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# Plant vascular development: mechanisms and environmental regulation

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## Abstract

1  
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3 Plant vascular development is a complex process culminating in the generation  
4 of xylem and phloem, the plant transporting conduits. Xylem and phloem arise  
5 from specialized stem cells collectively termed (pro)cambium. Once developed,  
6 xylem transports mainly water and mineral nutrients and phloem transports  
7 photo-assimilates and signaling molecules. In the past few years, major  
8 advances have been made to characterize the molecular, genetic and  
9 physiological aspects that govern vascular development. However, less is known  
10 about how the environment re-shapes the process, which molecular mechanisms  
11 link environmental inputs with developmental outputs, which gene regulatory  
12 networks facilitated the genetic adaptation of vascular development to  
13 environmental niches, or how the first vascular cells appeared as an evolutionary  
14 innovation. In this review, we (i) summarize the current knowledge of the  
15 mechanisms involved in vascular development, focusing on the model species  
16 *Arabidopsis thaliana*, (ii) describe the anatomical effect of specific environmental  
17 factors on the process, (iii) speculate about the main entry points through which  
18 the molecular mechanisms controlling of the process might be altered by specific  
19 environmental factors, and (iv) discuss future research which could identify the  
20 genetic factors underlying phenotypic plasticity of vascular development.  
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## Introduction

Land plants can be divided into two main groups: those that possess a vascular system (vascular plants) and those that do not (non-vascular plants). This division demonstrates that possessing a vascular system is not an absolute requirement for plants to survive on land. However, it is a clear advantage. Indeed, in evolutionary terms, the appearance of the vascular system was key in the massive colonization of land by plants, and represented an enormous impact not only on the biological, but also on the ecological and geological history of our planet.

The first land plants appeared 450 million years ago, evolving from an ancestral charophycean alga [1]. Two main characteristics of such plants were (i) the rudimentary cell-to-cell transporting system that forced them to live, exclusively, in moist zones where water was easily accessible, and (ii) their small size. As plants in moist habitats increased in population, fierce competition for water and light began. Two innovations coincided to influence the success in this competition: lignification and the emergence of new interconnected cell types that form the vascular tissue [2]. Lignin, a polymer deposited in cell walls, provides strong rigidity. Lignified cells can resist high water pressure and mechanical stress. As a consequence, the ground was laid to implement efficient long-distance communication systems, and to support the weight of larger body sizes without losing stability. With respect to the vascular system, three main tissues are produced in extant plants: the xylem (wood) transporting water and solutes from the soil upwards, the phloem (bark) transporting nutrients and signaling molecules throughout the plant body in multiple directions, and the (pro)cambium, a pool of stem cells programmed to generate xylem and phloem. Xylem and phloem are formed by several cell types, each fulfilling specific functions within each of the tissues. The spatial organization and relative abundance of xylem, phloem and cambium is characteristic of each species and of each organ within a given species. The same happens at the tissue-type level: the arrangement and relative abundance of the different cell types that compose the xylem or the phloem are also characteristic of each species and of each organ within a given species [3]. Due to adaptation, ecotypes within a single species usually display fine-tuned versions of such characteristics. Furthermore, developmental plasticity (the capacity to convert environmental inputs to developmental outputs) also

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adjusts the vascular developmental program of single individuals to the specific environmental conditions encountered during the life cycle [4].

In this review, we first summarize current knowledge about the genetic and molecular mechanisms controlling plant vascular development and phenotypic plasticity and then discuss potential environmental regulation of the process. We argue that understanding the molecular mechanisms by which the environment regulates vascular development may shed light on the adaptive and evolutionary history of the plant vascular system. In addition, we suggest that, by discerning how the environment regulates the molecular control of plant vascular development, we can better understand its plasticity and design biotechnology approaches to adapt vasculature-derived biomass production to climate change.

## **Vasculature develops throughout the plant life cycle**

From a developmental point of view, it is convenient to distinguish between primary and secondary vascular tissues [5]. The primary vascular tissues, found in all vascular plants, are initially specified when the body plan is established – during embryogenesis–, and maintained through the activity of the apical meristems during post-embryonic development [5] (Figure 1). Secondary vascular tissues are found in most plant species, although some lineages (i.e, monocots) lost the capacity to develop these tissues during evolution [6].

### ***Establishment and development of primary vascular tissues during embryogenesis***

The first pro-vascular cells are established during the globular stage of embryo development [7] (Figure 2a). After the embryonic regions giving rise to the aerial or underground parts of the plant are formed, four inner cells within the lower tier of the embryo divide to create a central zone of elongated cells [8]. Eventually, these cells acquire provascular identity in a process that involves a local accumulation of auxin to induce the expression of a central regulator of vascular identity specification: the Auxin Response Factor MONOPTEROS (MP/ARF5) [7-13] (Figure 2b). The *mp* mutant is defective in the early divisions of provascular cells and in body axis patterning [10], indicating how vascular specification is intimately connected to embryo development. Strikingly, MP induces the

1 expression of the polar auxin transporter *PIN1* [14], likely resulting in enhanced  
2 local auxin signaling through a positive feedback loop [13]. The MP inhibitor  
3 BODENLOS (BDL) limits MP activity [14, 15]. BDL belongs to the Aux/IAA family  
4 of transcriptional repressors (AUX/IAA12) which, upon auxin accumulation, are  
5 degraded by the 26-S proteasome [15-17]. Therefore, auxin-induced MP  
6 activation occurs via BDL degradation, and the phenotype of the *bdl* gain-of-  
7 function mutant mimics that of *mp* [15]. Thus, to better understand the  
8 mechanisms controlling the MP-mediated regulation of vascular specification or  
9 the root-pole formation (a process also controlled by MP), MP targets (TARGETS  
10 OF MONOPTEROS, TMOs) were identified [18]. In the study, an elegant  
11 transcriptomic assay used *bdl* mutants and an inducible version of *bdl* [18]. This  
12 approach identified several direct targets of MONOPTEROS (TMOs) involved  
13 either in the regulation of root or vascular development. Among them, *TMO3*  
14 (previously termed *CRF2* [19]), *TMO5* and *TMO6* were found to be MP-controlled  
15 regulators of vascular development. *TMO5* is considered a crucial regulator in  
16 the process [18]. Through heterodimerization with the atypical bHLH transcription  
17 factor LONESOME HIGHWAY (LHW), *TMO5* induces *LOG3* and *LOG4*  
18 expression [20], encoding cytokinin (CK) biosynthesis enzymes [21].  
19 Subsequently, CK induces periclinal cell divisions within (pro)cambial cells  
20 (Figure 2b). *TMO6* encodes a DOF transcription factor (DOF5.3) that is  
21 expressed not only in the provascular embryonic cells [18] but also in the  
22 protophloem sieve element in the root [22]. Indeed, *TMO6* belongs to the early  
23 phloem markers PHLOEM EARLY DOF (PEAR) family [22]. These PEAR factors  
24 are of high relevance to prime cambial growth [22]. *TMO3* is thought to regulate  
25 CK signaling in the embryo [7, 18]. However, its specific role during vascular  
26 development is currently less clear. The involvement of *TMO5* and *TMO3* in CK  
27 biosynthesis and signaling, respectively, is not the only proof of direct CK  
28 involvement in embryo provascular tissue establishment and patterning. Indeed,  
29 the strong expression of the *WOODENLEG* (*WOL*) CK receptor within the  
30 embryonic provascular cells also supports this [23]. In brief, the first vascular  
31 initials are specified during the globular stage of embryogenesis in a process that  
32 involves auxin and CK signaling together with a number of already identified  
33 genetic regulators (Figure 2b).  
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1 After specification, the vascular initials proliferate concomitantly with  
2 embryonic development. In some species, xylem and phloem can be  
3 anatomically identified rather easily at late embryonic stages, but in many others,  
4 this is not possible until after germination [5, 8, 11]. In *Arabidopsis*, embryonic  
5 cells displaying overlapping expression of *TMO5* and *LHW* are xylem initials [20].  
6 Given that *TMO6* is a PEAR factor, it might be the case that embryonic cells  
7 expressing *TMO6* are phloem initials, but no experimental data is available in this  
8 respect.  
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### 16 ***Postembryonic vascular patterning: roots as model system***

17 After germination, the primary vasculature that was pre-established during  
18 embryogenesis starts maturing, and new vascular tissue forms and develops as  
19 the plant grows. Depending on the species, the primary vasculature can organize  
20 into different patterns [5]. For simplicity sake, here we will focus on that of the  
21 model species *Arabidopsis thaliana*.  
22

23 A general trend in all vascular species is that the arrangement of primary  
24 vasculature differs between the aerial and the underground organs. In  
25 *Arabidopsis*, the root primary vasculature is structured following a diarch  
26 bisymmetric organization [24, 25] in which the xylem axis occupies the center  
27 (Figures 1c; 3a). A pole of phloem cells (typically composed of 4 cells) is found  
28 in each extreme of the perpendicular axis to the xylem, and procambial cells are  
29 located between the phloem poles and the xylem axis. The entire vasculature is  
30 surrounded by a layer of pericycle cells [26] (Figure 3a). At the seedling stage,  
31 this organizational pattern expands longitudinally throughout the root and the  
32 hypocotyl, connecting the shoot apical meristem (SAM) and the root apical  
33 meristem (RAM).  
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35 As is the case during embryogenesis, the prevailing models propose that  
36 patterning is initially established by the interplay between high CK and high auxin  
37 levels, which define distinct domains in the root procambium [27].  
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### 54 ***Xylem specification***

55 Metaxylem and protoxylem can be identified by their anatomical position in the  
56 root, the metaxylem being composed of three central cells and the protoxylem of  
57 the two cells located at each of the poles of the xylem axis [26]. The two xylematic  
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cell types are established through a complex mechanism involving the transcription factors SHORTROOT (SHR) and SCARECROW (SCR) as well as the miRNA165/166, which, in turn, targets the *HD-ZIP III* transcription factors [28] (Figure 3b). In this model, *SHR* is expressed in the procambium and SHR moves to the endodermis. Once there, SHR is recruited to the nucleus, where it interacts with SCR. Such interaction leads to the biosynthesis of miRNA165/166 [28], which also needs basic levels of ABA [29]. The miRNA diffuses to the neighbouring cells, creating a miRNA gradient that negatively correlates with *HD-ZIP III* transcript accumulation and metaxylem identity acquisition. Movement of miRNA165/166 to protoxylem cells causes *HD-ZIP III* expression to remain low, preventing protoxylem cells from differentiating into metaxylem.

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Protoxylem specification is dependent on the expression of the CK signaling inhibitor *ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 6 (AHP6)* [23, 30, 31]. *AHP6* expression is induced by the LHW-TMO5 heterodimer [32], meaning that it is, at least indirectly, auxin inducible (Figure 3b). Remarkably, procambial cells adjacent to the xylem facilitate polar auxin transport towards the xylem cells by relocating the PIN polar auxin transporters at the plasma membrane that faces the xylem in a CK-dependent manner (Figure 3b) [33]. Accordingly, in *wol* mutants the PIN proteins are mislocalized and protoxylem development is abnormal in *pin1pin3* [34]. CK is also locally synthesized in the xylem as a result of TMO5-LHW-dependent induction of *LOG3* and *LOG4* expression, but the auxin-induced expression of AHP6 counteracts CK signaling [35]. In brief, the mutual negative feedback loop of CK and auxin signaling establishes and maintains the bisymmetric patterning of root primary vasculature. Although CK signaling is diminished by AHP6 in xylem developing cells, LHW-TMO5-mediated local CK synthesis is still instrumental to promote the periclinal cell divisions that ensure the continuous supply of new xylem developing cells during postembryonic growth [21]. However, a safety mechanism prevents overproliferation by restricting LHW-TMO5 activity via an incoherent feed-forward loop that operates as follows: first, auxin and the HD-ZIP III factor ATHB8 promote the expression of *ACAULIS5 (ACL5)*, encoding a thermospermine synthase) [36-38]; then, the polyamine thermospermine promotes the translation of the atypical bHLH factors in the SACL family [21, 39, 40], which compete with TMO5 for heterodimerization with LHW, thereby preventing *LOG4* induction,



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reducing CK production, and controlling cellular proliferation. Accordingly, *acl5* mutants – in which the LHW dimerization with TMO5 is greatly enhanced- display cellular overproliferation, resulting in aberrant xylem development [41]. This effect is suppressed by overexpression of *SACL* genes or by reduction in LHW activity [21]. Ectopic expression of *ACL5* in *Populus* (*POPACAULIS5*) leads to repression of auxin biosynthesis, although the *Populus HD-ZIP III PthB8* induces *POPACAULIS5* expression, suggesting a negative feedback loop between thermospermine biosynthesis and auxin signalling. It is worth mentioning that, even though the only thermospermine function revealed to date is in xylem development, *ACL5* and thermospermine are also found in all non-vascular plants lineages [42]. Thermospermine function in such species remains to be elucidated

### ***Phloem specification***

The MYB transcription factor ALTERED PHLOEM DEVELOPMENT (*APL*) was the first phloem developmental regulator to be identified [43]. *APL* acts dually by inducing phloem identity while repressing xylem identity acquisition. Consequently, *apl* mutants feature xylem elements where phloem should be present. This leads to extremely reduced transport to the root apical meristem and, as a result, a short root phenotype and eventual seedling lethality [43]. However, phloem formation is normal in *apl* embryos [44], indicating that *APL* activity is most likely required for the later stages of phloem differentiation. Indeed, the *NAC45* and *NAC86* transcription factors were shown to act downstream from *APL* and regulate the activity of particular exonucleases (termed *NAC-DEPENDENT EXONUCLEASES*, *NENs*) which mediate enucleation during the last steps of sieve element formation [45]. Approaches through the *VISUAL* in-vitro system, by which mesophyll cells can artificially transdifferentiate into vascular cells (via “intermediate” procambial status), indicated that *NAC20* act as a negative upstream regulator of *APL* [46]. Recent studies identified the *PHLOEM EARLY DOF* (*PEAR*) *DOF* transcription factors as protophloem sieve elements markers [22]. Functional analyses revealed that *PEAR* proteins integrate positional information to prime cambial growth [22].

*BREVISRADIX* (*BRX*) and *OCTOPUS* (*OPS*) are two proteins required for protophloem specification [47-49]. The *ops* and *brx* mutants display the same

1 phenotype, both macroscopically (short root) and microscopically (discontinuities  
2 within the phloem strand). BRX, which was found through a natural variation  
3 approach [48], is auxin inducible. Protophloem differentiation depends on auxin  
4 concentration and, recently, it has been reported that BRX interacts with  
5 PROTEIN KINASE ASSOCIATED TO BREVISRADIX (PAX) to regulate auxin  
6 concentration during protophloem differentiation [50]. Both PAX and BRX locate  
7 at the basal zone of cells, where PIN auxin efflux carriers also locate. PAX  
8 activates PIN-mediated auxin efflux while BRX dampens it and, at the same time,  
9 PAX mediates BRX efficient plasma membrane localization, which is also  
10 negatively regulated by auxin. BRX inactivates PAX through direct interaction  
11 [50]. High auxin intracellular concentration induces PAX activity, leading to  
12 enhanced PIN-mediated auxin efflux, which depletes the cell of auxin, permitting  
13 BRX to associate with the plasma membrane and inactivate PAX, blocking PIN  
14 activity and, thus, favoring intracellular auxin accumulation [50]. Therefore, BRX  
15 and PAX are members of a molecular rheostat that modulates auxin flux during  
16 protophloem differentiation [50]. Protophloem specification is negatively  
17 regulated by a signaling pathway mediated by the leucine-rich repeat receptor  
18 like-kinase (LRR-RLK) BARELY ANY MERISTEM 3 (BAM3), whose activity  
19 depends on the binding to its ligand (the small peptide CLE45) in a dose-  
20 dependent manner [49]. *BRX* restricts the expression of *BAM3* [51] and the  
21 negative effect of the signaling, activated through CLE45 binding to BAM3, is  
22 amplified by MEMBRANE-ASSOCIATED KINASE REGULATOR 5 (MAKR5),  
23 which acts downstream from BAM3 [52]. Recently, it was shown that the positive  
24 regulation of the protophloem specification mediated by OPS acts as an insulator  
25 against CLE45 signaling by interfering with its binding to BAM3 [53].

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45 A screen for *brx* suppressors, *COTYLEDON VASCULAR-PATTERN 2*,  
46 identified mutants which accumulate abnormal levels of phosphoinositol and  
47 display discontinuous protophloem cell files in the root [54]. This and subsequent  
48 research indicate that phosphoinositides levels are important for the  
49 differentiation of protophloem [55]. Furthermore, the *SUPPRESSOR OF MAX2*  
50 *1-LIKE 3* (*SMXL3*), *SMXL4* and *SMXL5*, which were found to be strigolactone  
51 (SL)- and karrikin-independent, control protophloem specification in a redundant  
52 manner, since the double (*smxl4 smxl5*) and, specially, the triple *smxl3 smxl4*  
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*smx15* mutants, but not the single ones, show discontinuities in the root phloem strands and reduced root length [56].

### **Leaf venation**

From germination until floral transition (when the floral stem is developed), the only aerial organs found are cotyledons and leaves. In such flat organs, the vasculature is organized in so-called veins, which constitute a continuous, connected, reticulated system of vascular bundles. In these bundles the xylem usually faces the adaxial (upper) part of the leaf and the phloem, the abaxial (lower) part. The pattern formed by the veins is usually called venation and is characteristic of lineages and species. For example, monocots usually feature a parallel organization of their venation, while dicots often develop a reticulated structure [57].

A fundamental characteristic of the leaf vascular system is the close spatial relationship between the mesophyll (the photosynthetic cells) and the actual vascular tissues [5]. Physiologically, this is of great relevance, since photosynthesis implies water loss (which needs rapid supply by the xylem) and photoassimilates production that must be transported through the phloem.

In cotyledons and leaves, the vascular tissue starts developing as soon as the primordium is established. Preprocambial tissue differentiates from ground cells when the leaflet emerges and continues maturing into procambium while the leaf expands and matures into a fully-developed organ. In accordance with the auxin canalization model summarized by Sachs [58, 59], auxin signaling locates in specific ground cells that subsequently give rise to the veins even before they are anatomically different to their neighboring cells [60]. Both early induction of the auxin response factor *MP* in these cells [13, 61] and the discontinuity of vascular strands in *mp* mutants [10] suggest an early function of *MP* in leaf vein formation. The auxin efflux carrier PIN1 accumulates in the same preprocambial cells in which *MP* expression is detected, and which, later on, will define the vein pattern [13]. Interestingly, in *mp* mutants, the PIN1-GFP signal is greatly reduced and only detected in the cells where *MP* expression remains, implying that the *MP*-mediated auxin signaling precedes PIN1 accumulation [13].

In the initial steps of leaf primordia development, *MP* expression and PIN1 accumulation are widespread [62]. Gradually, both get confined to narrow regions

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in a process mediated by the activity of the HD-ZIP III transcription factor ATHB8 [62]. In brief, the procambial cells are determined and become specialized for PIN1-mediated auxin transport in a MP- and ATHB8-dependent manner. In turn, since *MP* expression is stimulated by auxin, the PIN1-mediated auxin transport leads to enhanced MP and ATHB8 activity in a feedback loop [61, 62].

### ***Vascular bundle formation in stems***

In Arabidopsis, the elongation of the floral stem starts upon the transition to the reproductive phase. Primary vasculature in stems develops in the form of discrete, not interconnected, vascular bundles that are distributed radially (Figure 1c). In Arabidopsis, stems typically develop between 5 and 8 vascular bundles in which the phloem faces the external part of the stem and the xylem, the internal one. Between xylem and phloem, the number of procambium cell layers can vary. Procambial cells give rise to extra xylem or phloem depending on the plant's necessities. Furthermore, these cells are key in the initiation of secondary vascular tissue growth (see below). To date, little is known about how vascular bundles are determined and patterned. While the HD-ZIP III transcription factors are key to establish the xylem and phloem polarity [63], the relative position of phloem and xylem is controlled, at least partly, by the signaling pathway mediated by the LRR-RLK PHLOEM INTERCALLATED WITH XYLEM (PXY)/TDIF RECEPTOR (TDR) [64, 65]. The dodeca-CLE peptides TDIF/CLE41/42/44 are synthesized in the phloem and then move to the procambium, where they meet their receptor, PXY, activating a signaling cascade that controls the xylem/phloem relative position [64-68] (see below). It is thought that such control on patterning might occur through an interaction with ERECTA [66, 67].

The stem vascular bundles function is double: they provide an apical/basal axis to transport water, solutes, nutrients and signaling molecules, and they serve to connect the stem with the veins of the lateral organs [69].

### **Vascular cell differentiation**

Xylem is mainly formed by conductive cells known as "tracheary elements" (TEs). In gymnosperms, the TEs are called tracheids. Such tracheids fulfill two roles: support and transport. In eudicots, two TEs cell types can be found, each with specialized functions and anatomy: the vessels, specialized in water and

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solutes transport, and the fibers, specialized in providing mechanical support and stability [5]. Vessels and fibers undergo secondary cell wall formation, lignification and programmed cell death (PCD) during their maturation [70].

Vessels start forming at very early stages of xylem development (Figure 4). The NAC transcription factors VND6 and VND7 are fundamental for the formation of this cell type [71] (Figure 5). The timing for fiber initiation is controlled by *ERECTA* (*ER*) and *ERECTA-LIKE1* (*ERL1*) in a GA-dependent manner [72]. In order for the cambium and developing xylem cells to become sensitive to GA and, therefore, differentiate fibers instead of parenchyma, the activity of the class I KNOX transcription factor KNAT1/BREVIPEDICELLUS (KNAT1/BP) is needed [72, 73]. Indeed, KNAT1/BP activity has been shown to be negatively regulated by DELLA through physical interaction [74], and *bp* mutants do not display fiber formation [75]. Such a defect cannot be rescued by GA application [74]. Recently, research has shown that the leucine-rich repeat receptor like-kinase SUPPRESSOR OF BIR 1/EVERSHED (*SOBIR1/EVR*) prevents the precocious initiation of xylem fiber differentiation by a mechanism that involves BP and *ERECTA* [76]. BP binds the promoter of *SOBIR1/EVR*, and *SOBIR1/EVR* expression is diminished in *bp* mutants, indicating a negative effect of BP on the expression of *SOBIR1/EVR* [76]. Furthermore, *SOBIR1/EVR* physically interacts with *ERECTA* and the effect of *sobir1/evr* mutation on the initiation of fiber differentiation is enhanced in *erecta* mutant backgrounds [76]. BP activity in fiber development occurs by activating the expression of *NST1* and *NST3/SND1*, two master regulators of fiber formation [75]. *NST1* and *NST3* are found upstream from *BLADE ON PETIOLE 1* (*BOP1*) and/or *BOP2*, with which KNAT1/BP genetically interacts [75], implying that *BOP1* and *BOP2* might be highly relevant for fiber differentiation. Although the *nst1*, *nst3* and even *nst1 nst3* double mutants do not show any obvious defective phenotype, *NSTs* are expressed in xylem cells undergoing secondary cell wall biosynthesis, and the overexpression of any of the *NSTs* leads to ectopic xylem formation [77, 78]. While BP controls the expression of *SOBIR1/EVR*, the interaction between *SOBIR1/EVR* and *ERECTA* controls KNAT1/BP levels by reducing GA signaling [76]. Presumably, this occurs by enhancing the levels of DELLA, which would limit KNAT1/BP activity through direct interaction. Thus, *SOBIR1/EVR* transcription would also be

1 limited, and a feedback loop that would maintain KNAT1/BP abundance and, as  
2 a result, NST activity in check would be generated [76].

3 The function of VND6, VND7, NST1 and NST3/SND1 is conserved across  
4 angiosperms [79]. Large scale yeast-one hybrid analyses identified numerous  
5 targets of such transcription factors [80]. Indeed, the authors used these results  
6 to construct a network regulating secondary cell wall formation during  
7 xylogenesis [80].

8 In addition to TEs, many species develop xylem parenchyma: a cell type that  
9 remains alive, stores reserves in the form of starch and provides physiological  
10 support to TEs during and after their maturation. To date very our knowledge  
11 about the mechanisms by which such xylem parenchyma cells develop is scarce.

## 21 **Regulation of cambial activity and secondary growth**

22 Secondary vascular tissues develop radially in stems, roots and hypocotyls in a  
23 process known as secondary growth which results in thickening. Secondary  
24 growth provides enhanced transport capacity as well as mechanical support and  
25 stability. In Arabidopsis hypocotyls, secondary xylem development resembles  
26 that of trees and, therefore, this organ has become a reference for research in  
27 secondary growth development in general and secondary xylem development in  
28 particular [81]. Secondary xylem occurs in two phases in Arabidopsis hypocotyls:  
29 phase I in which vessels and parenchyma (but not fibers) are formed and phase  
30 II, in which no parenchyma is formed, but vessels and, especially, fibers  
31 differentiate massively [81]. The transition from phase I to phase II is coordinated  
32 with flowering and is mediated by the activity of gibberellic acid, which is precisely  
33 synthesized during flowering [82].

34 Secondary growth occurs through the activity of the vascular cambium, a  
35 meristem that is found in a ring-like domain, internally to stems, roots and  
36 hypocotyls [83]. In Arabidopsis, the vascular cambium forms differently in the  
37 stem, the hypocotyls and the root. In the stem, the vascular cambium develops  
38 through two complementary processes: the lateral expansion of the procambial  
39 cells located between xylem and phloem in the vascular bundles and the  
40 formation of new cambial cells through proliferation and transdifferentiation of the  
41 interfascicular regions of the starch sheath, the innermost cortical cell layer in  
42 stems [4, 83] (Figures 1c; 6a). However, in the hypocotyl and the root, there is no  
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transdifferentiation and, indeed, the vascular cambium arises from massive cellular proliferation of the procambial and a few pericycle cells [26, 84]. Soon after the onset of this proliferation, cells in contact with the primary xylem start differentiating into secondary xylem [84]. Subsequently, more cambial cells in contact with the new xylem cells also start differentiating into secondary xylem. The process goes on to result in an accumulation of secondary xylem in the central part of the root and the hypocotyl. This new xylem forces the cambium towards the external part in a radial manner, leading to the formation of the classical cambial ring. In later stages, the cambium develops secondary phloem, which also organizes as a ring [24] (Figure 1d).

Similar to that described above for the vascular bundle development, PXY also controls both the relative disposition of the xylem and phloem during secondary growth [64]. In addition, PXY controls the proliferation of cambial cells as well [64, 66] (Figure 6b). The PXY-mediated control of cellular proliferation occurs by activating the WUSCHEL RELATED HOMEODOMAIN 4 (WOX4) and WOX14 proteins [64, 66]. The pathway is conserved between Arabidopsis and Populus [85]. Independently of PXY, two more receptor-like kinases (RUL1 and MOL1) play opposing roles to regulate cambial cell proliferation; RUL1 being a positive and MOL1 a negative regulator in the process [83] (Figure 6b). Recently, it was shown that the glycogen synthase kinase 3 BIN2-LIKE1 (BIL1) phosphorylates MP, which acts as a negative regulator during secondary growth by upregulating CK signaling repressors [86]. The phosphorylated MP enhances its negative activity [87]. However, PXY inhibits BIL1, maintaining secondary growth in homeostasis [87]. In brief, these results demonstrate that BIL1 connects peptide signaling with auxin/cytokinin signaling [87] (Figure 6b).

Which molecular mechanisms lead cambial cells to enter the phloem or xylem developmental programs is still an open question. Strikingly, a recent and elegant report based on lineage-tracing and molecular genetics discovered a role for cells with newly acquired xylem identity within the cambial zone as organizer/quiescent center for the cambium directing adjacent cambial cells to divide [88]. This is dependent on auxin concentration and expression of HD-ZIP III factors, and the quiescent center also maintains phloem identity in a non-cell autonomous manner [88]. Remarkably, it is within the early protophloem-sieve-element cell files of the procambial tissue where radial growth begins [22]. In such

1 cellular domain, CK induces the expression of the above mentioned PEAR1 and  
2 PEAR2, and their four homologues DOF6, TMO6, OBP2 and HCA2. Short-range  
3 movement of the PEAR proteins establishes a concentration gradient that is key  
4 for the initiation of radial growth [22]. Interestingly, the PEAR proteins promote  
5 the transcription of the HD-ZIP III factors, which antagonize the activity of the  
6 PEAR proteins, establishing a negative-feedback loop [22]. Thus, the module  
7 created by the CK-induced PEAR and auxin-induced HD-ZIP III transcription  
8 factors integrates the spatial information and hormonal signaling in the definition  
9 of the quiescent center and cells that are actively dividing, which is fundamental  
10 for radial growth [22, 88]. Apart from auxin and CK, other hormones such as  
11 ethylene, gibberellic acid, jasmonic acid, abscisic acid and strigolactone are  
12 known to play a role in secondary growth. Auxin action on cambial activity is well  
13 established [89]. Indeed, auxin peaks were found in cambium in *Populus* and  
14 *Arabidopsis*, and auxin signaling is enhanced in parenchymatic tissues  
15 transforming into interfascicular cambium [90-95]. Accordingly, in-vitro auxin  
16 treatment on isolated immature *Arabidopsis* stems lead to interfascicular  
17 cambium formation [83] and both *Populus* and *Arabidopsis* undergo reduced  
18 secondary growth when auxin signaling is reduced, as is the case in mutants  
19 such as *axr1* [90-95]. However, the regulation seems to be more complicated,  
20 since different *AUXIN RESPONSE FACTORS* (*ARFs*) seem to play different  
21 roles and, while *ARF3* and *ARF4* stimulate cambium activity, *ARF5* (*MP*)  
22 counteracts such induction, suggesting an auxin-controlled, *ARFs*-mediated  
23 homeostasis of cambial activity [86].

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42 Auxin signaling also results in the induction of *WOX4* activity independently  
43 of *PXY* -although *PXY* is required to keep the induction stable over time, as  
44 deduced from unstable auxin-induced *WOX4* expression in *pxy* mutants-,  
45 indicating that auxin-induced cambial activity does not require *PXY* signaling [96].  
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47 Auxin transport plays a central role in cambium activity [97, 98] and, indeed, at  
48 least *pin1* and *pin3* mutants undergo reduced cambium activity, reflected in  
49 decreased secondary growth in *Arabidopsis* [99]. As in the case of other  
50 developmental processes, auxin interacts with strigolactones (*SLs*) to regulate  
51 secondary growth; moreover, genetic, molecular and physiological analyses  
52 proved that *SLs* act downstream from auxin to control the process, adding a new  
53 step in the hormonal regulation of secondary growth initiation [99].  
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1 CKs are known to stimulate cellular proliferation in meristems [7]. In  
2 cambium, several reports indicate a clear correlation between CK signaling and  
3 cambial cell proliferation both in *Arabidopsis* and *Populus* [100]. The above  
4 mentioned heterodimerization of LHW with TMO5, leading to CK biosynthesis via  
5 *LOG3* and *LOG4* expression [21], is also crucial to stimulate cellular proliferation  
6 during radial growth. Recently, DOF2.1 was found to control such cellular  
7 proliferation downstream from the LHW-TMO5 heterodimer [101].  
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12 As explained above, gibberelins are essential for the formation of xylem  
13 fibers, which are associated to the expansion phase of secondary growth [81].  
14 Thus, they play a positive role in wood accumulation, thereby positively regulating  
15 secondary growth [102]. Jasmonic acid [103], ethylene [103, 104] and ABA [105]  
16 have also been shown to play a positive role in secondary growth.  
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21 Little is known about the regulation of secondary phloem formation (Figure  
22 4). However, it is known that most of it is parenchyma and that a few sieve  
23 elements and companion cells arise randomly through it.  
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## 29 **Environmental regulation of vascular differentiation**

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31 Vascular development is modulated by an array of environmental factors, ranging  
32 from temperature and light regimes to water availability, mechanical stress, or  
33 salt concentration in the soil. While light and temperature signals are interpreted  
34 mostly as seasonal cues by plants, other factors may represent transient,  
35 adverse situations that challenge the plant's ability to acclimate. In those cases,  
36 plasticity in vasculature development is part of an integral plan to optimize the  
37 use of resources. As described below, acclimation strategies include the  
38 alteration of cambial cell proliferation rates, xylem to phloem proportion, relative  
39 abundance of specific cell types within xylem or phloem, and the patterns and/or  
40 properties of such cell types, including cell wall thickness, cell length, cell  
41 diameter, cell wall pitting or cell wall reticulation. It must be noted that, in the case  
42 of xylem, fully differentiated cells are dead, preventing any post-developmental  
43 plasticity [106]. Therefore, xylogenesis plasticity must occur entirely during xylem  
44 specification and differentiation, and cell properties of fully-developed xylem cells  
45 are not reversible. Consequently, the environmental conditions during  
46 xylogenesis are critical for the general performance of plants.  
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## **Drought**

1 Water is absorbed by roots and transported acropetally through the plant by the  
2 xylem water conduits: vessels in angiosperms and tracheids in gymnosperms  
3 [107]. Thus, water availability constitutes an essential environmental input for  
4 xylogenesis plasticity, the result of which is crucial for plant acclimation to  
5 drought. Mechanisms underlying such xylogenesis plasticity for acclimation to  
6 water availability require hormone activity (Figure 7). In this regard, the role of  
7 ABA is the most frequently studied one, although CK and JA have been shown  
8 to play important roles as well.

9  
10 Drought is known to enhance the biosynthesis and accumulation of ABA  
11 [108]. Among other actions, ABA regulates various aspects of the differentiation  
12 and patterning of primary and secondary xylem [29, 105]. ABA treatments in  
13 Arabidopsis roots induced extra meta and protoxylem cells, the latter with  
14 reticulated, rather than pitted, secondary cell walls [29]. By contrast, *abi2-1* and  
15 *abi3-1* mutants, impaired in the last steps of ABA biosynthesis, displayed  
16 discontinuous or absent xylem strands [29]. An effect very similar to the *abi2-1*  
17 and *abi3-1* mutations was observed when WT was treated with the ABA  
18 biosynthesis inhibitor fluridone [29]. Further, provascular and endodermis cellular  
19 differentiation was accelerated in ABA-treated roots in both Arabidopsis and  
20 Tomato [109]. The mechanism by which ABA regulates the determination of the  
21 xylem cell-types is non-cell-autonomous [29]. Indeed, it occurs in the endodermis,  
22 where ABA induces miRNA165/166 biosynthesis while reducing the expression  
23 level of the miRNA165/166 repressor ZWILLE/ARGONAUTE 10 (ZLL/AGO10)  
24 [29, 109]. The miRNA165/166, as mentioned above, moves to the developing  
25 xylem cells, and its levels determine the proto- or metaxylem identity by  
26 controlling the mRNA accumulation of certain HD-ZIP III factors [28]. Accordingly,  
27 ABA treatments failed to induce extra xylem strands in miRNA165/166 resistant  
28 lines such as *phb1-d* or in mutants with considerably reduced miRNA165/166  
29 accumulation such as *scr* or *shr* [109]. Drought treatments –shown to induce  
30 ABA in roots [110, 111]- affected miRNA165/166 levels and led to the same  
31 phenotypic effects induced by ABA, linking drought with the ABA-mediated  
32 mechanism of regulation in miRNA165/166 levels to control xylem cell-type  
33 specification [29].

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In trees, long-term drought reduces secondary growth and modifies the pattern of vessel formation [112, 113]. Despite reducing general levels of secondary growth, drought induces the formation of extra vessel cells with reduced cellular diameter [112, 113]. Thus, this response represents an acclimation mechanism to drought that diminishes cavitation without losing vessel transport capacity [112, 113]. This alteration seems to be maintained, at least partially, across seed plants, since tracheid diameter in the gymnosperm *Picea abies* was also smaller in individuals subjected to severe drought [114]. Further observations also indicated drought-induced increases in vessel cell wall thickness, at the expense of the vessel lumen [115], which results in reduced conducting surface. The precise mechanism that regulates cell wall deposition in response to stress is not understood, but experiments with *Arabidopsis* in which osmotic stress was stimulated by high mannitol content in the growth medium [116] revealed a substantial decrease in microRNA857 abundance. Given that miR857 is a postranscriptional inhibitor of *LAC7*, which encodes a laccase involved in lignin deposition in the secondary xylem [117], the drought-mediated regulation of the machinery controlling cell wall properties is likely to be a widespread mechanism to modulate xylem conduits properties under drought. This view is also supported by work with *P. trichocarpa*, in which drought differentially affected the expression of a large number of genes involved in cell wall remodeling, including four master switch regulators orthologous to the *Arabidopsis* NST1 transcription factor, and 29 laccase-encoding genes [118].

Drought has also been shown to reduce secondary growth by hampering the accumulation of CK and its root-to-shoot transport [119-121]. Decreased levels of CK in well-watered plants increased ABA sensitivity [121], suggesting a cross-talk between the two hormones. Moreover, jasmonic acid (JA) is thought to induce xylem differentiation in *Arabidopsis* roots from meristematic procambial cells by counteracting the CK-dependent promotion of cell division [122]. Given that JA signaling is necessary for consistent ABA accumulation in roots in response to water limitation [123], current knowledge indicates that the interplay between ABA, JA and CKs modulates xylem differentiation as part of the acclimation strategy to varying water levels.

### **Salt stress**

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Although the effects of high salinity on vascular development are similar to those caused by drought, there are indeed differences in the responses of plants to each type of stress. For example, while drought induced extra protoxylem differentiation [29], this effect was not observed in roots grown under high salinity, even when incubating plants in increasing NaCl concentrations [109]. This observation constitutes a clear difference between the responses of plants to drought or salt stress in terms of vascular development. Therefore, it is plausible that salt stress, while inducing ABA biosynthesis, might trigger some signaling pathway preventing extra protoxylem differentiation. On the other hand, like drought, salt stress has been shown to reduce lumen in xylem vessels in *Arabidopsis*, tomato and *Populus* [124]. This reduction in overall xylem lumen development seems to be necessary to enhance salt tolerance, since the *ac15* *Arabidopsis* mutants, with excessive, premature, xylem development, display salt hypersensitivity [125]. Several possible mechanisms may be responsible for this effect. For instance, the reduction in xylem vessel volumes in poplar trees subjected to 2-week salt treatments was associated to an increase in ABA levels in leaves, which caused stomata closure and subsequent diminished nutrient uptake by the cambium, causing indirectly the decrease in vessel lumina [126]. Alternatively, enhanced lignin deposition has been observed in developing xylem vessels of plants growing under high salinity in *Aeluropus littoralis*, as well as in tomato roots [127, 128]. To support this hypothesis, studies have focused on two transcription factors whose expression is induced by salt, and whose overexpression promotes secondary cell wall deposition during xylem differentiation: *ERF139* in *Arabidopsis* [129] and *BpIMYB46* in birch [130].

### **Temperature**

The most widely studied effects of temperature on vascular development are those associated to the alteration of cambial cell proliferation and secondary cell wall deposition in xylem cells. In seasonal climates, the main environmental factor affecting cambial activity is temperature. This is reflected by the correlation between the timing of cambial reactivation after dormancy and the early onset of the growth season [131]. Studies in *Populus*, revealed extensive transcriptomic and proteomic changes in the cambium during the activity-dormancy cycle [132]. More importantly, these changes included the activation of cell cycle genes

1 during cambium activity and their repression during dormancy. These changes  
2 are primarily temperature-dependent, according to water culture experiments,  
3 which show that *PtoCDKB* and *PtoCYCB* transcripts are clearly expressed in  
4 cambium at warm temperatures but undetectable at low ones [133].  
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7 Given that cold induces MT de-polymerization in plant cells [134], it was  
8 thought that temperature-mediated cambial activity regulation might depend on  
9 the MT polymerization state [131]. However, it is not clear whether changes in  
10 MT polymerization are the cause or a readout of cambial activity state. Indeed,  
11 MT de-polymerization has been observed in the cambium when temperatures  
12 transiently descended during the warm growth period [131], but transient warm  
13 temperatures during the winter dormancy period did not re-polymerize MT [131].  
14 By contrast, the detection of enhanced accumulation of transcripts encoding MT-  
15 associated proteins (i.e: MAP20) in Poplar during the formation of secondary cell  
16 walls suggests the involvement of MT in vascular development [135].  
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19 Arabidopsis leaves have been shown to acclimate to high temperatures by  
20 increasing vein density [136]. This response might be related to a general shift  
21 from sugar transport at cool temperatures to water transport through tracheary  
22 elements at warmer temperatures, perhaps due to enhanced transpiration.  
23 Intriguingly, the expression of the *PXY* paralog encoded by *AtPXL1* is patently  
24 induced both by cold and heat stress, and the *atpxl1* mutant line showed a  
25 temperature hypersensitive phenotype [137]. These results suggest that *PXY*  
26 activity at the cambium might be important for acclimation when temperatures  
27 fluctuate. It remains to be seen whether temperature can control *PXY* expression  
28 or *PXY* activity, or whether there is a yet-to-be discovered *PXY* upstream  
29 regulator whose activity is temperature dependent.  
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32 As mentioned above, temperature is also a major modulator in the  
33 composition of xylem secondary cell walls, as shown by work with *ESKIMO1*, a  
34 gene encoding a 2-O- and 3-O-acetyl transferase that targets xylosyl residues in  
35 xylan [138]. *ESK1* expression is regulated by the secondary cell-wall regulator  
36 *SND1/NST3* and, accordingly, its expression is stronger in vascular tissues than  
37 in other tissues, especially in cells undergoing xylem differentiation [139]. Vessels  
38 collapse in the loss of function *esk1* mutants. However, the mutation allows  
39 resistance to freezing and cold temperatures in general, as well as to salt stress  
40 [140]. The defects in *esk1* xylem vessels appear to be linked to a reduction in  
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1 esterified pectins, which is in agreement with the observed downregulation of a  
2 gene encoding a Pectin Methyl Esterase Inhibitor in the *esk1* mutant [139].  
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### 5 ***Mechanical stress***

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7 The proliferation of cambial cells and the differentiation of the cell types that form  
8 the xylem and the phloem occur in the context of intense mechanical forces [103].  
9 On the one hand, tensions originate from endogenous processes, such as the  
10 increasing weight of the plant from growth, the pressure caused by the  
11 proliferation and accumulation of new cells or the changes in turgor caused by  
12 cell differentiation or water conductance through tracheids or vessels. On the  
13 other hand, external environmental conditions, including wind or the presence of  
14 obstacles in the growth axis, also generate mechanical forces. Due to  
15 lignification, the vascular cells can counteract such mechanical stress. Therefore,  
16 a common response of plants to mechanical forces is the generation of new  
17 vascular cells. In other words: mechanical stress induces vascular development.  
18 A clear example of this is the stimulation of secondary growth as a response to  
19 increased plant weight [97]. Such response has been shown to be accompanied  
20 by the enhanced expression of secondary cell wall regulatory genes [141],  
21 changes in the expression of auxin signaling elements and stimulation of polar  
22 auxin transport [97]. However, despite the dominant role of auxin in weight-  
23 induced secondary growth, other factors (perhaps some of them auxin-  
24 independent) also contribute to controlling the mechanical stimulation of  
25 secondary growth. For example, ABA, ethylene and JA signaling are induced  
26 during secondary growth in Arabidopsis and control several aspects of the  
27 process, most likely in an auxin-independent manner [103-105]. Additionally,  
28 ethylene was found to control cambial cellular proliferation both in normal  
29 conditions and during tension wood development in Populus [142]. During the  
30 process, no change in auxin levels was found in tension wood forming tissues  
31 [143], although the establishment of an auxin gradient –most likely led by auxin  
32 redistribution- seems to be necessary [144]. Furthermore, GA was shown to  
33 control all major aspects of tension wood formation when applied to Populus  
34 stems [145], and a role for it during the process cannot be excluded. As previously  
35 discussed, KNAT1/BP regulates xylem fiber production [75]. SHOOT  
36 MERISTEMLESS (STM), another class I KNOX transcription factor, was found to  
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act redundantly with BP in this process [75] and its expression is induced when there is mechanical stress in the shoot apical meristem [146]. The expression of *SOBIR1/EVR*, which, as described above, participates in the BP-mediated mechanism regulating fiber formation, was enhanced in the cambium upon mechanical stress induction [97]. All in all, current knowledge suggests a potential scenario in which the mechanical stimulation of secondary growth may act through auxin-dependent pathways regulating cambial cell proliferation and parallel auxin-independent pathways to control specific aspects in the differentiation of the vascular cell types.

### **Conclusions and future prospects**

In recent decades, our understanding of the genetic and molecular control of vascular development has increased considerably. In parallel, work with both herbaceous and woody species has revealed the effect that environmental factors have, anatomically, on the vascular tissues. Therefore, studies should now focus on identifying the molecular mechanisms underlying the environmental modulation of vascular development. Previous attempts to connect vascular development with environmental factors have been based mostly on field studies with woody plants, often comparing species. Given the relatively high level of understanding of the molecular mechanisms involved in vascular development in model plants, like *Arabidopsis* and *Populus*, it seems necessary to approach the study of all aspects of vascular development under the controlled combined action of different environmental signals. Such an approach is likely to identify “entry points” through which each environmental factor alters the molecular control of vascular development.

Classical forward genetic screens are extremely tedious when performed for vascular development because they usually entail microtom-based sectioning. This is of especial relevance when performing screens aimed identifying new genes involved in adjusting the process to specific environmental factors. However, reverse genetic approaches may be useful and, by including experimental approaches based on natural variation and Genome-Wide Association Studies (GWAS), it might be possible to identify not only new regulators but also natural alleles for already known vascular developmental regulators key for adapting vascular development to specific habitats. In this

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respect, recent works that used GWAS-guided reverse genetics approaches have identified XYLEM NAC-DOMAIN1 (XND1) as a negative regulator of hydraulic conductivity that also affects vascular anatomical variation [147] and, as mentioned above, SOBIR1/EVR as a new regulator of the timing for fiber differentiation [97]. These are two clear examples of how natural variation-mediated identification of either new genes or allele variants for previously known genes may provide new tools for biotechnological approaches as well as insights into our understanding about the adaptation mechanisms in the process. At a higher order scale, experimenting with key species in the evolutionary clades of vascular plants may reveal which environmental factors have led to specific evolutionary aspects of the vascular development process and what genes have been relevant for such evolution. All this information, especially when validated in real natural conditions, might provide new, essential knowledge that can be used to artificially adapt our forest trees and crops to future environmental conditions or even to expand their geographical crop domain.

In brief, we propose that understanding how plants adjust their vascular developmental program to environmental changes will enhance our understanding about the plasticity, adaptability and evolutionary history of the process. This knowledge may be a starting point for biotechnology applications aimed at ensuring biomass formation in a climate change context.

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## Figure legends

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4 Fig. 1. Organization of plant vascular tissues. **a** Appearance of the provascular  
5 tissue at the end of embryo development. **b** Localization of the procambial,  
6 phloem and xylem tissues within primary vasculature in veins and in the root of  
7 young seedlings. **c** Secondary growth in stems. In Arabidopsis (and other  
8 dicotyledoneous plants), primary vasculature appears in bundles with the phloem  
9 facing outwards and the xylem inwards, separated by a layer of procambial cells.  
10 Secondary growth requires the sequential formation of a cambial ring between  
11 bundles and the stimulation of periclinal cell divisions. **d** Secondary growth in  
12 roots also involves expansion of xylem at the expense of the cambium.  
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22 Fig. 2. Mechanism for provascular specification during embryo development. **a**  
23 Illustration of the localization of (i) the initial cells in the globular-stage embryo  
24 from which all vascular tissues originate and (ii) the periclinal divisions that give  
25 rise to additional vascular cells in a heart-stage embryo. **b** Gene regulatory  
26 network that determines vascular cell identity and cell divisions during embryo  
27 development. In response to local accumulation of auxin, the MP auxin-  
28 dependent transcription factor enhances auxin accumulation through the  
29 upregulation of the PIN1 auxin transporter, establishes vascular identity by  
30 inducing the ATHB8 HD-ZIP III transcription factor and promotes periclinal cell  
31 divisions via TMO5-LHW-mediated increase in CK synthesis.  
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42 Fig. 3. Mechanism for xylem specification and maintenance in the primary root. **a**  
43 Tissue layer organization surrounding the vascular cylinder of the primary root.  
44 The xylem axis in the center is surrounded by vascular-competent stem cells  
45 (procambium), and with two phloem poles on opposite ends. **b** Gene regulatory  
46 network that coordinates CK-dependent cell proliferation in the procambium, with  
47 auxin-mediated xylem cell-type specification. Xylem specification depends on  
48 high local auxin signaling via MP, and is also achieved by high HD-ZIP III  
49 localization in the xylem precursor cells. MP activity is not only responsible for  
50 the attenuation of CK signaling in xylem precursor cells, but also for triggering the  
51 ACL5-dependent inhibitory loop that maintains appropriate levels of the LHW-  
52 TMO5 complex.  
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2 Fig. 4. Cell differentiation pathways which produce the diverse phloem and xylem  
3 cell types. Illustration of the formation of different phloem and xylem cell types  
4 from unique vascular stem cells. Xylem cells include the dead vessels (tracheary  
5 elements) and fiber cells with extensive secondary cell wall deposition, along with  
6 live xylem parenchyma cells. Phloem is composed of the sieve elements and the  
7 phloem companion cells. Pink circles depict cell nuclei, and white ellipses are  
8 vacuoles.  
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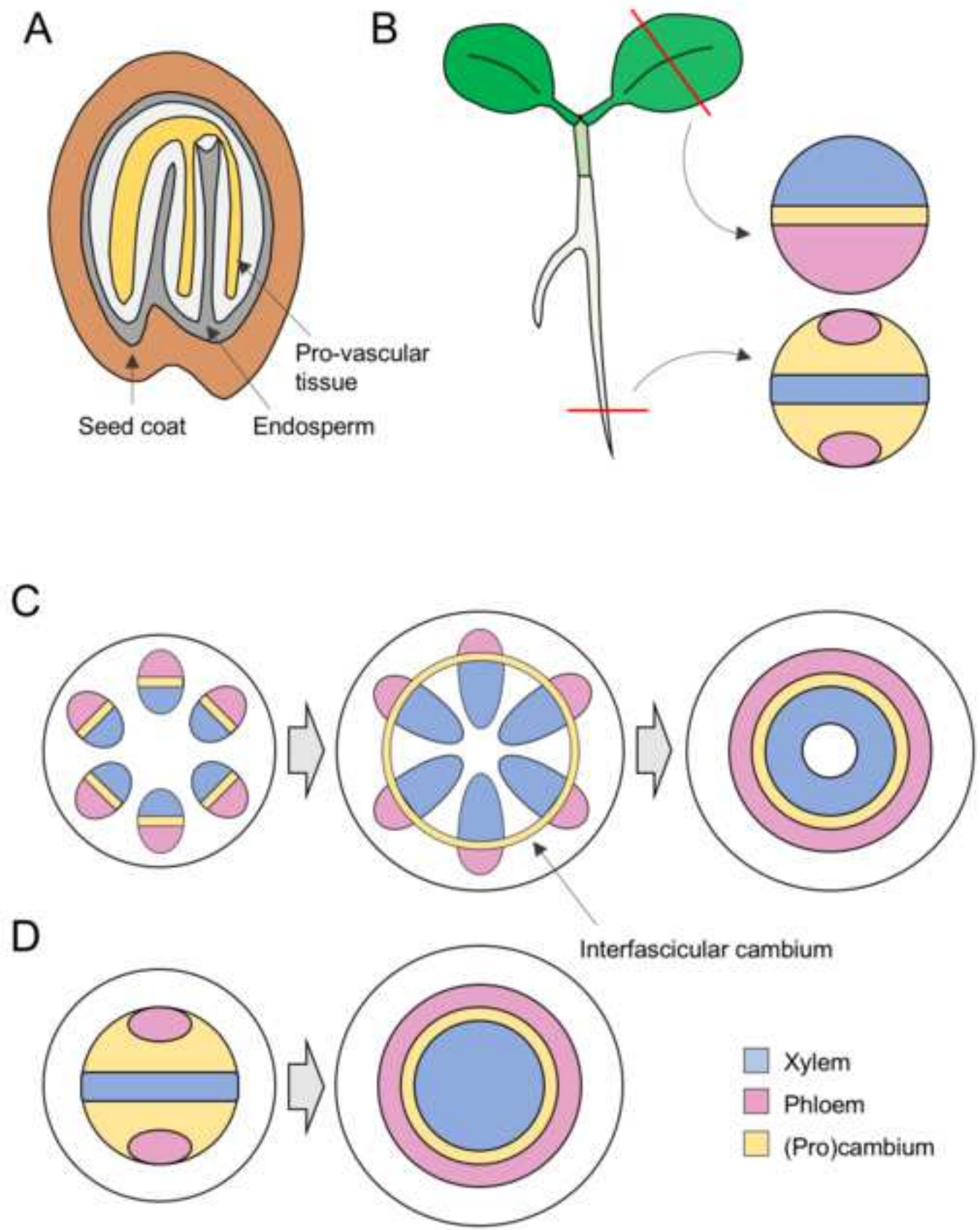
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16 Fig. 5. Gene regulatory network for the differentiation of xylem fibers and the two  
17 types of vessels. The different cell identities are established by specific  
18 expression of VND6 (metaxylem, MX), VND7 (protoxylem, PX), and SND1/NST3  
19 (fibers) transcription factors. SCW synthesis is regulated by the second tier of  
20 MYB transcription factors, while programmed cell death is regulated by the ACL5  
21 thermospermine synthase. Additionally, fiber formation is absolutely dependent  
22 on STM and KNAT1 activity, and this is stimulated by low DELLA levels achieved  
23 by high GA production; which is thought to be regulated by Receptor-Like  
24 Kinases such as ER and ERL1.  
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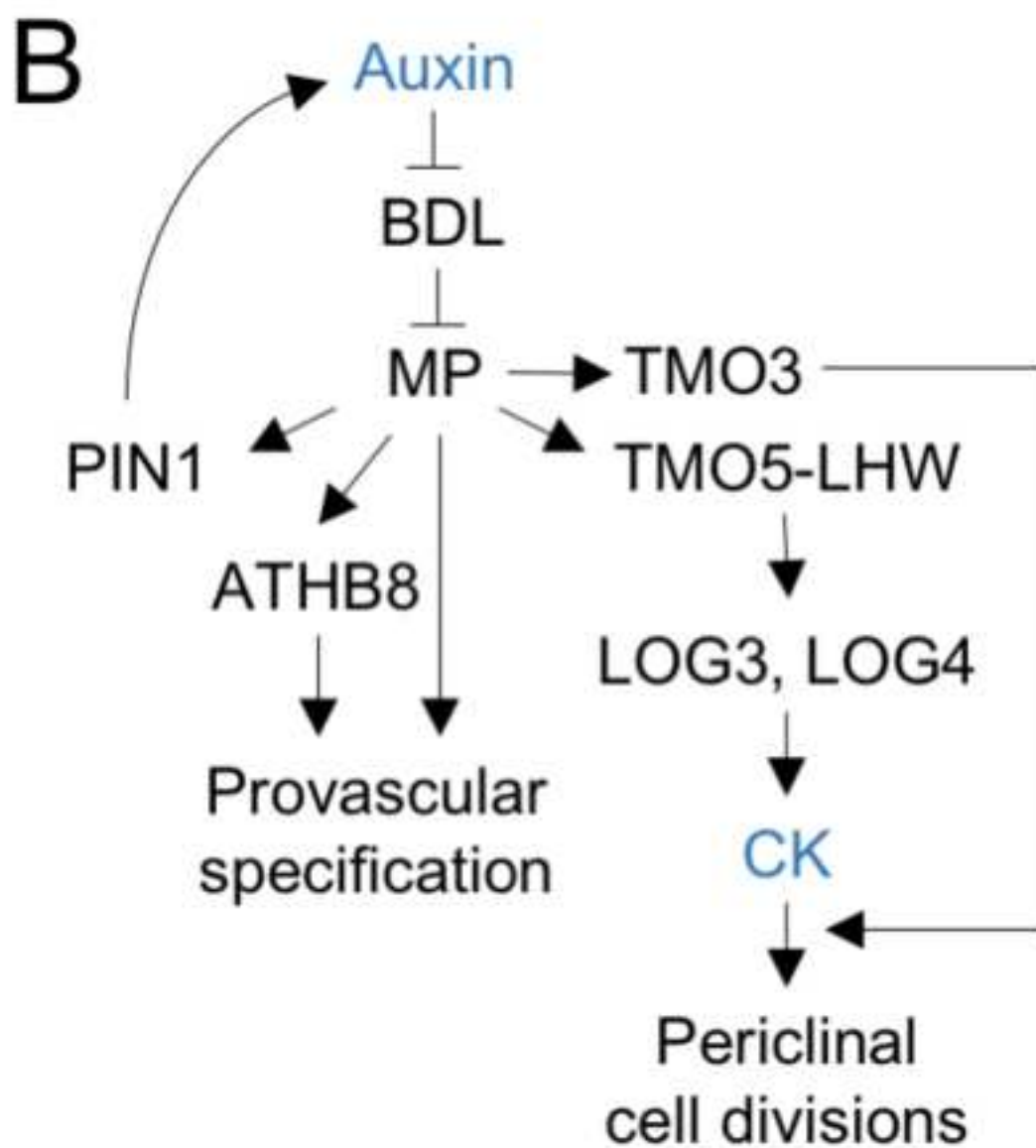
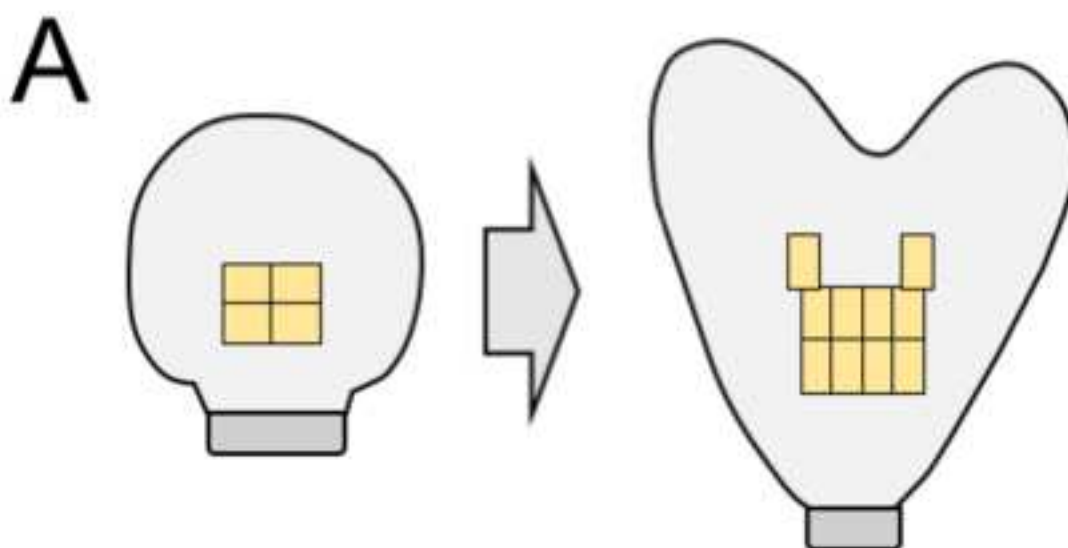
34 Fig. 6. Regulatory mechanism of cambial activity and secondary growth. **a**  
35 Scheme of stem vascular bundles showing the bifacial cambium. **b** Gene  
36 regulatory network coordinates cambial cell proliferation and the production of  
37 phloem and xylem on opposite sides of the cambium. This organization is the  
38 result of several regulatory interactions: (i) the PXY receptor-like kinase is  
39 activated by the phloem-generated TDIF signal (CLE41, 42, 44 peptides) and  
40 induces the WOX transcription factors which, in turn, promote cell proliferation in  
41 the cambium; (ii) auxin modulates cell proliferation with opposing effects that  
42 involve the attenuation of CK signaling and the upregulation of WOX genes; (iii)  
43 xylem specification is promoted by the PXY-dependent repression of  
44 brassinosteroid signaling and by the positive effect of auxin on HD-ZIP III  
45 expression in xylem precursor cells; (iv) phloem is regulated by the mobile PEAR  
46 transcription factors that are upregulated by CK in the cambium.  
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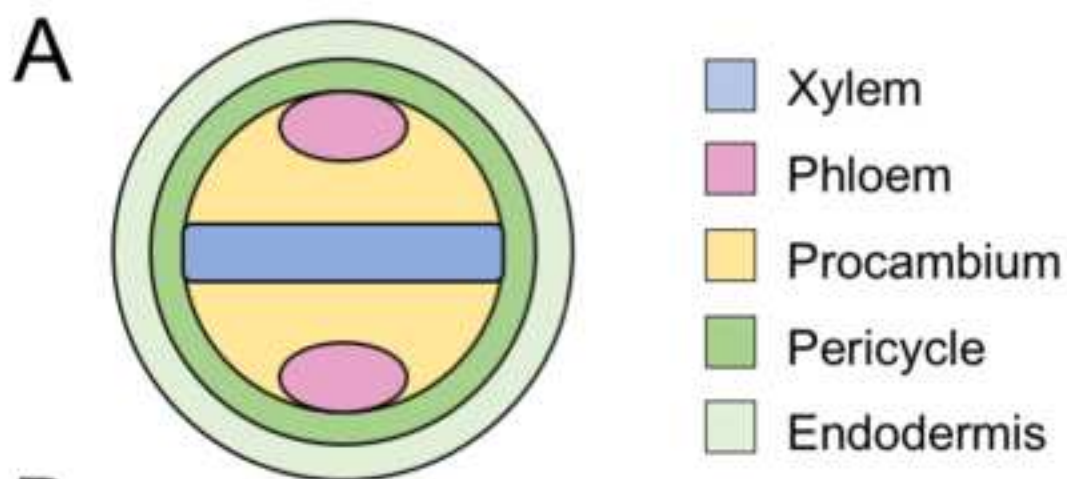


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Fig. 7. Possible mechanisms for environmental regulation of vascular development. Three processes have been reported to be under environmental control: (i) cell proliferation in the cambium, which is inhibited by drought via the reduction in root-to-shoot CK transport, and stimulated by mechanical stress via auxin upregulation; (ii) proliferation of xylem cells to increase water transport potential or counteract possible consequences of vessel cavitation; this is activated by drought through the ABA-dependent upregulation of miR165 (allowing for the reduction in HD-ZIP III activity), and also by mechanical stress in an auxin-dependent manner and (iii) the production of secondary cell wall components, which is increased by drought and salt through the upregulation of specific cell-wall enzymes; moreover, the relative composition of SCW material is also modulated by mechanical stress (with an increase in fiber cell production) and by temperature (which affects the activity of ESK, an enzyme that modifies xylan).







**B**

