protoxylem differentiation. This mechanism of HD-ZIP III–mediated xylem patterning is predicted to be similarly reflected in the Arabidopsis embryo (De Rybel et al., 2016).

Beyond HD-ZIP IIIIs, heterodimers of basic helix–loop–helix (bHLH) transcription factors were found to be necessary for the establishment of primary vasculature in Arabidopsis embryos (Schlereth et al., 2010). These heterodimers comprise LONESOME HIGHWAY (LHW), or LHW-LIKE 1 (LHL1) proteins, and MP-induced TARGET OF MONOPTEROS 5 (TMO5), or TMO5–LIKE (TSL) proteins (Schlereth et al., 2010; De Rybel et al., 2013). In a screen for mutants with compromised cell fate specification, lhw-deficient primary roots had a reduced number of vascular cells and displayed an abnormal monarch pattern with only one protoxylem strand (Ohashi-Ito and Bergmann, 2007). A similar phenotype was later observed in tmo5 t5l1 roots, and the formation of heterodimers between proteins of the LHW and TMO5 clades was confirmed in xylem precursor cells (De Rybel et al., 2013). In the embryo and primary root, the LHW–TMO5
module stabilizes growth and patterning of vascular tissue via regulation of cytokinin signalling. LHW–TMO5 up-regulates expression of cytokinin biosynthesis genes, LONELY GUY3 (LOG3) and LOG4, generating a cytokinin gradient that peaks in the xylem-adjacent procambium (De Rybel et al., 2014). Here, cytokinin promotes expression of the transcription factor gene, DNA-BINDING ONE FINGER2.1 (DOF2.1), which drives procambial cell division alongside its two homologues,
TMO6 and DOF6 (Smet et al., 2019). At the same time, ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 6 (AHP6), a repressor of cytokinin signalling, is cell autonomously induced by LHW–TMO5 which act downstream of auxin signalling to maintain the primary xylem in a non-dividing state (Bishopp et al., 2011; Ohashi-Ito et al., 2014).

Recently, TMO6 and DOF6 were assigned to a group of cytokinin-inducible PHLOEM EARLY DOF (PEAR) proteins, which promote the division of protophloem sieve elements in young roots (Miyashima et al., 2019). PEARs move intercellularly and up-regulate the expression of HD-ZIP IIIs in neighbouring cells. In turn, these inhibit PEAR expression to form a negative feedback loop, inhibiting periclinal division in the phloem-adjacent internal region of the root (Miyashima et al., 2019). Overall, the interlinking of auxin and cytokinin signalling via HD-ZIP IIIs, LHW–TMO5, and DOF proteins facilitates the establishment of a robust primary vascular pattern. This is essential for defining domain boundaries for subsequent cell division.

**Procambial cells divide at the onset of radial growth**

Soon after the establishment of diarch vascular bundles, radial growth initiates in the procambium, and secondary xylem is formed opposite the phloem poles (Esau, 1977; Baum et al., 2002). Pioneering cell lineage tracing experiments in the Arabidopsis root have pin-pointed secondary growth initiation to the divisions of xylem-adjacent procambial cells that occur 5–6 d after germination (Smetana et al., 2019) and ~15–18 mm from the root tip (Ye et al., 2021), although the exact timing of this transition is dependent on growth conditions (see, for example, Thamm et al., 2019; Fig. 1C, D). HD-ZIP III transcription factors are known to promote this early vascular proliferation. Expression of these genes, together with auxin signalling via MP, ARF7, and ARF19, defines a 'stem cell organizer' (yellow cells in Figs 1C and 2B) that is continually renewed as the procambium divides. In support of this model, suppression of HD-ZIP III function by chemical induction of MIR163, thus targeting HD-ZIP III transcripts for degradation, resulted in scattered cambial divisions, erratic xylem formation, and mitotic re-entry of previously quiescent xylem (Smetana et al., 2019). Following the initiation of procambial divisions, phloem-adjacent pericycle cells divide, pushing out the phloem region to generate an oblong-shaped stele (Fig. 1C; Baum et al., 2002). Divisions in the pericycle adjacent to the xylem poles subsequently contribute cells to generate a continuous cylinder of vascular cambium, a process that is similarly observed in young hypocotyls (Fig. 1D, E; Lehmann and Hardtke, 2016).

In contrast to the diarch patterns of immature roots and hypocotyls, primary vascular tissues in the Arabidopsis inflorescence stem are arranged in discrete vascular bundles (or fascicles). In a wild-type stem, 5–8 fascicles are derived from procambial cells below the SAM and arranged around a central pith (Fig. 3A), as is typical in dicots and gymnosperms (Fischer et al., 2019). Secondary growth initiates in the procambium of vascular bundles, forming fascicular cambium. Meristematic activity also extends to the interfascicular regions, where stem cells are formed de novo from differentiated parenchyma cells or those in the inner endodermal layer (known as the starch sheath) (Agusti et al., 2011b; Fig. 3B). The precise location of interfascicular cambium initiation is dependent on the stem's developmental stage, with a gradual shift towards the cortex being observed as the stem apex is approached (Sehr et al., 2010). Clusters of fascicular cambia are subsequently connected to form a continuous cambial ring in mature stems, from which secondary xylem and phloem are derived.

**Intercellular signalling via TDIF–PXY organizes secondary growth**

Following the initiation of secondary growth, cell to cell communication remains integral for maintaining the activity of the vascular cambium. Coordinated regulation of cambial cell division and differentiation requires integration of multiple external signals, including those from the extracellular matrix and adjacent cell layers (Busch et al., 2010; De Rybel et al., 2016; Trewavas, 2021). The molecular mechanisms governing cambial maintenance are topics of ongoing research (for reviews, see Fischer et al., 2019; Bagdassarian et al., 2020; Wang, 2020).

Peptide perception by transmembrane receptor(-like) kinases (RK/RLK) is a key signalling mechanism underpinning vascular cambium activity. The ligand–receptor pair comprising TRACHEARY ELEMENT DIFFERENTIATION INHIBITORY FACTOR (TDIF) and PHLOEM INTERCALATED WITH XYLEM (PXY), also known as TDIF RECEPTOR (TDR), is an extensively studied and multifunctional signalling module in this context (Fig. 2C). As part of the largest RLK family in Arabidopsis (Becraft, 2002), PXY possesses an extracellular ligand-binding domain with 21 leucine-rich repeats (LRRs), a single-helix transmembrane domain, and a cytoplasmic kinase domain that is activated upon TDIF perception (Fisher and Turner, 2007; Hirakawa et al., 2008). PXY has two close homologues in Arabidopsis, PXY-LIKE 1 (PXL1) and PXL2 (Fisher and Turner, 2007; Etchells et al., 2013), collectively referred to as the PXY family (PXf).

PXY is expressed in vascular tissues throughout the plant body, including in leaf veins, inflorescence stems, hypocotyls, and root steles. This expression specifically localizes to the xylem side of the vascular cambium (Hirakawa et al., 2008; Etchells and Turner, 2010a, b; Shi et al., 2019; Smetana et al., 2019; Wang et al., 2019). In contrast, the genes encoding the PXY ligand, GLAVATA3/ENDOSPERM SURROUNDING REGION 41 (CLE41), CLE42, and CLE44 (Ito et al., 2006),
are expressed in the phloem (Hirakawa et al., 2008; Etchells and Turner, 2010a). CLE41/42/44 peptides are 88–101 amino acids in length and are cleaved by a currently unknown mechanism to yield the TDIF dodecapeptide (Ito et al., 2006). X-ray crystallography demonstrated that TDIF specifically bound PXY by interacting with the inner concave surface of the receptor’s LRR domain (Morita et al., 2016; Zhang et al., 2016a), following earlier identification of this interaction by photoaffinity labelling (Hirakawa et al., 2008). Further structural and genetic studies revealed that, like other RLKs, PXY activation required SOMATIC EMBRYOGENESIS RECEPTOR KINASE (SERK) co-receptors, which associated with PXY in a ligand-dependent manner at the plasma membrane (Zhang et al., 2016a, b). An identifying feature of pxy loss-of-function mutants is their lack of a continuous cambial zone and resulting intercalation of xylem and phloem (Fisher and Turner, 2007; Wang et al., 2019). Meanwhile, phloem-specific overproduction of TDIF in SUC2::CLE41 stems resulted in enhanced, yet organized, vascular proliferation (Etchells and Turner, 2010a). In the same study, overexpression of CLE41 via the 35S or xylem-specific IRREGULAR XYLEM 3 (IRX3) promoter triggered disorganized vascular development (Fig. 4A, C). Considering this, a phloem-derived TDIF signal is thought to convey positional information to PXY in order to maintain the activity and bifacial nature of cambial stem cells (Etchells and Turner, 2010a, b; Etchells et al., 2016). Presently, this is understood to occur via three distinct pathways.

Firstly, PXY has an established role as a suppressor of cell differentiation. The TDIF peptide was originally identified as an inhibitor of xylem vessel differentiation when applied to Zinnia cell cultures (Ito et al., 2006), a finding that was later replicated in Arabidopsis hypocotyls (Whitford et al., 2008) and leaf disc cultures (Kondo et al., 2015). Consistently, 35S::CLE41 plants (in which TDIF–PXY signalling is enhanced) showed reduced expression of the xylem development marker, IRX3 (Etchells and Turner, 2010a). In addition, ectopic xylem differentiation and lignification of parenchyma cells were evident in pxy inflorescence stems (Etchells et al., 2016). In wild-type plants, activation of glycogen synthase kinase 3 (GSK3) proteins actively suppresses xylem differentiation downstream of TDIF–PXY. Förster resonance energy transfer (FRET)-based analyses revealed PXY association with

Fig. 3. Secondary growth in the stem, and contrasting LBD3/4 expression patterns in stems and roots. (A, B) Transverse sections of fascicles in Arabidopsis stems at 36 d. (A) Section from 2 cm above the rosette showing a discrete vascular bundle with no secondary growth. (B) Section adjacent to the rosette showing secondary initiation, including cambial cell divisions (red arrowheads) adjacent to the fascicle. (C) In situ hybridization with the antisense LBD4 probe marking the phloem–procambium boundary (Smit et al., 2020). (D–G) LBD3::YFP and LBD4::YFP throughout the vascular cylinder in both the presence (E, G) and absence (D, F) of a 24 h cytokinin treatment. Expression is higher in cytokinin-treated roots. Reprinted from Current Biology 31. Ye L, Wang X, Lyu M, Siligato R, Eswaran G, Vainio L, Blomster T, Zhang J, Mähönen AP. Cytokinins initiate secondary growth in the Arabidopsis root through a set of LBD genes. 3365–3373, Copyright (2021), with permission from Elsevier. Scales are 50 µm (A, B), 30 µm (C) and 10 µm (D–G). ph, phloem; pc, procambium; and x, xylem.
Growing thick and fast: secondary growth in Arabidopsis

the GSK3, BRASSINOSTEROID INSENSITIVE 2 (BIN2),
at the plasma membrane (Kondo et al., 2014). In response
to brassinosteroid perception, BIN2 is known to phosphorylate
the transcription factors BRASSINAZOLE RESISTANT 1
(BZR1) and BRASSINOSTEROID INSENSITIVE 1–EMS–SUPPRESSOR 1 (BES1), targeting
them for proteolytic degradation (He et al., 2002). These tran-
scription factors redundantly promote xylem and phloem
differentiation in cell culture systems (Saito et al., 2018). PXY-
mediated enhancement of BIN2 kinase activity is thus thought
to promote phosphorylation and subsequent destabilization of
BES1/BZR1, which is consistent with the reduced nuclear lo-
calization of BZR1–green fluorescent protein (GFP) observed
in TDIF-treated seedlings (Kondo et al., 2014). Through this
BIN2–BZR1/BES1 pathway, PXY signalling protects cambial
stem cells from differentiation, thereby maintaining a pool of
dividing cells for secondary growth.

Secondly, TDIF–PXY promotes vascular proliferation by
targeting the cambium-expressed WUSCHEL-RELATED
HOMEÖBOX (WOX) transcription factor genes, WOX4 and
WOX14 (Ji et al., 2010; Etchells et al., 2013). Such CLE–RLK–
WOX signalling modules have been repeatedly observed in
plants and are known to regulate SAM and RAM mainten-
ance (Schoof et al., 2000; Stahl and Simon, 2009; Lee and Torii,
2012). Wild-type Arabidopsis seedlings incubated with TDIF
exhibited an increase in procambial cell number and PXY-
dependent up-regulation of WOX4 and WOX14 expression
(Hirakawa et al., 2010; Etchells et al., 2013). Furthermore,
WOX4-deficient plants had a reduced number of cells in their
root and stem vascular bundles—a phenotype that was en-
hanced by simultaneous knockout of WOX14 (Etchells et al.,
2013; Zhang et al., 2019). Given that wox4 and wox4 wox14
mutants showed no defects in vascular organization (Fig. 4E, F),
the TDIF–PXY–WOX4/14 module for regulation of cambial
cell division is suggested to act in parallel to those regulating
xylem differentiation (Etchells et al., 2013).

Thirdly, a function for TDIF–PXY in defining the bound-
aries between vascular tissues was recently uncovered.
Through loss– and gain-of-function approaches, LATERAL
ORGAN BOUNDARIES DOMAIN 4 (LBD4) and
WOX14 were identified as positive regulators of procambium activity
in Arabidopsis roots (Zhang et al., 2019). Later, these tran-
scription factors were found to be part of a PXY-regulated
feedforward loop that mediated cell proliferation and vascular
bundle shape in inflorescence stems (Smit et al., 2020).
Strikingly, lbd4 was found to be required for IRX3::CLE41
phenotypes (Fig. 4C, D). Downstream of PXY, WOX14 func-
tions with the auxin- and cytokinin-inducible DOF tran-
scription factor, TMO6 (Schlereth et al., 2010; Miyashima
et al., 2019) to promote the expression of LBD4 along the
phloem–procambium boundary (Smit et al., 2020). Disruption
of this pattern in lbd4 IRX3::LBD4 stems (in which LBD4
expression was confined to the xylem) resulted in a loss of the

Fig. 4. wox4 and lbd4 suppression of IRX3::CLE41 phenotypes. (A) Wild type, (B) lbd4, (C) IRX3::CLE41, (D) IRX3::CLE41 lbd4, (E) wox4, and (F)
IRX3::CLE41 wox4 hypocotyls. Upper and middle panels show thin sections, stained with toluidine blue; the lower panel shows aniline blue hand
sections. In aniline blue-stained sections, callose in the sieve plates fluoresces green, marking phloem. Lignin in secondary cell walls autofluoresces blue,
marking the xylem. Red asterisks in (D) (IRX3::CLE41 lbd4) denote a file of differentiated xylem absent from IRX3::CLE41 (C). Thus lbd4 suppresses the
organization defects in IRX3::CLE41. IRX3::CLE41 wox4 lines (F) lack organization, but differentiated cell types are close together, suggesting a reduction
in stem cells (lower panel). Thus wox4 suppresses stem cell overproliferation observed in IRX3::CLE41. Scale bars are 50 μm in upper and lower panels; 20 μm in middle panels. X, xylem; ph, phloem; c, cambium.
characteristic phloem arc and reduced expansion of fascicles along the radial axis (Smit et al., 2020). Through this indirect regulation of LBD4, PXY is thought to regulate vascular organization by defining boundaries and amplifying cell divisions on the phloem side of the procambium.

Cytokinin and the LBD family drive vascular development

Cytokinin is critical for cambium initiation. Indeed, simultaneous loss of four ATP/ADP ISOPENTENYLTRANSFERASE (IPT) enzymes, required for cytokinin biosynthesis (Miyawaki et al., 2006), abolished secondary growth initiation in primary roots (Matsumoto-Kitano et al., 2008). The vascular cambium was restored to ipt mutants by exogenous cytokinin application. This rescue was dependent on the presence of LBD3 and LBD4, the expression of which is rapidly induced upon cytokinin treatment (Ye et al., 2021; Fig. 3D–G). The expression of additional homologues, LBD1 and LBD11, increased subsequently. Induction of the former pair occurred in the presence of protein synthesis inhibitors, demonstrating that these genes were primary cytokinin targets as their induction did not rely on the synthesis of intermediates. Thus, LBD4 and its close relatives, LBD3, LBD1, and LBD11, act together to regulate radial growth by controlling cell division in the cambium. In support of this, loss of these four transcription factors resulted in considerable reductions in root secondary growth (Ye et al., 2021).

Evidence suggests that a subset of LBD genes also influence cell size. Induction of LBD1, LBD3, or LBD11 in young roots resulted in a marked increase in radial area of vascular cells, while their loss was characterized by a reduction (Ye et al., 2021). One explanation for this combination of phenotypes is that cellular growth is closely interlinked with cell cycle progression (Sablowski and Carnier Dornelas, 2014). Therefore, these three LBD genes may drive division by first promoting cell enlargement. While cytokinin is primarily associated with cell division, increases in cell size have yet to be reported following its application during secondary growth.

Also, evidence from the stem suggesting that LBD4 expression marks the phloem–cambium boundary (Fig. 3C) in a PXY-mediated mechanism (Smit et al., 2020) does not hold for cambium initiation in the root vascular cylinder, where LBD3 and LBD4 promoter activity is widespread (Fig. 3D–G). Perhaps the simplest explanation for these contradictions is that LBD4 is multifunctional, with modified outputs depending on the developmental context.

ERECTA receptors regulate vascular proliferation and fibre formation

Since the characterization of PXY, further RLKs with roles in vascular development have been identified (Agusti et al., 2011b; Uchida and Tasaka, 2013; Wang et al., 2013; Gursansky et al., 2016), although the components and interactions of their corresponding signalling pathways in vascular development are less well understood. Among these, ERECTA (ER) receptors are known to regulate cell division and xylem fibre formation during secondary growth (Ragni et al., 2011; Uchida and Tasaka, 2013; Ikematsu et al., 2017; Milhinhos et al., 2019). ER encodes an RLK with 20 LRRs in its extracellular domain (Torii et al., 1996), and possesses two close homologues, ER-LIKE 1 (ERL1) and ERL2 (Shpak et al., 2004). This trio is collectively referred to as the ER family (ERf). ER was first cloned over two decades ago (Torii et al., 1996), and has subsequently been implicated in a surprisingly diverse array of processes, including stomatal patterning (Shpak et al., 2005), elongation of aerial organs (Woodward et al., 2005; Chen et al., 2013), reproductive development (Shpak et al., 2004; Pillitteri et al., 2007), meristem maintenance (Uchida et al., 2012), leaf morphogenesis (Tameshige et al., 2016), and responses to necrotrophic pathogens (Llorente et al., 2005; Jordá et al., 2016; Cai et al., 2021). The ligands for ERf receptors reside within the family of EPIDERMAL PATTERNING FACTOR (EPF)/EPFL-LIKE (EPFL) peptides. These range from 45 to 75 amino acids in length and are encoded by 11 genes in Arabidopsis (Kondo et al., 2010; Sugano et al., 2010; Takata et al., 2013). However, only a few receptor–ligand combinations have been studied in depth.

The influence of ERf signalling on plant development is seemingly dependent on the organ and ER/ERL genes in question. In inflorescence stems, the ER promoter is active in the xylem, phloem, and endodermis (Uchida et al., 2012). erf triple mutants contained fewer cells within their stem vascular bundles, implicating ER/ERL receptors as positive regulators of vascular proliferation (Ettchells et al., 2013). Similarly, er lrl1 stems showed defects in procambium maintenance, a phenotype that was rescued by phloem-specific (but not xylem-specific) expression of ER (Uchida and Tasaka, 2013). The candidate ligands perceived by the ERf in this context are EPFL6 and EPFL4, also known as CHALLAH (CHAL) and CHAL-LIKE 2 (CLL2), respectively, which are highly expressed in inflorescence stems (Abrash et al., 2011; Uchida et al., 2012). The physical binding of ER to EPFL4/6 was confirmed by co-immunoprecipitation, while endodermis-specific expression of EPFL4 or EPFL6 rescued the e-like dwarf and compact inflorescence phenotypes of epfl4 epfl6 double mutants (Uchida et al., 2012). Thus, EPFL peptides secreted from the endodermis are hypothesized to signal to ERf receptors in the phloem to promote both inflorescence elongation and vascular development. Conversely, a role for ERf receptors as suppressors of secondary growth has been uncovered in hypocotyls. ER, ERL1, and ERL2 expression is observed in most hypocotyl cell types and peaks in the cambium, xylem initials, and periderm, although ERL2 promoter activity is only evident in mature hypocotyls (Ikematsu et al., 2017; Wang et al., 2019). In contrast to the stem, EPFL4/6 expression in hypocotyls is
greatest in the xylem parenchyma and differentiating xylem, suggesting that most active EPFL4/6–ERf complexes reside in xylem initials (Wang et al., 2019). While er etd2 hypocotyls were indistinguishable from those of the wild type (Wang et al., 2019) and those of er etf triple mutants were reduced in diameter (Etchells et al., 2013), er erl1 hypocotyls exhibited a dramatic enhancement of secondary growth (Ikematsu et al., 2017). In wild-type Arabidopsis, radial growth in this organ proceeds in two distinct phases. During the initial ‘proportional phase’, the rate of xylem and phloem formation is roughly equal. The subsequent ‘xylem expansion’ (Fig. 1F) phase is induced by bolting (Sibout et al., 2008), which generates a shoot-derived gibberellic acid (GA) signal (Ragni et al., 2011). The arrival of GA triggers the release of BREVIPEDICELLUS (BP), ARF6, and ARF8 transcription factors from DELLA-mediated repression, shifting the balance of secondary growth to favour xylem production and differentiation of fibres (Ben-Targem et al., 2021). Interestingly, er erl1 mutation enhanced lignification and expansion of the xylem area to roughly three times that of the wild type (Ikematsu et al., 2017). This suggests that ER and ERL1 ordinarily suppress precocious xylem fibre differentiation during the proportional phase of secondary growth.

Currently, our understanding of signalling components acting downstream of ER in the vasculature is less complete in comparison with stomata. Stomatal clustering in epidermal tissues is regulated by ERf receptors binding to EPF1, EPF2, or EPFL9, in association with SERK co-receptors and the receptor-like protein, TOO MANY MOUTHS (TMM) (Shpak et al., 2005; Lee et al., 2012, 2015; Shpak, 2013). Following ligand perception in the epidermis, a MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) cascade is initiated. This culminates in the phosphorylation and destabilization of the transcription factor, SPEECHLESS (SPCH), which specifies the initiation and proliferation of stomatal cells (Jewaria et al., 2013; Meng et al., 2015). In contrast, signalling via EPFL4/6–ERf does not require TMM (Lin et al., 2017). Genetic loss-of-function and expression analyses have highlighted likely transcription factors downstream of this module involved in the regulation of pathogen responses (Cai et al., 2021), inflorescence architecture (Uchida and Tasaka, 2013; Wang et al., 2019), and ovule development (Pillitteri et al., 2007). In hypocotyls, genes encoding the transcriptional regulators of xylem differentiation, NAC SECONDARY WALL THICKENING PROMOTING FACTOR 1 (NST1) and NST3, were up-regulated in er erl1 mutants (Ikematsu et al., 2017), making these likely downstream targets of ER/ERL1 signalling in the xylem. Beyond this suppression of NAC transcription factors, the molecular signalling events underlying ER-mediated inhibition of secondary growth remain elusive. Further targets acting downstream of EPFL4/6–ERf complexes may help explain, for example, the contrasting vascular phenotypes of erf and er etl1 hypocotyls.

**The PXF and ERECTA families genetically interact**

In addition to their individual characterization, understanding the crosstalk between RLK-mediated signalling pathways is an important focus of ongoing vascular development research (Fukuda and Hardtke, 2020). ER was recently found to physically interact with another LRR RLK, SUPPRESSOR OF BIR-1 (SOBIR1), and, together, these two receptors signal to suppress precocious fibre development in hypocotyls (Milhinhos et al., 2019). The EPFL4/6–ERf module is also known to genetically interact with TDIF–PXf to control cell proliferation, cell size, and organization within the plant vasculature (Etchells et al., 2013; Uchida and Tasaka, 2013; Wang et al., 2019). When the functions of PXY receptors were removed in px y single or px f triple mutants, hypocotyl vascular tissue developed in a disorganized manner, as exemplified by the occurrence of non-periclinal cell divisions (Etchells et al., 2013; Wang et al., 2019). In these same studies, the vasculature of er or erf hypocotyls exhibited wild-type organization. Interestingly, when both PXf and ERf function were simultaneously compromised, disruption to vascular organization became more pronounced and mean hypocotyl radius was reduced beyond that of pxf, to the extent that secondary growth initiation was absent in pxf erf sextuple mutants (Etchells et al., 2013; Wang et al., 2019). Similarly, pxf or er stem vascular bundles carried fewer cells than the wild type and displayed a dramatically altered shape owing to reduced expansion along the radial axis—a phenotype that was absent in px y and er mutants (Uchida and Tasaka, 2013; Wang et al., 2019). Given that the introduction of erf mutation(s) in a px f background resulted in non-additive enhancement of organizational defects, this is indicative of a synergistic interaction between the two receptor families.

The PXF-ERF genetic interaction could be underpinned by multiple non-mutually exclusive phenomena at the molecular level. Firstly, PXF signalling may regulate the expression of ERf signalling components and vice versa, which could result in compensatory expression when receptors from one family are removed. In corroboration of this hypothesis, the expression of ERL1 and ERL2 was up-regulated in pxf or erf hypocotyls, suggesting that PXf and ER may ordinarily interact to suppress ERL gene expression (Wang et al., 2019). In surprising contrast, evidence suggests that PXf and ER jointly promote EPFL6, EPFL4, ERL1, and ERL2 expression in inflorescence stems, highlighting the organ-specific nature of this interaction (Wang et al., 2019). Secondly, PXf and ERf receptors may form protein complexes at the plasma membrane, and the activity of these complexes may be unequally perturbed when different receptor combinations are removed. The expression patterns of ER and PXF consistently overlap on the xylem side of the cambium in hypocotyls (Hirakawa et al., 2008; Ikematsu et al., 2017; Wang et al., 2019). Furthermore, a recent high-throughput screen for in vitro interactions between RLK LRR domains supported the binding of ER to PXY and PXL1, as
well as binding of PXL2 to ERL2 (Smakowska-Luzan et al., 2018; Mott et al., 2019).

Thirdly, it is possible that PXf and ERf interact via convergence of their signalling pathways on common genes or proteins. In this scenario, PXf- or ERf-mediated regulation of these hypothetical targets persists in the absence of one receptor family, yet removal of both families abolishes this regulation and gives rise to an enhanced phenotype. With the identification of SERK co-receptors (Zhang et al., 2016b) and GSK3 proteins (Kondo et al., 2014; Han et al., 2018), the molecular signalling components transducing PXY signalling to the nucleus are partially understood. Additionally, the NAC transcription factor XYLEM DIFFERENTIATION, DISRUPTION OF VASCULAR PATTERNING (XVP) was recently shown to negatively regulate TDIF–PXY outputs by binding BRI1-ASSOCIATED RECEPTOR KINASE1 (BAK1/SERK3) at the plasma membrane and thus the BAK1–PXY heterodimer (Yang et al., 2020a). In contrast, components acting downstream of ER to regulate vascular proliferation are unknown. It is therefore possible that PXY and ER could have shared targets within their signalling cascades, and/or at the level of transcription. The low number of genes known to play a role in the regulation of cambial morphogenesis and maintenance suggests that further regulatory components await discovery (Nieminen et al., 2015; Lehmann and Hardtke, 2016). Indeed, only a few genetic repressors of vascular development have been identified to date (Gursanscky et al., 2016; Ikematsu et al., 2017; Zhang et al., 2019; Wallner et al., 2020; Yang et al., 2020a). Given that interactions between the PXf, Erf, and phytohormones evidently make a significant contribution to cambial regulation (Qiang et al., 2013; Wang et al., 2019; Smit et al., 2020), identification of the molecular mechanisms linking these components will be an interesting focus for future research.

**Signalling crosstalk in cambium development requires further study**

Crosstalk and interactions between developmental regulators has emerged as a feature of cambium development (Fig. 2C). LBD4, described above, is a prime example of this, being regulated by both cytokinin and PXY signalling, as is the genetic interaction between PXf and ERf. Further examples include ethylene signalling converging on WOX4 (generally considered to act downstream of PXY) to regulate cambial proliferation, while simultaneously inhibiting xylem fibre development through suppression of NAC transcription factors (Yang et al., 2020b). In stems, overexpression of another PXY target, WOX14, promoted accumulation of bioactive GA, inducing strong lignification of secondary xylem (Denis et al., 2017). Crosstalk between TDIF–PXY and auxin is prevalent in the cambium, as auxin-induced promotion of cell division was reduced in wox4 stems (Suer et al., 2011). Expression analysis suggested that PXY and WOX4 were transcriptionally regulated by both auxin-responsive MP and HD-ZIP III transcription factors in the root procambium (Smetana et al., 2019). However, MP activity in stems ordinarily inhibits cytokinin biosynthesis and vascular proliferation via activation of ARABIDOPSIS RESPONSE REGULATOR 7 (ARR7) and ARR15 expression (Han et al., 2018), and suppression of WOX4 (Brackmann et al., 2018). PXY signalling is thought to remove this inhibition by repressing the kinase activity of the GSK3, BIN2-LIKE 1 (BIL1), a positive regulator of MP function (Han et al., 2018). Overall, it is evident that the coordinated action of multiple factors is crucial for regulating the rate and organization of secondary growth. In future, additional phytohormones such as strigolactone and jasmonic acid, which have been independently identified as positive regulators of secondary growth (Sehr et al., 2010; Agusti et al., 2011a), will probably be drawn in to complete the picture.

**Mechanisms of cambial regulation are conserved**

As previously discussed, some factors (e.g. MP and ERf) reportedly have organ-specific roles in the Arabidopsis stem and root. Nevertheless, key signalling components, such as PXY–TDIF and cytokinin, function similarly across the vascular system and may therefore represent conserved organizers and drivers of secondary growth. Indeed, there is increasing evidence that these components also regulate wood formation in divergent plant lineages. Tree species in the genus *Populus* and their hybrids (encompassing poplars, aspens, and cottonwoods) have been employed as models for understanding wood formation owing to their relatively rapid growth and availability of genomic resources (Tuskan et al., 2006). Through *in vitro* propagation and transformation of *Populus* spp. (for examples, see Takata and Eriksson, 2012; Maheshwari and Kovalchuk, 2016; Li et al., 2017), combined with tissue-specific transcriptomics and hormone profiling (Immanen et al., 2016; Sundell et al., 2017), researchers have dissected the roles of vascular development regulators in trees.

The three major phytohormone classes implicated in Arabidopsis secondary growth (auxin, cytokinin, and GAs), are also implicated in wood formation. For instance, GA signalling is crucial for triggering the xylem expansion phase of secondary growth of Arabidopsis hypocotyls (Ragni et al., 2011; Ben-Targem et al., 2021). Bioactive GAs peaks in the developing xylem of *Populus trichocarpa* (Immanen et al., 2016), and expression of *Pinus densiflora* GA20-oxidase (*PdGA20ox1*) under constitutive or xylem-specific promoters triggered increased xylem width and cell number in hybrid poplar (Jeon et al., 2016). Together, this highlights a conserved role for GA in regulating secondary xylem expansion.

Reminiscent of models in the Arabidopsis root, auxin and HD-ZIP III transcription factors drive cambial proliferation in *Populus*. In cryosections of hybrid aspen stems (*Populus tremula×troumluloides*), a gradient of IAA (a major bioactive
auxin) was detected in developing vascular tissue, with a peak in the cambium and decreasing concentration on either side (Tuominen et al., 1997). Disruption of auxin signalling by ectopic expression of IAA biosynthesis genes or constitutive reduction of auxin responsiveness led to reduced cambial cell division (Tuominen et al., 1997; Nilsson et al., 2008). In addition, tissue-specific transcriptomic analysis identified auxin-responsive genes whose expression patterns correlated with the phytohormone gradient (Nilsson et al., 2008; Immanen et al., 2016). Among these was PtIPT8, an orthologue of the Arabidopsis HD-ZIP III transcription factor gene, ATHB8. Interestingly, popREVOLUTA (PRE), the Populus orthologue of Arabidopsis REV, was up-regulated following the transition of stems to secondary growth, while expression of an miRNA-resistant form of PRE resulted in aberrant vascular patterning, including polarity defects and ectopic cambium initiation (Robischon et al., 2011). This is consistent with the role of the HD-ZIP III class in cambial stem cell organization (Smetana et al., 2019). Auxin gradients with peak concentrations in the vascular cambium were similarly detected in Pinus sylvestris (Uggla et al., 1996), suggesting that auxin may be important for defining the zone of cambial cell division in both gymnosperms and angiosperms.

Unsurprisingly, auxin’s role in wood formation is interlinked with that of cytokinin. Hormonal profiling and RNA-sequencing of Populus stems revealed a peak in cytokinin concentration, biosynthesis, and signalling in the developing phloem (Immanen et al., 2016; Fu et al., 2021). Transgenic hybrid aspen overexpressing the Arabidopsis cytokinin biosynthesis gene, AtIPT7, in the cambium and developing xylem contained more cambial stem cells than the wild type and displayed an increased cambial auxin concentration (Immanen et al., 2016). Conversely, cytokinin catabolism was achieved in Populus vascular tissue by localizing expression of Arabidopsis AtCKX genes to either the cambium or the phloem (Immanen et al., 2016; Fu et al., 2021). In both cases, this resulted in a reduction in cambial cell division. Phloem- and cambium-localized cytokinin signalling are therefore thought to drive wood formation synergistically.

While cytokinin is a conserved positive regulator of secondary growth, the observation that cytokinin signalling peaks in the tree phloem does not align fully with Arabidopsis root models, in which the cytokinin response is greatest in the procambium (Bishop et al., 2011). Indeed, the regulation of cytokinin signalling is hypothesized to be more complex in Populus spp. owing to the expansion of gene families associated with negative regulation of cytokinin responses (Tsukam et al., 2006). Downstream of cytokinin in Arabidopsis, LBD1, LBD3, LBD4, and LBD11 drive cambial proliferation in roots (Ye et al., 2021). LBD genes were independently identified as positive regulators of wood formation in trees, as P. tremula×alba trees overexpressing PtLBD1 had thicker stems and enhanced secondary phloem production compared with the wild type (Yordanov et al., 2010). In fact, four PtLBD family members are highly expressed in Populus stems undergoing secondary growth, and PtLBD4 expression specifically localized to the phloem and adjacent region of the cambial zone (Yordanov et al., 2010), a pattern resembling that of AtLBD4 (Fig. 3C). Whether or not PtLBD genes are cytokinin responsive or mediate cell growth like their Arabidopsis orthologues remains to be determined.

Alongside those of phytohormones, the roles of TDIF–PXY in promoting and organizing secondary growth are conserved between Arabidopsis and Populus spp. Within the P. trichocarpa genome, six genes encode putative TDF-like peptides and four of these (encoded by PtCLE41a–d) are specifically expressed in secondary phloem (Kucukoglu et al., 2017). Genes encoding the LRR RLKs, PtPXYa and PtPXYb, and homeodomain transcription factors, PtWOX4a and PtWOX4b, were also identified as orthologues of AtPXY and AtWOX4, respectively. Promoter activity of these four genes peaked in the vascular cambium and positively correlated with cambial activity throughout cycles of growth and dormancy (Kucukoglu et al., 2017). Furthermore, overexpression of PtCLE41b and PtPXYa in the phloem and cambium, respectively, resulted in a dramatic increase in xylem cell numbers, stem thickening, and biomass formation in hybrid aspen (Etchells et al., 2015). In contrast, hybrid aspen expressing an RNAi construct targeting PtWOX4a/b suffered severely compromised radial growth, to the extent that they failed to support their own weight (Kucukoglu et al., 2017). Combined with observations that 35S::PtPXYa constructs complemented Arabidopsis pxy mutants (Etchells et al., 2015), this suggests that TDIF–PXY signalling functions similarly in both Arabidopsis and trees to maintain the vascular cambium and promote secondary growth.

Beyond Populus, orthologues with similar spatial expression patterns to AtCLE41, AtPXY, and AtWOX4 were identified in the gymnosperm tree, Pinus abies (Kucukoglu et al., 2017). Additionally, tissue-specific transcriptomics in radish (Raphanus sativus), followed by in situ hybridization, revealed restricted expression of RsHB8, RsWOX4a, and RsPXY to a subset of root cambial cell layers (Hoang et al., 2020a). Further orthologues of established vascular development regulators, including RsMP, RsTM05, RsLHWS, RsDOF2.1, and RsTM06, showed cambium-enriched expression. It is thus possible that both TDIF–PXY and auxin-responsive regulatory pathways coordinate radial growth in herbaceous weeds, trees, and domesticated root crops.

Perspectives

In the last few decades, our understanding of plant radial growth has been greatly enhanced by pioneering studies that have revealed a complex web of interacting phytohormones and peptide signalling modules. However, several outstanding questions remain. What are the downstream targets of ERF signalling in the vasculature? What is the molecular basis of the PXY–ERF genetic interaction? How is TDIF processed and exported from phloem cells? How is vascular development regulated during times of stress? Interestingly, within their
gene regulatory network of Arabidopsis and radish cambium-enriched transcription factors, Hoang et al. (2020a) noticed over-representation of several stress-responsive genes, including those in the WRKY and ETHYLENE RESPONSE FACTOR (ERF) family. As ethylene positively regulates secondary growth (Etchells et al., 2012; Yang et al., 2020b) and acts as an abiotic and biotic stress signal (Dubois et al., 2018; Sadidharan et al., 2018; Riyazuddin et al., 2020), the ethylene response represents a potential pathway whereby secondary growth regulation and stress signalling could be integrated. Future exploration of these open questions may lead to the identification of novel regulatory components and promising intervention points for targeted enhancement of plant secondary growth.

There is increasing evidence that studies of Arabidopsis secondary growth are informative when seeking to understand and manipulate wood formation in trees. However, gene family expansion has been a major contributor to Populus biology, and these plants therefore carry considerably more protein-encoding genes than Arabidopsis (Tuskan et al., 2006). For instance, there are 57 LBD family genes in P. trichocarpa (Zhu et al., 2007), while only 43 are present in Arabidopsis (Shuai et al., 2006). For instance, AtCLE25 peptides are expressed in phloem cell lineages and signal via CLAVATA 2 (CLV2) receptors to promote phloem initiation (Ren et al., 2019). Surprisingly, PtCLE47 is expressed in the cambium and drives the formation of secondary xylem (Kucukoglu et al., 2020). It is thus important to consider that both increased genetic redundancy and the evolution of novel regulators may underpin secondary growth in trees. Collectively, plants contribute a huge proportion (~80%) of the world’s biomass, with stems and tree trunks alone thought to contribute 70% of this (Bar-On et al., 2018). The secondary vascular tissues of trees therefore represents a significant carbon sink and must play a major part in efforts to slow global warming (Bastin et al., 2019). Radial growth also underlies the swelling of root and tuber crops, which provide vital carbohydrates in human diets worldwide (Chandrasekara and Kumar, 2016). While this review has focused on a somewhat esoteric weed, studies in trees and root vegetables demonstrate that discoveries made in Arabidopsis may nevertheless contribute to the enhancement of forestry and agricultural outputs.

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### Conflict of interest

The authors declare that there are no conflicts of interest.

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