From primary to secondary growth: origin and development of the vascular system

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Abstract

Vascular tissue differentiation is essential to enable plant growth and follows well-structured and complex developmental patterns. Based on recent data obtained from Arabidopsis and Populus, advances in the understanding of the molecular basis of vascular system development are reviewed. As identified by forward and/or reverse genetics, several gene families have been shown to be involved in the proliferation and identity of vascular tissues and in vascular bundle patterning. Although the functioning of primary meristems, for example the shoot apical meristem (SAM), is well documented in the literature, the genetic network that regulates (pro)cambium is still largely not deciphered. However, recent genome-wide expression analyses have identified candidate genes for secondary vascular tissue development. Of particular interest, several genes known to regulate the SAM have also been found to be expressed in the vascular cambium, highlighting possible overlapping regulatory mechanisms between these two meristems.

Key words: Arabidopsis, cambium, phloem, Populus, primary growth, secondary growth, vascular system development, xylem.

Introduction

Higher plants have acquired unique developmental mechanisms enabling them to cope with their sessile status. The mature plant embryo comprises structures required for seedling outgrowth: the cotyledon(s), the embryonic axis, the shoot apical meristem (SAM), the root apical meristem (RAM), and a network of procambial cells. The development of tissues and organs required for further plant growth occurs post-embryonically through the activity of both primary (SAM and RAM) and secondary meristems (phellogen and vascular cambium) (reviewed by Jürgens, 2001). Meristems, the driving forces of plant growth, are niches of cells responding to the two criteria defining stem cells, self-maintenance and the ability to give rise to daughter cells capable of differentiating into at least one specialized cell type (Laux, 2003; Sablowski, 2004; Scheres, 2007). Stem cells in the SAM divide infrequently and produce either daughter stem cells or cells that will divide more rapidly and undergo differentiation when displaced at a certain distance from the stem cells (Stewart and Dermen, 1970; Grandjean et al., 2004; Reddy et al., 2004). The indeterminate growth resulting from the activity of these primary meristems allows the spatial deployment of both aerial and underground organs, thereby providing plants with an efficient photosynthetic activity and a functional translocation of water, nutrient, and signalling molecules throughout their life. However, as plants grow, the expansion of their assimilation surfaces requires adapted growth features, including the implementation of appropriate supporting and conducting tissues. This developmental adaptation is of particular importance for perennial species such as trees since it contributes to their longevity and robustness. During evolution, these requirements were largely met by the acquisition of fundamental changes in cell wall ultrastructure and plant architecture, ensuring long-distance conduction and mechanical support.

Firstly, the incorporation of lignin, resulting from the polymerization of monolignols into the cell wall of specialized cells (such as fibres and tracheary elements), has strengthened the supporting system of plants. Lignin is considered to be absent from algae, but the presence of lignin-like compounds has been reported in Coleochaete, an algal model for land plant ancestry (Delwiche et al.,...
and Populus or overexpression on vascular tissue development are expression pattern and the consequences of their mutation involved in the setting up of the vascular system. Several genes have been found to be consisting of sieve elements, companion cells, fibres, and parenchyma cells. Phloem is also a complex tissue different cell types including tracheary elements, fibres, and parenchyma cells. Phloem is also a complex tissue respectively, led to the enlargement of the girth of plant axes. Secondary growth occurs in gymnosperms and in most dicotyledonous species, but not in monocotyledons nor in ferns. The appearance of lignification in the Lower Devonian (-409 to –386 My) (Ewbank et al., 1996), followed by vascular cambium emergence during the Middle Devonian (-386 to –377 My) (Rowe and Speck, 2005), represent key innovations for vascular plants as they allowed plants to adapt both their size and their growth forms to environmental land conditions.

During the last decade, significant progress has been achieved in the knowledge of the molecular mechanisms that are involved in the establishment of the vascular system. In this review, the focus is on data collected mainly from Arabidopsis, which is widely used to study primary growth, and Populus, which is a model plant to study secondary growth. Advances in the identification of molecular determinants for vascular development are presented. Finally, based mainly on expression data, emphasis is laid on the hypothesis that some regulatory mechanisms acting on SAM are co-opted by the vascular cambium.

Molecular determinants of vascular development during primary growth phase

In Arabidopsis, procambium precursor cells can be discerned during the transition from the globular to the heart stage of embryogenesis (West and Harada, 1993). In the Arabidopsis embryo, no mature or differentiating vascular elements have been identified and the vascular system is composed of a continuous network of procambial cells distributed along the hypocotyl–root axis and the cotyledons (Busse and Evert, 1999a). After seed germination and during primary growth of the stem, the procambium produces xylem centripetally and phloem centrifugally, leading to the formation of vascular bundles that are arranged along a ring passing through the ground tissue (Esau, 1960) (Fig. 1B). Xylem is composed of different cell types including tracheary elements, fibres, and parenchyma cells. Phloem is also a complex tissue consisting of sieve elements, companion cells, fibres, and parenchyma cells. Several genes have been found to be involved in the setting up of the vascular system. Their expression pattern and the consequences of their mutation or overexpression on vascular tissue development are described in the following sections and summarized in Table 1.

Genes involved in adaxial/abaxial specification

The class III homeodomain-leucine zipper (HD-ZIP III) gene family comprises five members in Arabidopsis, ATHB8, PHABULOSA/ATHB14 (PHB), PHAVOLUTA/ATHB9 (PHV), CORONA/ATHB15 (CNA), and REVOLUTA/INTERFASCICULAR FIBERLESS1 (REV/IFL). These genes have been associated with several developmental processes including embryo patterning, meristem initiation, meristem regulation, organ polarity, and vascular development (Talbert et al., 1995; McConnell and Barton, 1998; McConnell et al., 2001; Emery et al., 2003; Prigge et al., 2005) (Fig. 1). During Arabidopsis embryogenesis, ATHB8 expression is restricted to procambial cells of torpedo stage embryos and of developing organs, as demonstrated by in situ mRNA localization (Baima et al., 1995). In Arabidopsis seedlings, the expression of an ATHB8 promoter–GUS fusion (pATHB8::GUS) was localized in the region where the vasculature will be formed in cotyledons and leaflets as well as in the procambium of cotyledons and roots (Baima et al., 1995; Ohashi-Ito and Fukuda, 2003) showed, by promoter–GUS analysis, that CNA had an expression pattern similar to the one of ATHB8 in young leaves and in roots. In situ mRNA localization, the expression of PHV, PHB, REV, and CNA was detected in the adaxial region of embryos including procambial cells starting from the heart/torpedo stage (McConnell et al., 2001; Otsuga et al., 2001; Emery et al., 2003; Prigge et al., 2005; Williams et al., 2005).

In cross-sections of Arabidopsis inflorescence stems, Zhong and Ye (1999) showed by both in situ mRNA localization and promoter–GUS analysis that REV is expressed in interfascicular fibres and in vascular bundles. Pineau et al. (2005) reported that pATHB8::GUS is expressed in parenchyma cells surrounding vessel elements with only a trace amount of expression in the fascicular cambium. In longitudinal sections of Arabidopsis inflorescence stems, in situ mRNA localization revealed that each of the HD-ZIP III genes is expressed in vascular tissues, although PHV expression was only weakly detected in pith cells (Prigge et al., 2005).

Forward and reverse genetic studies of HD-ZIP III function in Arabidopsis have indicated their possible role in the development of the vascular system. Single loss-of-function mutants have been analysed for each family member and whereas no phenotype has been observed for athb8, phb, and phv (Baima et al., 2001; Emery et al., 2003; Prigge et al., 2005), alterations of vascular differentiation were observed for cna and rev mutants. In single cna mutants, vascular bundles are less well distributed around the stem periphery and expanded lignified tissues are observed (Prigge et al., 2005).
similar phenotype was obtained in antisense CNA transgenic *Arabidopsis* (Kim et al., 2005). Mutation of REV not only abolished the differentiation of interfascicular fibres but also reduced the number of fibres and vessels in vascular bundles as well as secondary xylem differentiation (Talbert et al., 1995; Zhong et al., 1997; Zhong and Ye, 1999; Prigge et al., 2005). According to Zhong and Ye (2001), the polar auxin transport (PAT) was reduced by 70% in the rev mutant. PAT is known to be essential for the patterning of procambium and vascular tissues (Sachs, 1981; Mattsson et al., 2003; reviewed by Scarpella and Meijer, 2004). In *Arabidopsis*, several potential PAT candidate genes were shown to be essential for vascularization such as *PIN-FORMED* (*PIN1*)
<table>
<thead>
<tr>
<th>Gene family</th>
<th>Gene name</th>
<th>Post-embryonic expression in vascular tissues</th>
<th>Loss-of-function phenotype</th>
<th>Gain-of-function phenotype</th>
<th>Downregulation</th>
<th>Overexpression</th>
<th>References</th>
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<tbody>
<tr>
<td>1. HD-ZIP III (adaxial specification)</td>
<td>ATHB8</td>
<td>Procambium, parenchyma cells surrounding vessels, induced by wounding and IAA</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>Wider VB, more xylary procambial cells, more SXy production and more Ph fibres, lignin in the pith</td>
<td>Baima et al., 1995, 2001; Prigge et al., 2005; Pineau et al., 2005</td>
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<td></td>
<td>PHV</td>
<td>Pith</td>
<td>No phenotype</td>
<td>Amphivasal VB</td>
<td>No phenotype</td>
<td>–</td>
<td>McConnell et al., 2001; Prigge et al., 2005</td>
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<tr>
<td></td>
<td>PHB</td>
<td>Vascular tissues</td>
<td>No phenotype</td>
<td>Amphivasal VB</td>
<td>No phenotype</td>
<td>–</td>
<td>McConnell and Barton, 1998; Prigge et al., 2005; Williams et al., 2005</td>
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<tr>
<td></td>
<td>CNA</td>
<td>Procambium</td>
<td>VB less well distributed</td>
<td>Reduced lignified tissue, reduced number of VB</td>
<td>Dwarf plants, stem fasciation, increased SXy production, increased number of VB</td>
<td>No phenotype</td>
<td>Ohashi-Ito and Fukuda, 2003; Prigge et al., 2005; Kim et al., 2005; Green et al., 2005</td>
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<tr>
<td></td>
<td>REV</td>
<td>IF, VB</td>
<td>Pendent stems, no IF fibres, reduced cell number in VB, reduced SXy differentiation, reduced PAT</td>
<td>Stem fasciation, amphivasal VB, ectopic VB</td>
<td>No phenotype</td>
<td>No phenotype</td>
<td>Emery et al., 2003; Zhong et al., 1997, 1999; Zhong and Ye, 2001, 2004; Prigge et al., 2005;1999, Talbert et al., 1995</td>
</tr>
<tr>
<td>Double and triple mutants in HD-ZIP III genes</td>
<td>cna phv</td>
<td>NA</td>
<td>Ectopic VB</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Green et al., 2005</td>
</tr>
<tr>
<td></td>
<td>rev phb/rev phv</td>
<td>NA</td>
<td>Enhanced rev phenotype</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Prigge et al., 2005</td>
</tr>
<tr>
<td></td>
<td>cna phb phv</td>
<td>NA</td>
<td>Ectopic amphivasal VB</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Green et al., 2005; Prigge et al., 2005</td>
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<tr>
<td></td>
<td>rev phb phv</td>
<td>NA</td>
<td>Amphi-vasal VB</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Emery et al., 2003</td>
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<td></td>
<td>rev cna athb8</td>
<td>NA</td>
<td>Reduced rev phenotype</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Prigge et al., 2005</td>
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<td>2. miRNA (abaxial specification?)</td>
<td>miR166</td>
<td>Adaxial region of cotyledons and provascular tissues in embryos</td>
<td>–</td>
<td>Stem fasciation, disturbed radial patterning, ectopic amphivasal VB in stem</td>
<td>–</td>
<td>Stem fasciation</td>
<td>Kidner and Martienssen, 2004; Kim et al., 2005; Jung and Park, 2007; Williams et al., 2005</td>
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<tr>
<td>Gene family</td>
<td>Gene name</td>
<td>Post-embryonic expression in vascular tissues</td>
<td>Loss-of-function phenotype</td>
<td>Gain-of-function phenotype</td>
<td>Downregulation</td>
<td>Overexpression</td>
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<td></td>
<td>miR165</td>
<td>Detected throughout the entire embryo</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Stem fasciation, reduced leaf venation, abnormal leaf polarity, affected SAM formation, anthocyanin accumulation, pendent stems, reduced cell number in VB, reduced number of IF</td>
</tr>
<tr>
<td>3. KANADI (abaxial specification)</td>
<td>KAN1</td>
<td>Limited to root Ph</td>
<td>No phenotype</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Lack of vasculature</td>
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<tr>
<td></td>
<td>KAN2</td>
<td>Ph</td>
<td>No phenotype</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Lack of vasculature</td>
</tr>
<tr>
<td></td>
<td>KAN3</td>
<td>Ph</td>
<td>No phenotype</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Lack of vasculature</td>
</tr>
<tr>
<td>Double and triple mutants in KANADI genes</td>
<td>kan1 kan2</td>
<td>NA</td>
<td>Amphivasal VB</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>kan1 kan2 kan3</td>
<td>NA</td>
<td>Amphivasal VB</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>4. Histidine kinase/Cytokinin receptor (procambial cell division)</td>
<td>WOL</td>
<td>Procambium</td>
<td>Reduction in number of procambial cells, absence of Ph in root and lower part of Hy</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Scheres et al., 1995; Mähönen et al., 2000</td>
</tr>
<tr>
<td>Gene family</td>
<td>Gene name</td>
<td>Post-embryonic expression in vascular tissues</td>
<td>Loss-of-function phenotype</td>
<td>Gain-of-function phenotype</td>
<td>Downregulation</td>
<td>Overexpression</td>
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<td>5. <strong>MYB</strong> (phloem identity)</td>
<td><strong>APL</strong></td>
<td>Ph</td>
<td>Ectopic formation of Xy in position of Ph</td>
<td>–</td>
<td>–</td>
<td>Repressed Xy differentiation (WOL promoter)</td>
<td>Bonke et al., 2003</td>
</tr>
<tr>
<td>6. <strong>NAC</strong> (vessel cell fate)</td>
<td><strong>VND6</strong></td>
<td>Metaxylem in root</td>
<td>No phenotype</td>
<td>–</td>
<td>No phenotype</td>
<td>Ectopic metaxylem</td>
<td>Kubo et al., 2005</td>
</tr>
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<td></td>
<td><strong>VND7</strong></td>
<td>Immature protoxylem in root, vascular cells in shoot</td>
<td>No phenotype</td>
<td>–</td>
<td>No phenotype</td>
<td>Ectopic protoxylem</td>
<td>Kubo et al., 2005</td>
</tr>
<tr>
<td>7. <strong>COV1</strong> (ordered patterning of vascular bundles)</td>
<td><strong>COV1</strong></td>
<td>Not described</td>
<td>Stunted growth and wrinkled leaves, increased vascular development in place of IF tissue, loss of defined VB</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Parker et al., 2003</td>
</tr>
<tr>
<td>8. Spermine synthase (polyamine biosynthesis pathway)</td>
<td><strong>ACL5/TKV</strong></td>
<td>Procambium, VB</td>
<td>Dwarf plants, reduction in internode length, increase in Xy and Ph, reduced PAT, increased <strong>HD-ZIP III</strong> transcript level</td>
<td>–</td>
<td>Semi-dwarf plants</td>
<td>–</td>
<td>Hanzawa et al., 1997, 2000; Clay and Nelson, 2005</td>
</tr>
<tr>
<td>9. <strong>KNOX1</strong> (lignin biosynthesis pathway)</td>
<td><strong>BP/KNAT1</strong></td>
<td>Cortex adjacent to vascular cells, Ph occasionally</td>
<td>Short internodes and pedicels, VB closer to each other, premature lignin deposition in IF tissue, less lignin in VB, defect in VB organization, gaps of non-lignified cells</td>
<td>–</td>
<td>–</td>
<td>Delayed lignin deposition</td>
<td>Lincoln et al., 1994; Venglat et al., 2002; Douglas et al., 2002; Smith and Hake, 2003; Haskins and Riggs, 2005</td>
</tr>
<tr>
<td>10. Sterol methyltransferase (sterol biosynthesis pathway)</td>
<td><strong>CVP1(SMT2)</strong></td>
<td>Cotyledon vascular cells</td>
<td>Defects in venation, more Xy and lignified sclerenchyma</td>
<td>–</td>
<td>–</td>
<td>No phenotype</td>
<td>Carland et al., 1999, 2002</td>
</tr>
<tr>
<td>11. <strong>Brassinosteroid receptor</strong> (Xy/Ph patterning)</td>
<td><strong>BRL1</strong></td>
<td>Procambium</td>
<td>More Ph, less Xy</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Caño-Delgado et al., 2004</td>
</tr>
<tr>
<td></td>
<td><strong>BRL3</strong></td>
<td>Ph in leaves</td>
<td>No phenotype</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Caño-Delgado et al., 2004</td>
</tr>
</tbody>
</table>

Hy, hypocotyl; IF, interfascicular; NA, not applicable; PAT, polar auxin transport; Ph, phloem; SXY, secondary xylem; VB, vascular bundle; Xy, xylem; -, not described; ? not confirmed by Li et al. (2005).
encoding a putative auxin efflux carrier (Gälweiler et al., 1998) and GNOM encoding a guanine-nucleotide exchange factor for ADP-ribosylation factor G protein known to be responsible for the polar localization of PIN1 (Steinmann et al., 1999; Geldner et al., 2003). Mutation in either gene hampers normal basipetal transport of auxin and results, in addition to the failure to develop lateral organs, in dramatic alterations of vascular differentiation, such as localized proliferation of vascular tissue and the formation of discontinuous vascular bundles (Gälweiler et al., 1998; Steinmann et al., 1999; Koizumi et al., 2000). Similar alterations have been observed in plants in which PAT was chemically inhibited (Mattsson et al., 1999) or in plants that overproduce auxin (Klee et al., 1987; Uggla et al., 1996) supporting a role for auxin gradients in the specification of vascular differentiation sites during plant development.

PHB and PHV gain-of-function mutants (McConnell and Barton, 1998; McConnell et al., 2001) present amphivasal bundles in leaves, with xylem surrounding the phloem (Fig. 1C). Similarly, the REV gain-of-function mutant, also called amphivasal vascular bundle1 (avb1), is characterized by amphivasal bundles in leaves as well as in stems where, in addition to the ring-like arrangement of vascular bundles, extra ectopic vascular bundles are present in the pith (Zhong et al., 1999; Emery et al., 2003; Zhong and Ye, 2004) (Fig. 1C). These three gain-of-function mutations are located in a microRNA target sequence (recognized by miR165 or miR166), indicating that HD-ZIP III genes are under the control of microRNA regulation through mRNA cleavage (McConnell et al., 2001; Mallory et al., 2004; Zhong and Ye, 2004). By in situ hybridization using pre-miR165 and pre-miR166 antisense probes, miR165 and miR166 have been shown to be expressed in the abaxial domain of developing leaves, suggesting that the occurrence of these microRNAs could specify abaxial fate (Juarez et al., 2004; Kidner and Martienssen, 2004) (Fig. 1A). However, by using a 4-concatenate miR165 and a pre-miR165 antisense probe, Li et al. (2005) revealed a non-polar miR165/166 distribution pattern in leaf primordia. These authors suggested that miR165/166 may be required for the development of the entire leaf and not only for the abaxial domain. These divergent data, probably due to the occurrence of several members of miR165/166, should be clarified by localizing their expression by other methods, such as pmiRNA-reporter gene fusions (Jung and Park, 2007). Overexpression of a miR166-resistant CNA cDNA resulted in a reduced proportion of lignified tissues (Kim et al., 2005). These results indicate a role for CNA, as negative regulator, in the production of xylem (Kim et al., 2005). Moreover, the overexpression of ATHB8 resulted in an increased production of xylem (Baima et al., 1995, 2001), Kim et al. (2005) suggested that ATHB8 and CNA could have antagonistic roles during xylem development.

In the miR166a gain-of-function mutant men1 (meristem enlargement 1) (Kim et al., 2005) and in the miR166g gain-of-function mutant jabba-1D (jba-1D) (Williams et al., 2005), levels of CNA, PHV, and PHB transcripts were reduced and the mutants had fasciated inflorescence stems with disrupted radial vascular patterning, including amphivasal ectopic bundles within the pith (Fig. 1C). The overexpression of miR165 led to a reduction of the transcript level of the five HD-ZIP III genes (Zhou et al., 2007) (Fig. 1A). These transgenic lines were affected in SAM formation, had impaired vein development and alteration in organ polarity. In cross-sections of inflorescence stems of miR165 overexpressors, a reduced number of cells was observed in the vascular bundles and fewer interfascicular fibres developed between the vascular bundles when compared with the wild type (WT), a phenotype reminiscent of the rev mutant (Zhou et al., 2007). As suggested by these authors, miR165 and miR166 seem to differentially regulate the expression of HD-ZIP III genes, probably as a consequence of distinct effectiveness of miR165 and miR166 on the cleavage of their target genes and/or different specificities in tissue expression.

The REV gene seems to be the principal determinant in vascular development since a single mutation of this gene confers a pendent stem phenotype, probably due to the lack of interfascicular fibres (Zhong and Ye, 1999). Moreover, since rev phb or rev phv double mutants have phenotypes similar to each other, corresponding to enhanced vascular defects of the rev single mutants (Prigge et al., 2005), and rev phb phv triple mutant displays amphicribal vascular bundles with phloem surrounding the xylem (Emery et al., 2003), it can be assumed that these three genes interact in a complex and not-yet-elucidated regulation network acting on vascular system development. cna phb phv triple loss-of-function mutants display ectopic amphivasal bundles within the pith (Prigge et al., 2005; Green et al., 2005), indicating that CNA and REV have distinct roles in vascular development and/or patterning.

The KANADI gene family, belonging to the GARP family of transcription factors, has been shown to promote the differentiation of abaxial tissues (Eshed et al., 2001; Kerstetter et al., 2001; Emery et al., 2003) (Fig. 1A). By using promoter–GUS fusions, Emery et al. (2003) showed that KAN2 and KAN3 are expressed in the developing phloem along the entire plant whereas KAN1 expression is limited to the root phloem. kan1 kan2 double mutants (Eshed et al., 2004) and kan1 kan2 kan3 triple mutants (Emery et al., 2003) exhibit amphivasal bundles, a phenotype also observed for several HD-ZIP III mutants (Table 1; Fig. 1C). Ectopic expression of KAN1, KAN2, or KAN3 using the constitutive CaMV 35S (35S) promoter resulted
Identification of other vascular development-related genes

The wooden leg (wol) mutant is characterized by a reduction in the number of procambial cells in the root and these cells differentiate into xylem but not into phloem (Scheres et al., 1995). These authors observed a similar phenotype in the lower part of the hypocotyl of wol seedlings but not in the upper part of the hypocotyl where phloem differentiation occurs. The absence of phloem in the primary root of wol mutant was apparently due to a deficiency in procambial cell division during embryogenesis (Scheres et al., 1995; Mähönen et al., 2000). In addition, the narrow vascular cylinder of the wol primary root consists solely of protoxylem whereas the WT vascular cylinder is made of both proto- and metaxylem (Caño-Delgado et al., 2000). By in situ mRNA localization in Arabidopsis embryos, WOL was found to be expressed in the precursors of the procambium and in the procambium (Mähönen et al., 2000). WOL [also known as CYTOKININ RESPONSE 1 (CRE1) and ARABIDOPSIS HISTIDINE KINASE 4 (AHK4)] encodes a putative histidine kinase believed to function as a cytokinin receptor (Inoue et al., 2001; Suzuki et al., 2001).

ALTED PHLOEM DEVELOPMENT (APL), belonging to the MYB coiled-coil-type transcription factor family, has been shown to be involved in the determination of phloem identity in Arabidopsis (Bonke et al., 2003). The mutation of APL results in the formation of xylem in the phloem position in both roots and aerial organs (Bonke et al., 2003). When APL was expressed throughout the procambium, under the control of the WOL promoter, cells close to the root tip that normally differentiate into protoxylem remained undifferentiated and metaxylem elements in the vascular cylinder of the root differentiated later than in the WT plants. These data indicate that APL promotes phloem differentiation but also represses xylem differentiation in phloem poles. In mature embryos, APL expression was detected in the prospective phloem tissue of cotyledons and hypocotyls. At later stages of plant development, APL was shown to be expressed in phloem of all organs (Bonke et al., 2003). As suggested by these authors, APL could be required for the asymmetric division of sieve element mother cells as well as for the differentiation of the derived sieve elements and companion cells.

The VASCULAR-RELATED NAC-DOMAIN PROTEIN 6 and 7 (VND6 and VDN7), encoding NAC transcription factors, have been shown to be regulators of vessel cell fate (Kubo et al., 2005). These genes were identified by microarray analysis as being up-regulated during in vitro xylem vessel element differentiation in Arabidopsis suspension cell cultures. Expression of pVND7::YFP-NLS was detected in the immature protoxylem vessels just above the root meristem and expression of pVND6::YFP-NLS was restricted to the metaxylem vessels in the middle part of the root (Kubo et al., 2005). When overexpressed in Arabidopsis or poplar under the control of the 35S promoter, these authors showed that VND7 and VND6 induced transdifferentiation of various cells into protoxylem- and metaxylem-like elements, respectively. No phenotype was noticed for vnd6 and vnd7 loss-of-function mutants or for transgenic plants carrying antisense VND6 and VND7. By contrast, the overexpression of the translational fusion of VND7 and VND6 to the SRDX strong repression domain inhibited metaxylem and protoxylem formation, respectively, suggesting that these two VND transcription factors control the expression of specific target genes required for the differentiation of each type of vessel element (Kubo et al., 2005).

The continuous vascular ring (cvr1) mutant is characterized by an increased vascular development in the stem in place of the interfascicular tissue resulting in a continuous ring-like pattern of xylem and phloem and the loss of defined vascular bundles (Parker et al., 2003). Although the function of COV1 is still unknown, this predicted integral membrane protein, possibly involved in signalization or transport, may play a role in the maintenance or in the initiation of a defined pattern of vascular bundles within the stem (Parker et al., 2003).

An increased number of vascular cells has been detected in the Arabidopsis acaulis5 (ACL5)/thickvein (tkv) mutant (Hanzawa et al., 2000; Clay and Nelson, 2005). The acl5 mutants exhibit severe reduction in the internode length of inflorescence stems compared with WT plants (Hanzawa et al., 1997, 2000). Cross-sections of veins or inflorescence stems of acl5 mutants showed a slight increase in the number of cells involved in the vascular system, including xylem, phloem, and cambial-like cells (Hanzawa et al., 1997; Clay and Nelson, 2005). By both in situ mRNA localization and promoter–GUS analysis, ACL5 expression was detected during embryogenesis, starting from early globular embryos and persisting until the bent cotyledon-staged embryos in which the ACL5 expression was limited to procambial cells (Clay and Nelson, 2005). This procambial specific expression pattern was also noticed during primary root development and at early leaf development as well as in axillary buds. In cross-sections of inflorescence stems, GUS expression was detected in vascular bundles. The acl5 mutant exhibited a 34% reduction in PAT, as measured by the basipetal transport of 14C-IAA in excised inflorescence stems (Clay and Nelson, 2005), and ACL5 is induced by
auxin (Hanzawa et al., 2000). ACL5 encodes a spermine synthase involved in polyamine biosynthesis (Hanzawa et al., 2000; Clay and Nelson, 2005). Interestingly, the transcript level of the five HD-ZIP III genes, and particularly the one of ATHB8, was increased in acl5 mutants, suggesting that the defect in PAT in the acl5 mutant may cause a local increase in auxin levels which, in turn, results in the induction of HD-ZIP III expression and in the increased number of vascular cells (Imai et al., 2006).

Mutants in the homeobox gene BREVIPEDECILLUS (BP), also named KNAT1 and belonging to the class-1 KNOTTED1-like homeobox (KNOX1) family, show defects in the organization of the vascular bundles in inflorescence stems (Smith and Hake, 2003). bp mutants are characterized by short internodes and pedicels due to reduced cell division compared with the WT (Douglas et al., 2002; Venglat et al., 2002). The vascular bundles in the bp mutants were closer to each other compared with the WT and the continuous ring of lignified cells was interrupted in the bp mutants by gaps of non-lignified cells. Epidermal and cortical cells adjacent to these gaps were lignified, suggesting a switch in cell fate (Douglas et al., 2002; Venglat et al., 2002; Mele et al., 2003; Smith and Hake, 2003). By in situ mRNA localization (Lincoln et al., 1994) and promoter–GUS analysis (Venglat et al., 2002; Douglas and Riggs, 2005), BP expression was localized in cortical tissue peripheral to the vascular bundle and occasionally in phloem cells adjacent to the stem cortex. Comparison of the genome expression in bp mutants and WT revealed differences in the expression of genes related to cell wall biosynthesis, and particularly those involved in the lignin biosynthetic pathway (Mele et al., 2003). As shown by these authors, BP seems to be involved in the regulation of lignin biosynthesis since a premature deposition of lignin was observed in the interfascicular region of the inflorescence stem basis in bp mutants. In addition, lignin deposition was delayed in BP overexpressing transgenic plants as compared to the WT.

The mutation of COTYLEDON VASCULAR PATTERN 1 (CVP1) results in defects in the normal pattern of vascular bundles in cotyledon (Carland et al., 1999, 2002). Histological analyses of stem cross-sections revealed an increased amount of xylem and lignified sclerenchyma in affected internodes of the mutant (Carland et al., 1999). Since CVP1 encodes a sterol methyltransferase (SMT2), a role for sterols in vascular differentiation and/or patterning can be suggested, but their precise function is still not elucidated.

In Arabidopsis, BRASSINOSTEROID-INSENSITIVE LIKE 1 (BRL1) and BRL3, encoding two brassinosteroid (BR) receptors, are specifically expressed in vascular tissues, as shown by promoter–GUS analyses (Caño-Delgado et al., 2004). These authors revealed that, in mature Arabidopsis stems, pBRL3::GUS expression was associated with the procambial cells of the vascular bundles whereas pBRL3::GUS expression was localized in the phloem of cotyledons and leaves but not in stems. Besides, the brl1 mutant displays an increased number of phloem cells and a decreased xylem differentiation in the vascular bundles, suggesting that BRL1 plays a role in phloem/xylem patterning (Caño-Delgado et al., 2004). BRs involvement in vascular development is further supported by the findings that inhibition of BRs biosynthesis causes an increased phloem/xylem ratio in Arabidopsis and prevents the differentiation of procambium-like cells into tracheary elements in the Zinnia elegans system (reviewed by Fukuda, 2004). Besides, BRs have been shown to up-regulate the expression of ZeHB-12, a Zinnia REV homologue (Ohashi-Ito et al., 2002). The expression of BRL3 and BR-ASSOCIATED RECEPTOR KINASE 1-like were shown to be up-regulated in Arabidopsis plants overexpressing ZeHB-12, reinforcing the role of BR in the regulation of xylem differentiation (Ohashi-Ito et al., 2005).

Molecular determinants of vascular development during secondary growth

Secondary growth is of great economical importance as it results in the production of wood, which is a valuable renewable source of energy and is a raw material for pulping and construction purposes. This developmental process takes place when the vascular cambium initials differentiate from procambium within the vascular bundles (fascicular cambium) and from parenchyma in the interfascicular regions (interfascicular cambium) (Esau, 1965) (Fig. 1B). Cambium activity results in the production of xylem and phloem, in its self-maintenance, in intercellular signal transmission as well as in stem radial expansion (Savidge, 2001). In Arabidopsis, the first histological evidence of vascular cambium formation, a periclinal division of the procambial cells, has been detected in the hypocotyl–root axis and the cotyledonary node of 6-d-old seedlings (Busse and Evert, 1999b). As shown by these authors, secondary growth was clearly visible after 14 d of growth. Recently, the Arabidopsis mutant high cambial activity (hca) was shown to exhibit alteration in cambium activity resulting in dramatic increase in vascular tissue development, but the genetic origin of this mutant is not known (Pineau et al., 2005).

The vascular cambium has typically two morphologically distinct types of initials: the axillary elongated fusiform initials that will lead to the formation of the axial system (including tracheids, vessel elements, fibres, axial parenchyma cells, sieve elements, and companion cells) and the smaller isodiametrical ray initials giving rise to the radially orientated parenchymatous rays (Iqbal and Gouse, 1990). The identification of putative regulatory genes
associated with cell type identity within the vascular cambium is of particular interest since they are probably specific to cambium and their characterization could help in understanding how cambium works and how secondary vascular tissue develops. For instance, an aspen gene encoding a member of the RING-H2 protein family, called PtRHE1, has been shown to be expressed in the ray initials and derivatives within the cambial zone, but not in their fusiform counterparts, suggesting a potential role for this gene in the determination and/or the maintenance of cambial cell identity (van Raemdonck et al., 2005). Rays are determinant for secondary growth in plants because they ensure the translocation of nutrients between the phloem and the xylem and the transmission of messenger molecules (Lachaud et al., 1999). The process of secondary growth has been studied in A. thaliana (Lev-Yadun, 1994; Altamura et al., 2001). Although Chaffey et al. (2002) reported the formation of secondary vascular tissues in the hypocotyls of short-day-grown Arabidopsis plants, they noticed a lack of rays, suggesting that there may be some developmental processes characteristic to wood formation in trees that cannot be approached in the Arabidopsis system.

A typical organization of secondary vascular tissues is shown in Fig. 2. Anticlinal divisions of the cambium initials cause enlargement of the circumference of the cambial cylinder, whereas periclinal divisions produce phloem or xylem mother cells, called derivatives, leaving initial cells in the meristem. Cambium derivatives may divide several times before differentiating into vascular tissues (Lachaud et al., 1999; Dengler, 2001; Mellerowicz et al., 2001). Because most anatomical criteria are often not sufficient to discriminate between cambial initials and derivatives, the term cambial zone is used to denote these cell types (reviewed by Samuels et al., 2006). However, differences in cell wall composition (Catesson et al., 1994), ultrastructural characteristics (Arend and Fromm, 2003), or in transcriptome profiles (Schrader et al., 2004) between cells within the cambial zone suggest that the differentiation process may occur early in cambial derivatives. Determination of the number and the location of anticlinal divisions within the cambial zone of aspen in the growing season allowed the vascular cambial stem cells to be located on the phloem side of the cambial zone (Schrader et al., 2004).

The strategies used to study molecular processes associated with vascular secondary growth are based on the identification of candidate genes expressed in particular tissues. Transcript profiling either across aspen cambial zones (Schrader et al., 2004) and along the vertical stem segments of a hybrid aspen tree (Prassinos et al., 2005; van Raemdonck et al., 2005) or loblolly pine (Lorenz and Dean, 2002) provided molecular support for candidate genes involved in the setting up of secondary growth and cambium functioning in woody plants. Other strategies such as gene and enhancer trap tagging of vascular expressed genes in poplar have been used to identify genes expressed in the cambial zone (Johansson et al., 2003) and during secondary vascular development (Groover et al., 2004).

Emerging from transcriptome analyses in Arabidopsis and poplar, some genes associated with vascular development during primary growth have been found to be expressed during secondary growth as well. For instance, Populus tremula×P. tremuloides PtkKAN1, a homologue of AtKAN1, was shown to have a higher expression toward the phloem side of the cambial zone (Schrader et al., 2004). In Arabidopsis, KAN2 and KAN3 expression were higher in the phloem or in non-vascular tissues than in the secondary xylem, whereas KAN1 expression was not detected in the root–hypocotyl junction, where secondary growth takes place (Zhao et al., 2005). APL expression was detected in the phloem and cambial zone, but not in the secondary xylem of Arabidopsis (Zhao et al., 2005). Besides, HD-ZIP III genes were shown to be expressed in the cambium region of aspen (Schrader et al., 2004; Ko et al., 2006). P. tremula×P. alba, the expression level of PtaHB1, a poplar REV homologue, was found to increase in the stem segment where primary to secondary growth transition occurs and to accumulate preferentially on the xylem side of the cambium (Ko et al., 2006). These authors showed that the transcript level of PtaHB1 was inversely correlated with that of Pta-miR166. Similarly, in Arabidopsis stems undergoing secondary growth, ATHB8 expression was increased (Ko and Han, 2004; Ko et al., 2004) and the five HD-ZIP III genes were up-regulated in the secondary xylem of Arabidopsis compared to the phloem and cambium (Oh et al., 2003; Zhao et al., 2005). Members of the HD-ZIP III gene family have been shown to play key roles in the establishment of SAM (Otsuga et al., 2001; McHale and Koning, 2004; Green et al., 2005; Prigge et al., 2005) (Fig. 1A). For instance, rev phb (Prigge et al., 2005) and rev phb phv (Emery et al., 2003) mutants lack a functional SAM. Although SAM and vascular cambium produce different structures, all these observations suggest that several regulatory mechanisms may be conserved in these two meristems.

Overlapping molecular mechanisms associated with SAM and vascular cambium functioning

The functional organization of the SAM relies on three main developmental states (i) maintenance of the stem cells, (ii) prevention from premature cell differentiation, and (iii) initiation of tissues and organs (Baïrle and Laux,
The number of stem cells within the SAM is maintained by a balance between cell division and cell differentiation. WUSCHEL (WUS) and CLAVATA (CLV) have been identified as key actors in the control of the size of stem cell population (Fig. 1A). WUS, encoding a putative homeodomain transcription factor, has been shown to confer stem cell identity (Mayer et al., 1998). In the Arabidopsis wus mutant, the SAM is formed during embryogenesis but is not maintained because the stem cells appear to undergo differentiation (Laux et al., 1996). By contrast, Arabidopsis mutants in any of the three CLV1–3 genes have a phenotype opposite to the wus phenotype as they formed enlarged meristems due to the generation of an excess number of cells in the SAM (Clark et al., 1993, 1995; Kayes and Clark, 1998). CLV1 encodes a leucine-rich repeat receptor kinase (Clark et al., 1997), CLV2 a similar protein without the kinase domain (Jeong et al., 1999) and CLV3 a secreted and processed polypeptide (Fletcher et al., 1999; Rojo et al., 2002; Kondo et al., 2006; Ni and Clark, 2006) belonging to the CLV3/EMBRYO-SURROUNDING REGION (ESR) related (CLE) protein family (Cock and McCormick, 2001). The signalling between CLVs and WUS is not totally understood, but it is presumed that CLV3 activates, as a ligand, a CLV1/CLV2 receptor complex which restricts WUS expression (Brand et al., 2000; Schoof et al., 2000). clv mutants, in addition to their enlarged SAM producing an increased number of lateral organs, exhibit also enlarged, flattened stems that contain increased numbers of stem veins (Brand et al., 2000). A comparable phenotype was reported for the cna phb phv triple mutant (Prigge et al., 2005) and the jba-1D mutant (Williams et al., 2005). The clv cna double mutant developed massively enlarged apices compared with the clv meristems (Green et al., 2005). On this basis, Green et al. (2005) suggested that HD-ZIP III genes and the CLV pathway contribute to the regulation of meristem size in a parallel manner. The analysis of WUS expression led Williams et al. (2005) to propose that, in WT plants, PHV, PHB, and CNA restrict SAM activity by down-regulating WUS transcription.

In Arabidopsis, other major genes involved in the maintenance of stem cells in an indeterminate state in the SAM are the KNOX1 genes SHOOT MERISTEMLESS (STM) (Barton and Poethig, 1993), BP (considered to be partially redundant to STM; Long et al., 1996; Byrne et al., 2002), and KNAT6 (Belles-Boix et al., 2006). Strong stm mutants fail in the establishment of the SAM indicating that STM is required to specify the SAM cells of the embryo and weaker stm mutants have a lower amount of undifferentiated cells in the SAM, revealing a role for this gene in the maintenance of the SAM as well (Barton and Poethig, 1993; Endrizzi et al., 1996; Long et al., 1996). BP and KNAT6 have been shown to contribute with STM to SAM maintenance (Byrne et al., 2002; Belles-Boix et al., 2006). The down-regulation of KNOX1 genes (STM, BP, KNAT2, and KNAT6) is considered as a key step in leaf initiation and organogenesis (reviewed by Hake et al., 2004; Scofield and Murray, 2006). STM, which is expressed throughout the meristem

![Figure 2](https://academic.oup.com/jxb/article-abstract/58/13/3485/492345?download=true)
but down-regulated in organ founder cells (Long et al., 1996; Long and Barton, 2000), has been proposed to restrict differentiation by repressing genes like ASYMMETRIC LEAVES (AS1), encoding a R2R3-MYB protein, and AS2, encoding a cysteine-repeat rich and leucine zipper protein (Byrne et al., 2000; Ori et al., 2000; Lenhard et al., 2002) (Fig. 1A). Alternatively, STM and AS1 may competitively regulate common target genes involved in meristem functioning (Scofield and Murray, 2006).

The aspen putative orthologues of CLV3 (PttCLV3) and WUS (PttWUS) are expressed in the aspen apex, but their expression was not detected in the vascular cambium (Schrader et al., 2004). In accordance, neither WUS nor CLV3 transcripts were detected in Arabidopsis stems undergoing secondary growth (Ko and Han, 2004; Zhao et al., 2005), but a mechanism similar to the SAM CLV–WUS signalling pathway regulating the functioning of the vascular cambium cannot be excluded. Indeed, the expression of PttHB3 and PttRLK3, two aspen genes respectively related to WUS and CLV1, was detected within the cambial zone and was found to be higher in the xylem than in the phloem. The putative poplar CLV1 orthologue, PttCLV1, was shown to have a higher expression in the phloem than in the cambial zone and the xylem (Schrader et al., 2004). Two other genes encoding CLE proteins, PttCLE;1 and PttCLE;3, had a higher expression in the phloem than in the cambial zone and the xylem (Schrader et al., 2004). In Arabidopsis, a gene related to CLV1 and two members of the CLE family, CLE6 and CLE26, were found to be more expressed in the phloem/cambial zone than in the xylem in the root–hypocotyl junction of Arabidopsis (Zhao et al., 2005). The GFP expression driven by the CLV1 promoter has been localized in the cambial zone and in secondary phloem of the root–hypocotyl junction of Arabidopsis (Zhao et al., 2005). Finally, CLV1 was up-regulated in Arabidopsis stems undergoing secondary growth (Ko and Han, 2004) and CLV2 expression was also detected in the root–hypocotyl junction of Arabidopsis (Zhao et al., 2005).

The Populus putative orthologue of STM [PttSTM, Schrader et al., 2004; ARBORKNOXI (ARK1), Groover et al., 2006] is expressed in shoot apices and in the cambial zone suggesting that similar mechanisms prevent cells from premature differentiation in both SAM and cambium. Accordingly, STM is expressed in Arabidopsis stems undergoing secondary growth (Ko and Han, 2004). The overexpression of either Arabidopsis STM or poplar ARK1 in aspen resulted in a similar bushy and highly branched phenotype, reflecting the formation and the outgrowth of ectopic meristem during primary growth (Groover et al., 2006). Both 35S::STM and 35S::ARK1 had thin stems and the onset of secondary growth in the stem was delayed compared with WT plants. Although a continuous ring of lignified secondary xylem was present at the stem base of 6-month-old trees, the boundary between the cambium and secondary xylem was wavy compared with WT and there were almost no lignified phloem fibres (Groover et al., 2006). Transcriptomic analysis of ARK1 overexpressors showed that 42% of the genes that were up-regulated are involved in extracellular matrix-linked functions including genes encoding fasciclin class arabinoalactan proteins, glycosyl hydrolases, and proteins involved in secondary cell wall biosynthesis (Groover et al., 2006).

The poplar KNOX1 genes PttKNOX1, PttKNOX2, and PttKNOX6 were found to be highly expressed in both aspen SAM and the cambial zone, where their expression level was slightly higher on the phloem side than on the xylem side (Schrader et al., 2004). As suggested by these authors, these data are compatible with a regulation model analogous in both the cambium and the SAM of aspen, in which STM expression in the cambial zone would repress the AS1-2 homologue. If xylem and phloem differentiation is regulated by a mechanism similar to the one controlling organ formation in the SAM, it can be expected that a balance exists between AS1-2 and STM homologue gene expression within the cambial zone to promote vascular tissue differentiation. The expression of the closest homologue of AS1 was not detected in the poplar cambial zone (Schrader et al., 2004), suggesting that the differentiation of secondary vascular tissues involves repressor genes different from known AS acting in leaf primordia initiation.

Concluding remarks

Vascular development is essential for plant growth and relies on a tight integration of cell proliferation, cell-fate determination, cell differentiation, and patterning, leading to xylem and phloem formation. In the last decade, several molecular determinants involved in the regulatory mechanisms controlling these developmental processes in Arabidopsis have been uncovered, thanks to reverse and forward genetics. For instance, the pendent phenotype of the rev mutants, the absence of phloem in the primary root of the wol mutant, the production of xylem in place of phloem in the apl mutant, the increased production of phloem in the brill mutant, or the opposite roles of the HD-ZIP III and KAN transcription factors illustrate the complexity of vascular system development in plants. Beside these genetic controls, integration of hormone signals (particularly auxin and BRs) appears to be equally important for the appropriate continuity and patterning of procambium and vascular tissues as manifested by the phenotype of Arabidopsis plants with reduced PAT.
Considerations on the functioning of primary (SAM) and secondary (vascular cambium) meristems suggest overlapping regulatory mechanisms between them, but also specific characteristics for each of these two plant meristems (Schrader et al., 2004; Groover, 2005). On the one hand, studies on Arabidopsis and Populus have shown that SAM and vascular cambium both have an indeterminate cell fate, and several genes such as STM, CLV1, KANADI, and members of the HD-ZIP III gene family are probably involved in the genetic regulation of both meristems, suggesting the occurrence of evolutionary conserved processes in the functioning of plant meristems. Important regulatory genes for the maintenance of the stem cells in the SAM, such as WUS and CLV3, have been found to be expressed in the SAM but not in the vascular cambium. Although homologue genes have been reported to be expressed in the cambium (Schrader et al., 2004), their precise role in the cambium still has to be demonstrated. On the other hand, SAM and vascular cambium have several specific features. One of the major differences between the SAM and the cambium is the ability of vegetative SAM to become determinate in order to ensure flower production. By contrast, the indeterminate state of the cambium is critical to ensure perennial growth. Although significant progress has been made in the identification of genes involved in the biosynthesis of cell wall components, including lignin (Boerjan et al., 2003), cellulose (Somerville, 2006), and hemicellulose (Farrokhi et al., 2006; Lerouxel et al., 2006), until now, the molecular determinants for vascular cambium initiation, maintenance, and functioning have not yet been identified, probably because existing developmental genetic analyses are difficult to perform on trees due to their large size and long generation time. The recent availability of the Populus genome sequence (Tuskan et al., 2006) should greatly facilitate the study of aspects linked to woody species, such as secondary growth. The comparison of Arabidopsis and Populus genomes revealed that the relative frequency of protein domains is similar in both genomes (Tuskan et al., 2006). However, these authors showed that Populus has more protein-coding genes than Arabidopsis and that some genes are overrepresented in Populus compared with the Arabidopsis genome, such as genes associated with meristem development and cell wall biosynthesis. Therefore, dissimilarities in growth patterns amongst taxa could result from variations in gene expression levels, but also from the expression of various sets of genes at different stages of growth. Functional approaches including silencing and/or over-expression of candidate genes should be achieved in the near future to take advantage of the results obtained from the transcriptomic analysis towards vascular cambium development and to identify those genes that concern more specifically the vascular cambium versus other plant meristems.

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