Arabidopsis thaliana as a model species for xylem hydraulics: does size matter?

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Abstract

While Arabidopsis thaliana has been proposed as a model species for wood development, the potential of this tiny herb for studying xylem hydraulics remains unexplored and anticipated by scepticism. Inflorescence stems of A. thaliana were used to measure hydraulic conductivity and cavitation resistance, whereas light and electron microscopy allowed observations of vessels. In wild-type plants, measured and theoretical conductivity showed a significant correlation (R2 = 0.80, P < 0.01). Moreover, scaling of vessel dimensions and intervessel pit structure of A. thaliana were consistent with structure–function relationships of woody plants. The reliability and resolution of the hydraulic methods applied to measure vulnerability to cavitation were addressed by comparing plants grown under different photoperiods or different mutant lines. Sigmoid vulnerability curves of A. thaliana indicated a pressure corresponding to 50% loss of hydraulic conductance (P50) between –3 and –2.5 MPa for short-day and long-day plants, respectively. Polygalacturonase mutants showed a higher P50 value (–2.25 MPa), suggesting a role for pectins in vulnerability to cavitation. The application of A. thaliana as a model species for xylem hydraulics provides exciting possibilities for (1) exploring the molecular basis of xylem anatomical features and (2) understanding genetic mechanisms behind xylem functional traits such as cavitation resistance. Compared to perennial woody species, however, the lesser amount of xylem in A. thaliana has its limitations.

Key words: Arabidopsis thaliana, bordered pit, cavitation resistance, hydraulic conductivity, inflorescence stem, xylem.

Introduction

Xylem functional traits play an important role in understanding plant distribution, primary productivity, growth rate, resistance to abiotic factors (e.g. drought and frost), and the capacity of plants to adapt to changing environmental conditions over time (Tyree and Zimmermann, 2002; McDowell et al., 2008; Brodribb and Cochard, 2009; Pittermann, 2010). Not surprisingly, there is a considerable amount of homoplasy in xylem anatomy of plants as a result of ecological adaptations for efficient water transport, hydraulic safety against embolism, and mechanical support (Sperry, 2003; Rowe and Speck, 2005). Ecological adaptations that determine these major xylem structure–function relationships are reflected in the large anatomical variation of the hydraulic network (Carlquist, 2001; Choat et al., 2008).

Understanding of functional traits associated with long-distance water transport in plants requires not only integration of hydraulics with anatomy and ecology, but also with plant genetics. However, genetic aspects of hydraulic parameters remain little studied. Firstly, there are practical problems impeding the study of genetic mechanisms in trees, in particular their slow growth and long generation times, which make the production and isolation of mutants laborious and time
consumption (Chaffey et al., 2002; Groover, 2005). Secondly, it remains difficult to link gene function with quantitative physiological traits such as hydraulic efficiency or drought tolerance, because these physiological traits are the result of integrated and balanced genetic processes. Besides, comparison of genotypic variability and phenotypic plasticity of cavitation resistance among natural populations show frequently high phenotypic plasticity and a lack of genetic differentiation in some species (Mencuccini and Comstock, 1997). As a result, insights into the genes controlling vulnerability to cavitation and xylem hydraulic conductivity are still lacking, despite their physiological importance in trees.

Transgenic trees make it possible to study the function of genes identified by genomic approaches and provide useful models to understand the genetic basis of xylem functional traits (Mellorwicz et al., 2001; Chaffey et al., 2002; Awad et al., 2012). Nevertheless, investigations on the genetic basis of xylem hydraulic traits using transgenic trees are rare and the approach by gene overexpression shows limitations in research on the genetic bases of traits. In order to overcome the above-mentioned problems associated with trees, Arabidopsis thaliana has been proposed as a model plant to investigate the genetic basis of wood formation (Chaffey et al., 2002; Nieminen et al., 2004; Zhang et al., 2011). Chaffey et al. (2002) emphasized the strong similarities in anatomy and development of secondary xylem between A. thaliana and Populus tremula L. × P. tremuloides Michx. A. thaliana has also been suggested as a model species for many other wood traits (Boerjan et al., 2003; Flashman et al., 2003; Ko et al., 2004; Wyatt et al., 2010; Haughn and Western, 2012; Lens et al., 2012b). Thus, it seems reasonable to question whether the inflorescence stem of A. thaliana can also be used as a model for xylem hydraulics despite its herbaceous and minuscule nature. Since there are strong differences in habit between perennial plants and short-living herbs, the xylem hydraulic characteristics of A. thaliana deserve special attention.

The main goal of this paper is to investigate if standard techniques applied in the field of xylem hydraulics are practically appropriate in inflorescence stems of A. thaliana, which are thought to have similar functions as stems of woody plants. Indeed, the inflorescence stem shows secondary growth (Altamura et al., 2001; Ko et al., 2004), allows long-distance water transport from the roots to leaves, flowers, seeds and fruits, and is also subject to similar mechanical constraints as a tree canopy, namely their weight, gravitropism, and various external perturbations (Telewski, 2006). Additionally, special attention is paid to hydraulically significant structures such as xylem vessels and bordered pits to evaluate the hydraulic properties of A. thaliana. Given various similarities in xylem structure between A. thaliana and woody eudicots (Chaffey et al., 2002; Lens et al., 2012b), the present study hypothesized that inflorescence stems of A. thaliana show a similar hydraulic behaviour as many shrubs and trees. If this assumption was correct and if measuring hydraulic parameters was feasible, a wide range of genetic techniques and approaches could be applied to facilitate links between xylem physiology, anatomy, and genetics.

The reliability and resolution of the hydraulic techniques were verified by measuring the effect of growth conditions and genetic differences on cavitation resistance. An interesting aspect of this annual herb is that xylem development is controlled by flowering time, which can be influenced by several environmental cues such as photoperiod (Melzer et al., 2008; Sibout et al., 2008). Differences in secondary xylem development between short-day plants and long-day plants were hypothesized to parallel differences in cavitation resistance. Overall, such work could offer new opportunities for understanding functional hydraulic traits (safety vs. efficiency) regarding the developmental and evolutionary shift form primary xylem towards secondary xylem. Moreover, this study explored potential differences in cavitation resistance in three mutant lines modified for genes involved in the primary wall metabolism.

**Materials and methods**

**Plant material**

Seeds from wild-type (WT) A. thaliana (L.) Heynh. Columbia (Col-0) and T-DNA insertion mutant lines were provided by the NASC (Nottingham Arabidopsis Stock Centre). The sequence-indexed A. thaliana T-DNA insertion mutant sequences were provided by the Salk Institute Genomic Analysis Laboratory. The polygalacturonase mutant (PG, SALK_100709, on locus At1g19170) was chosen because its locus had a stem-specific expression pattern (Kim et al., 2006) and it is an orthologue of the Populus tremula × tremuloides POPTR_0006s14170 gene encoding for a polygalacturonase and upregulated during xylem maturation (Hertzberg et al., 2001). The choice for the pectin methyltransferase loci (PME3, SALK_059908 on locus At2g45220 and PME5, SALK_012478 on locus At3g59010) was based on the availability of SALK mutants on PME genes and expression patterns. PME3 showed an inflorescence stem xylem expression that responded to changes in lignin biosynthesis in cinnamyl alcohol dehydrogenase (CAD) mutant lines (Sibout et al., 2005). PME5 (At3g59010) was expressed in inflorescence stem xylem (Winter et al., 2007) and was upregulated while promoting secondary growth (Koizumi et al., 2009). Insertion and T-DNA zygosity were checked in PG, PME3, and PME5, based on screening for kanamycin resistance.

Seeds were sown on a commercial soil mixture (Hunustar, Champex, France) and grown in a growth chamber under long-day (16/8 light/dark) or short-day (12/12 light/dark) conditions. A second set of WT plants with PG and PME mutants were grown in long-day conditions. The plants were cultivated at 22° C under light intensity 100–200 μmol m⁻² s⁻¹ and with relative humidity 60%. Phenotypes and genotypes were randomly distributed in the growth chamber in order to limit potential variability in light intensity. As soon as inflorescence stems showed a first silique ripening (i.e. after 7–8 weeks), they were harvested and immediately used for analysis.

**Xylem anatomy**

**Light microscopy**

Anatomical analysis was performed on long-day plants. Transverse sections (thickness 0.5 μm) were sectioned and stained with toluidine blue (0.5% in 2.5% carbonate buffer, pH 11). Toluidine blue stains cells in blue and fibres and parenchyma cells in purple. The sections were examined under an optical microscope (Axioplan 2, Zeiss, Jena, Germany), and images of whole sections were recorded using a digital camera (AxioCam, HR, Zeiss) and AxioVision digital imaging software. After their hydraulic conductivity was measured, vessel
areas were measured on 50 μm cross-sections of basal inflorescence stems from eight plants. After spatial calibration, the surface area of vessels was calculated using ImagePro Plus 6.1 (Media Cybernetics, Silver Spring, MD, USA). For each sample, a graphic tablet (Wacom Technology, Vancouver, WA, USA) was used to measure each vessel area. The circle diameter (D) with a surface area corresponding to the vessel surface area was then deduced (Christman and Sperry, 2010). The hydraulicly weighted vessel diameter (D_i) was calculated following Sperry et al. (1994) as \( D_i = \frac{\Sigma D_i^2}{\Sigma D_i} \). Metaxylem and secondary xylem were defined according to Altamura et al. (2001). Theoretical hydraulic conductivity (\( K_{ht} \)) of the lumen was calculated as 
\[
K_{ht} = \frac{N_i D_i}{\eta L}
\]
where \( N_i \) was the ratio between the number of silicon-filled vessels and the total number of vessels at the injection surface, \( L \) the distance from the injection point, and \( k \) the best fit extinction coefficient.

Electron microscopy
Samples for transmission electronic microscopy (TEM) were prepared according to Jansen et al. (2009). Observations were carried out on transverse sections using a JEM1210 microscope (Jeol, Tokyo, Japan) at an accelerating voltage of 80 kV. Digital images were taken using a Mega View III camera (Soft Imaging System, Münster, Germany) and analysed using Image software (Rasband, 1997–2012).

For scanning electron microscopy (SEM), several inflorescence stems of Arabidopsis thaliana were cut into 0.5–1 cm segments, split longitudinally, and dried at 65 °C overnight. Samples were fixed to aluminium stubs and coated with platinum using a BAF100 sputter coater (Balzers, Liechtenstein) for 3 min. Samples were observed with a S-5200 field-emission scanning electron microscope (Hitachi High Technologies, Tokyo, Japan) at an accelerating voltage of 2 kV.

Xylem hydraulics
Experimental specific conductivity
The hydraulic conductivity (\( K_s \)) was measured on 1-cm-long segments of basal inflorescence stem in order to decrease the pit resistivity and compared with \( K_{ht} \). \( K_s \) was measured from eight plants grown under long-day conditions using a Xyl’EM apparatus following procedures described for woody species (Sperry et al., 1988; Cruiziat et al., 2002). This technique involved measuring the hydraulic conductance of segments before and after flushing (\( k_s \) and \( k_{max} \), respectively). Two stem segments 1–2 cm long were cut under water from the middle part of the stem. The basal cut end was then connected to the hydraulic apparatus. A solution containing KCl (10 mM) and CaCl\(_2\) (1 mM) was used to perfuse the stem segments \( k_s \) and \( k_{max} \) were measured under 6–9 kPa on both segments separately in order to increase the number of repetitions for each plant. Before measuring \( k_{max} \), samples were flushed for 5 min at 0.1 MPa to remove embolism. To draw a single vulnerability curve, 15–46 samples from 15–24 different stems were required. This study also tentatively applied the bench drying method (Sperry et al., 1988), the Cavitation technique (Cochard, 2002; Cochard et al., 2005), and the single-ended pressure chamber approach (Cochard et al., 1992). The PLC curves were fitted using the sigmoid function of Pammenter and van der Willigen (1998), 
\[
PLC = 100 \left( 1+e^{\frac{5}{4} \cdot \frac{S}{P_0}} \right),
\]
where \( P_0 \) is the pressure causing 50% loss in conductance, and \( S \) the curve slope at this point.

Results
Anatomy of xylem vessels and pits
The xylem of A. thaliana was organized in vascular bundles (Fig. 1A), with (mean ± SE) 9.4 ± 0.9 bundles per transverse section and 19.5 ± 1.7 vessels per vascular bundle (Fig. 1B). The vessels can be distinguished from other cell types because of differences in the staining reaction of cell walls with toluidine blue (Fig. 1B). Additional evidence for vessel identification was based on silicon injection (Fig. 1C). Vascular bundles included both primary metaxylem and secondary xylem with differences in their respective proportions across vascular bundles (Fig. 1A). Primary xylem consisted of relatively wide vessels (16.0 ± 0.6 μm), while secondary xylem was characterized by narrower vessels (11.8 ± 0.2 μm) in combination with xylem fibres. Ray cells were absent in the xylem of the samples studied. The mean vessel diameter \( D_v \) was 11.9 ± 1.4 μm (Fig. 2A), with mean \( D_o \) of 17.7 ± 0.5 μm and mean vessel length (\( L \)) of 1.2 ± 0.2 cm (Fig. 2B).

Neighbouring vessels were connected to each other via bordered pit fields (Fig. 1D–H). Three different pit types that were associated with vessels occurred in A. thaliana: intersinus pits (Fig. 1E), vessel-fibre pits (Fig. 1D), and vessel-parenchyma pits (not shown). The pit dimensions are summarized in Table 1. A. thaliana had vented pits (Fig. 1E, F, H), showing branched protuberances from the secondary cell wall of the pit chamber. The ventricle partly occluded the pit aperture (Fig. 1F, H).

Xylem hydraulics
Hydraulic conductivities (\( K_s \)) were in the range of 1.10\(^{-11}\) to 12.10\(^{-11}\) m\(^3\) MPa\(^{-1}\) s\(^{-1}\). \( K_s \) was linearly correlated to the sample length effect on \( K_s \). Perfusion was performed following the same methods as above. Stem segments were perfused basipetally and shorted every 0.5 cm.

Xylem vulnerability to cavitation
The vulnerability to cavitation of inflorescence stems was assessed using the centrifugal technique (Alder et al., 1997; Cochard, 2002) on 14-cm-long samples. For this purpose, the embolism rate was measured using a Xyl’EM apparatus following procedures described for woody species (Sperry et al., 1988; Cruiziat et al., 2002). This technique involved measuring the hydraulic conductance of segments before and after flushing (\( k_s \) and \( k_{max} \), respectively). Two stem segments 1–2 cm long were cut under water from the middle part of the stem. The basal cut end was then connected to the hydraulic apparatus. A solution containing KCl (10 mM) and CaCl\(_2\) (1 mM) was used to perfuse the stem segments \( k_s \) and \( k_{max} \) were measured under 6–9 kPa on both segments separately in order to increase the number of repetitions for each plant. Before measuring \( k_{max} \), samples were flushed for 5 min at 0.1 MPa to remove embolism. To draw a single vulnerability curve, 15–46 samples from 15–24 different stems were required. This study also tentatively applied the bench drying method (Sperry et al., 1988), the Cavitation technique (Cochard, 2002; Cochard et al., 2005), and the single-ended pressure chamber approach (Cochard et al., 1992). The PLC curves were fitted using the sigmoid function of Pammenter and van der Willigen (1998), 
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\]
where \( P_0 \) is the pressure causing 50% loss in conductance, and \( S \) the curve slope at this point.
theoretical hydraulic conductivity ($K_{ht}$) predicted from the Hagen-Poiseuille equation ($R^2 = 0.80$). $K_h$ was on average 41% lower than $K_{ht}$ predicted for ideal capillaries. The measured ($K_{hs}$) and theoretical ($K_{hts}$) specific conductivities showed a strong linear relationship ($P < 0.01$; Fig. 3A). Fig. 3B showed no significant change in $K_h$ when shortening samples from 5 to 1 cm ($ANOVA, P < 0.05$).

This study was unable to obtain vulnerability curves following the bench drying or the single-ended cavitation chamber approach. Samples were flattened because of...
dehydration of the pith parenchyma, which made hydraulic measurements impossible because of leaks. This study was also unable to determine cavitation resistance using the Cavitron technique because the conductance of the inflorescence stems was too low to be measured accurately with this method.

Vulnerability curves were successfully obtained with the centrifuge approach (Fig. 4). *A. thaliana* had PLC values decreasing with xylem pressure, following a sigmoidal curve. Some PLC values appeared negative because of the centrifuge method employed (e.g. Adler *et al.*, 1997). The sigmoid regression analysis provided a strong fit with $R^2 > 90\%$. *A. thaliana* WT plants showed a $P_{50}$ value of $-2.53 \pm 0.053$ MPa and $-2.98 \pm 0.139$ MPa when grown under long-day and short-day conditions, respectively, with a significant difference between both conditions (Student’s t-test, $P < 0.05$).

The second set of WT plants grown under long-day conditions showed a $P_{50}$ value of $-2.54 \pm 0.080$ MPa. This value was not significantly different from the values obtained for *PME3* and *PME5* plants grown in same conditions, which were $-2.49 \pm 0.137$ MPa and $-2.44 \pm 0.108$ MPa, respectively (Fig. 4C, D). On the contrary, PG mutants showed a $P_{50}$ value of $-2.25$ MPa $\pm 0.033$, significantly different from WT plants (Student’s t-test, $P < 0.05$, Fig. 4B).

**Discussion**

This study demonstrates that inflorescence stems of *A. thaliana* can be used to measure specific conductivity and cavitation resistance in an accurate and reliable approach. The hydraulic data obtained support this study hypothesis that *A. thaliana* shares both structural and functional xylem characteristics with many eudicots, which opens important opportunities to utilize this species for investigating xylem hydraulics. While there are various advantages of using *A. thaliana* for studying water transport in plants, its limitations also need to be considered.

Given the small size of the inflorescence stems of *A. thaliana*, the hydraulic pathway of the xylem can more easily be studied in its totality on a cross-section as compared to trees with large sapwood areas that include various growth rings. Indeed, theoretical conductivities can be performed on the total number of vessels and shows a strong linear relationship with measured conductivities (Fig. 3A). This also demonstrates that the vessels that contribute to hydraulic conductance can accurately be identified on transverse stem sections. Silicone injection (Fig. 1C) and dye perfusion (data not shown) also confirmed the conductive nature of vessels in the primary and secondary xylem.

Measured conductivity includes lumen and end-wall conductivity, whereas theoretical conductivity estimates only lumen conductivity. Based on the silicone injection experiments, 80% of the vessels are not open at both ends in the 1-cm stem segments used for measuring conductivity (Fig. 3A), and thus there would be an end-wall resistance to the flow. In order to evaluate the contribution of this resistance on 1-cm stem segments, stem-shortening experiments were performed on six samples (Fig. 3B). The maximum vessel length

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**Table 1.** Intervessel pit morphology of *A. thaliana* inflorescence stem. Transverse sections of stems were observed under TEM and bordered pit characteristics and double intervessel wall thickness measured with image analysis. Values are mean $\pm$ SE for $n$ pits observed.

<table>
<thead>
<tr>
<th>Pit character</th>
<th>$n$</th>
<th>Size (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aperture diameter ($D_a$)</td>
<td>19</td>
<td>0.69 ± 0.23</td>
</tr>
<tr>
<td>Membrane diameter ($D_p$)</td>
<td>17</td>
<td>3.56 ± 0.53</td>
</tr>
<tr>
<td>Membrane thickness ($T_m$)</td>
<td>20</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>Chamber depth ($D_c$)</td>
<td>18</td>
<td>0.93 ± 0.66</td>
</tr>
<tr>
<td>Double intervessel wall thickness ($T_w$)</td>
<td>20</td>
<td>1.67 ± 0.69</td>
</tr>
</tbody>
</table>
measured with silicon injection (Fig. 2B) was smaller than 5 cm, meaning that all vessels had at least one end wall in 5-cm long samples. No significant difference in conductivity was found between 5- and 1-cm segments, suggesting that the specific conductivity can be estimated by working with 1-cm segments. In shorter segments, flow rates were higher and more precise compared to the theoretical specific conductivity. The 41% hydraulic efficiency of *A. thaliana* is in line with findings of both woody and herbaceous angiosperms, with measured conductivity values less than half of the theoretical values of ideal capillaries (Tyree and Zimmermann, 2002). Therefore, sap flow in *A. thaliana* seems to follow the Hagen–Poiseuille efficiency model developed on woody plants (Sperry et al., 2005; Hacke et al., 2006). End-wall resistivity generally accounts for 50% of the total hydraulic resistivity (Lancashire and Ennos, 2002; Sperry et al., 2005; Choat et al., 2008) and thus can explain the lower conductivity measured. Although measuring the xylem conductivity of inflorescences can be achieved easily, quantification of the end-wall resistivity remains a technical challenge in *A. thaliana* because of the very short (<1 cm) sample length that would be required for such measurements.

The reliability and resolution of the vulnerability curves obtained are supported by the differences found between the growing conditions or the mutant lines of *A. thaliana* (Fig. 4). A significant difference was observed in vulnerability to cavitation between plants grown under long days and short days (Fig. 4A). Changes in the photoperiod from long to short days were shown to stimulate and prolong xylem development in the hypocotyls and inflorescence stems (Lev-Yadun, 1994; Chaffey et al., 2002). According to the present findings, an increase in wood production would enhance the resistance to cavitation of the inflorescence stem. Since secondary xylem is characterized by narrower vessels than metaxylem vessels, the secondary xylem would be less vulnerable to cavitation than metaxylem. Indeed, wider vessels would show a larger pit area, which may increase the likelihood of air-seeding via the largest pit-membrane pore (Wheeler et al., 2005; Hacke et al., 2006; Christman and Sperry, 2010; Christman et al., 2012). Differences in vessel grouping between primary and secondary xylem could offer an additional explanation (Lens et al., 2011), as well as potential differences in pit characters.

The vulnerability curves for *A. thaliana* (Fig. 4) showed $P_{50}$ values ranging from −2.25 to −3 MPa. Meyre et al. (2001) showed that *A. thaliana* plants grown under short days were able to maintain an optimum transpiration rate for a soil water potential of −0.28 MPa and observed a complete decline in transpiration rate for a soil water potential of −1.3 MPa. In the present study, the predawn leaf water potential was −0.06 MPa and therefore within range of the optimal transpiration rate. Moreover, the pressure for stomata closure was a long way from the $P_{50}$ values found in here, which suggests that the plant has a high safety margin (Cochard et al., 2002).

Some mutant lines were analysed to test if the *A. thaliana* stem is suitable and the technique is reliable to investigate the genetic bases for the vulnerability to cavitation. Mutant lines for pectin genes represent good candidates because they have an important role in elongation processes, determining cell size, shape, and stiffness (Kim et al., 2006). PG and PME are known to catalyse disassembly and structural modification of pectins (Wolf and Greiner, 2012). Changes in expression of these enzymes could thus have an effect on conduit dimensions and thus on pit density (Christman et al., 2009). Moreover, they could be involved in pit-membrane maturation and thus in its properties, especially its porosity. Pectin methylesterases catalyse the demethylesterification of homogalacturonan (HGA). These demethylesterified HGA can form calcium bonds, promoting an ‘egg-box’ model structure, or become a target for pectin-degrading enzymes, affecting the texture and rigidity of the cell wall (Pelloux et al., 2007). Herbette and
Cochard (2010) proposed that the observed calcium effect on vulnerability to cavitation was due to calcium bridges in pectin chains of the pit membrane. Although no significant effect of the PME3 and PME5 mutations were detected, the present study cannot exclude PME as candidate genes involved in vulnerability to cavitation, especially when considering the size of this multigene family (67 genes, Micheli, 2001) and a potential compensation effect between isoforms. On the contrary, mutation of PG (multigene family of 66 genes, Kim et al., 2006) showed a clear effect on cavitation resistance. Anatomical observations remain to be performed to understand the role of PG.

The $P_{50}$ values for *A. thaliana* fall within the range of many tree species with a temperate distribution area. *Quercus robur*, for instance, has a $P_{50}$ around $-2.7$ MPa (Cochard et al., 1992). Although $P_{50}$ values have not been determined for many herbaceous species (Rosenthal et al., 2010), the xylem of *A. thaliana* is rather cavitation resistant compared to other herbaceous plants, which could be explained by the distribution of *A. thaliana* on dry and sandy soils (Koornneef et al., 2004). Examples are *Phaseolus vulgaris* L., which showed $P_{50}$ values from $-0.46$ to $-0.99$ MPa (Mencuccini and Comstock, 1999), and *Epilobium angustifolium* L. with a $P_{50}$ value around $-1.6$ MPa (Maherali et al., 2009). Although both species show vestured pits (Jansen et al., 2008), which are assumed to increase resistance to cavitation (Jansen et al., 2003, 2004; Choa et al., 2004), *A. thaliana* shows a higher cavitation resistance than these two species.

The hydraulic behaviour in *A. thaliana* appears to be paralleled by many similarities in xylem anatomy and ultrastructure between this species and woody eudicots. Despite the small scale of vessel dimensions in *A. thaliana*, the 3/2
scaling between average vessel length \((L)\) and vessel diameter corresponding to mean lumen resistivity \((D_{RL})\), reported by Hacke et al. (2006) on 29 woody angiosperm species, is also found for \textit{A. thaliana}. Vessel dimensions of \textit{A. thaliana} indicate a scaling of \(L = 0.044 \ D_{RL}^{3/2}\). This finding is in line with previous observations that narrow vessels are generally short, while wide vessels range from short to long (Sperry et al., 2005; Hacke et al., 2006; Christman and Sperry, 2010). However, Jacobsen et al. (2012) showed recently no relationship between these vessel traits among 130 species, and vessel length was hypothesized to be dependant of the species habit and diameter of samples. The low vessel dimensions in \textit{A. thaliana} could then be explained by the narrow diameter of the inflorescence stem.

Other structural similarities between \textit{A. thaliana} and woody plants include the presence of simple perforation plates and bordered pits. Simple perforation plates are characteristic of \textit{Brassicaceae} and very common in eudicots (Schweingruber, 2006; Wheeler et al., 2007). The mean pit-membrane thickness of \textit{A. thaliana} (170 nm) is within the range observed for angiosperms (Jansen et al., 2009; Lens et al., 2011). These observations suggest similar structure–function relationships between \textit{A. thaliana} and woody angiosperms at the pit-membrane level (Choat et al., 2008).

The xylem of \textit{A. thaliana} shows a rather limited amount of fibres, while woody plants generally show a high proportion of lignified ground tissue in their xylem. Based on pit morphology and silicon injection data, this study assumes that the fibres in \textit{A. thaliana} are hydraulically non-functional (Sano et al., 2011). Moreover, the lack of rays in xylem of \textit{A. thaliana} confirms observations by Chaffey et al. (2002), although ray cells have been detected in \textit{A. thaliana} plants in which secondary growth was induced (Mazure et al., 2012).

Hence, \textit{A. thaliana} has the genetic potential to develop ray cells, while rayless wood could be associated with paedomorphosis and secondary woodiness (Carlquist, 1962, 2009; Dulin and Kirchoff, 2010). Since the development of rays is delayed in a small number of eudicots, raylessness should be interpreted as a juvenile character and an example of heterochronic shift in xylem organization (Carlquist, 2009).

Lens et al. (2012b) described paedomorphic wood features in woody double mutants of \textit{A. thaliana} such as a continuously decreasing length-on-age curve of vessel elements and delayed periderm formation.

\textit{A. thaliana} provides many advantages as a model plant. Environmental effects on hydraulic parameters can be more easily applied from a practical point of view to small plants than large trees. Knock-out mutants of \textit{A. thaliana} allow the screening of a large number of genes putatively involved in xylem hydraulics. The irregular xylem (irx) \textit{A. thaliana} mutants have already brought various insights into the biochemical and genetic aspects of xylem structure (e.g. Turner et al., 1997). The \textit{A. thaliana} genome sequence (The Arabidopsis Genome Initiative, 2000) provides novel avenues for identification and systematic analysis of genes involved in cell-wall metabolism (Liepman et al., 2010). Plant cell walls are extremely complex in their composition, biosynthesis, assembly, and post-assembly modifications (Popper et al., 2011). Genes involved in cell-wall synthesis or modification represent 10\% of the genes in \textit{A. thaliana} and they mainly belong to multigene families (Farrokhi et al., 2006). Thus, \textit{A. thaliana} is an excellent plant model for screening the functional significance of genes involved in xylem hydraulics by working on already characterized genes and ready-to-use mutant lines.

Given the short-life cycle of \textit{A. thaliana}, its role as an ecophysiological model for trees remains questionable. Annual weeds with a short life cycle are often characterized by stress avoidance and show strong ecological differences with a tree habit. Considering the wide geographical range of \textit{A. thaliana}, it is not surprising that this species shows a large variation in physiological processes in order to deal with contrasting environments worldwide (Pigliucci, 2002). Meyre et al. (2001) proposed, for instance, an adaptive drought-tolerance strategy for the Columbia ecotype in contrast to the escape strategy of the Langsberg ecotype, which has a shortened life cycle. Moreover, the woody double mutant soc1 ful of \textit{A. thaliana} shows extensive secondary growth perennial-like features with prolonged plant lifetime (up to 18 months), co-occurrence of vegetative and reproductive meristems, and recurrent flowering cycles, and thus strong similarities with the habit of a tree (Melzer et al., 2008; Lens et al., 2012b). The use of such mutant could be of special interest for ecophysiological modelling. However, the use of xylem from reproductive organs is potentially problematic because genes affecting reproductive tissues could change the characteristics of the inflorescence xylem without being important for the systemic xylem of the vegetative part of the plant.

The ‘hidden woodiness’ of \textit{A. thaliana} is found in many lineages of herbaceous plants, which frequently retain the capacity to develop secondary meristems and xylem following similar developmental patterns as woody trees, without special innovations of their bauplan or hydraulic plumbing system (Spicer and Groover, 2010; Lens et al., 2012a; Rowe and Paul-Victor, 2012). A fuzzy boundary between morphological categories is common in many organisms, especially in plants (Sattler and Rutishauser, 1997), which has implications in the functional understanding of processes such as water transport. The traditional view of classifying species and features into mutually exclusive categories such as herbaceous or woody ignores the dynamic continuum of growth forms, and similarities in structure–function. From a functional and anatomical point of view, size does not matter and should not prevent the use of \textit{A. thaliana} as a hydraulic model species, although the limited amount of xylem in combination with its short life cycle and low hydraulic demands do have some limitations. Therefore, validation on transgenic trees will certainly be needed.

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References


Haugn GW, Western TL. 2012. Arabidopsis seed coat mucilage is a specialized cell wall that can be used as a model for genetic analysis of plant cell wall structure and function. Frontiers in Plant Science 3, 1–5.


