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RESEARCH PAPER

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 (R^2 = 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 (P_{50}) between –3 and –2.5 MPa for short-day and long-day plants, respectively. Polygalacturonase mutants showed a higher P_{50} 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

consuming (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 across populations (Lamy *et al.*, 2011; Wortemann *et al.*, 2011), although others have shown that there is no phenotypic plasticity in cavitation resistance of stem wood (Alder *et al.*, 1996; Kolb and Sperry, 1999) and 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 (Mellerowicz *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; Flaishman *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 to 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 methylesterase 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 inflorescent 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 inflorescent 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 (Humustar, Champeix, 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 22/20 °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 vessels 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 (Wacum 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). A hydraulically weighted vessel diameter (D_h) was calculated following Sperry *et al.* (1994) as $D_h = \Sigma D^5/\Sigma D^4$. Metaxylem and secondary xylem were defined according to Altamura *et al.* (2001). Theoretical hydraulic conductivity (K_{ht}) of the lumen was calculated as $K_{ht} = \Sigma\pi D^4/128\eta$, where η was the viscosity at 20 °C. The total theoretical specific conductivity (K_{hts}) was obtained by dividing K_{ht} by the total lumen area. This approach was chosen because it compensated for differences in vessel size and number, and it removed the contribution of non-conducting cells in xylem tissue of herbaceous plants (Kocacinar and Sage, 2003).

To determine the proportion of open vessels in stem segments of different lengths, the vessel-length distribution of three *A. thaliana* inflorescence stems grown under long-day conditions was measured. Stems were fixed in a solution containing (v/v) 3.7% formaldehyde, 5% acetic acid, and 50% ethanol, gradually dehydrated using solutions from 50 to 100% ethanol, and dried at 65 °C overnight. Then, one end of the stem segments was injected with the silicone Rhodorsil RTV-141 (Rhodia, Cranbury, NJ, USA), which was mixed with the fluorescent dye Uvitex OB (Ciba UK, Bradford, West Yorkshire, UK). Injection was conducted basipetally with a vacuum pump at 0.2 MPa for 30 min. After drying, thin sections were cut with a cryomicrotome at various distances from the injection point, and the ratio of vessels filled with silicone per total number of vessels (N_L) was determined for each distance. The conduit length distribution was calculated according to Sperry *et al.* (2005) as $N_L = N_0\, e^{(-kL)}$, where N_0 is 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 ImageJ software (Rasband, 1997–2012).

For scanning electron microscopy (SEM), several inflorescence stems of *A. 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 an BAF100 sputter coater (Balzers, Dietenheim, Germany) 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_h) was measured on 1-cm-long segments of basal inflorescence stem in order to decrease the pit resistivity and compared with K_{ht}. K_h was measured from eight plants grown under long-day conditions using a Xyl'em apparatus (Bronkhorst, Montigny-les-Cormeilles, France). Stem segments were cut under water and gently sealed to a tubing system using thread seal tape (polytetrafluoroethylene film) to avoid leaking and crushing of samples with clamps. A solution containing KCl (10 mM) and CaCl (1 mM) was used to perfuse stems basipetally and flush them at 21 °C for 5 min under 0.1MPa. The flow rate was measured 10 minutes after starting the perfusion under 6–9 kPa. The specific hydraulic conductivity (K_{hs}) was obtained by dividing K_h by the total vessel lumen area.

K_h was also measured on 5-cm-long basal inflorescence stems from six other plants grown under long-day, in order to evaluate the sample length effect on K_h. Perfusion was performed following the same methods as above. Stem segments were perfused basipetally and shortened 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_i 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_i 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 Cavitron 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), $PLC = 100/(1+e^{(S/25 \times (P-P_{50}))})$, where P_{50} 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 was 11.9 ± 1.4 µm (Fig. 2A), with mean D_h 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*: intervessel 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 vestured pits (Fig. 1E, F, H), showing branched protuberances from the secondary cell wall of the pit chamber. The vestures partly occluded the pit aperture (Fig. 1F, H).

Xylem hydraulics

Hydraulic conductivities (K_h) were in the range of 1.10^{-11} to 12.10^{-11} m^4 MPa^{-1} s^{-1}. K_h was linearly correlated to the

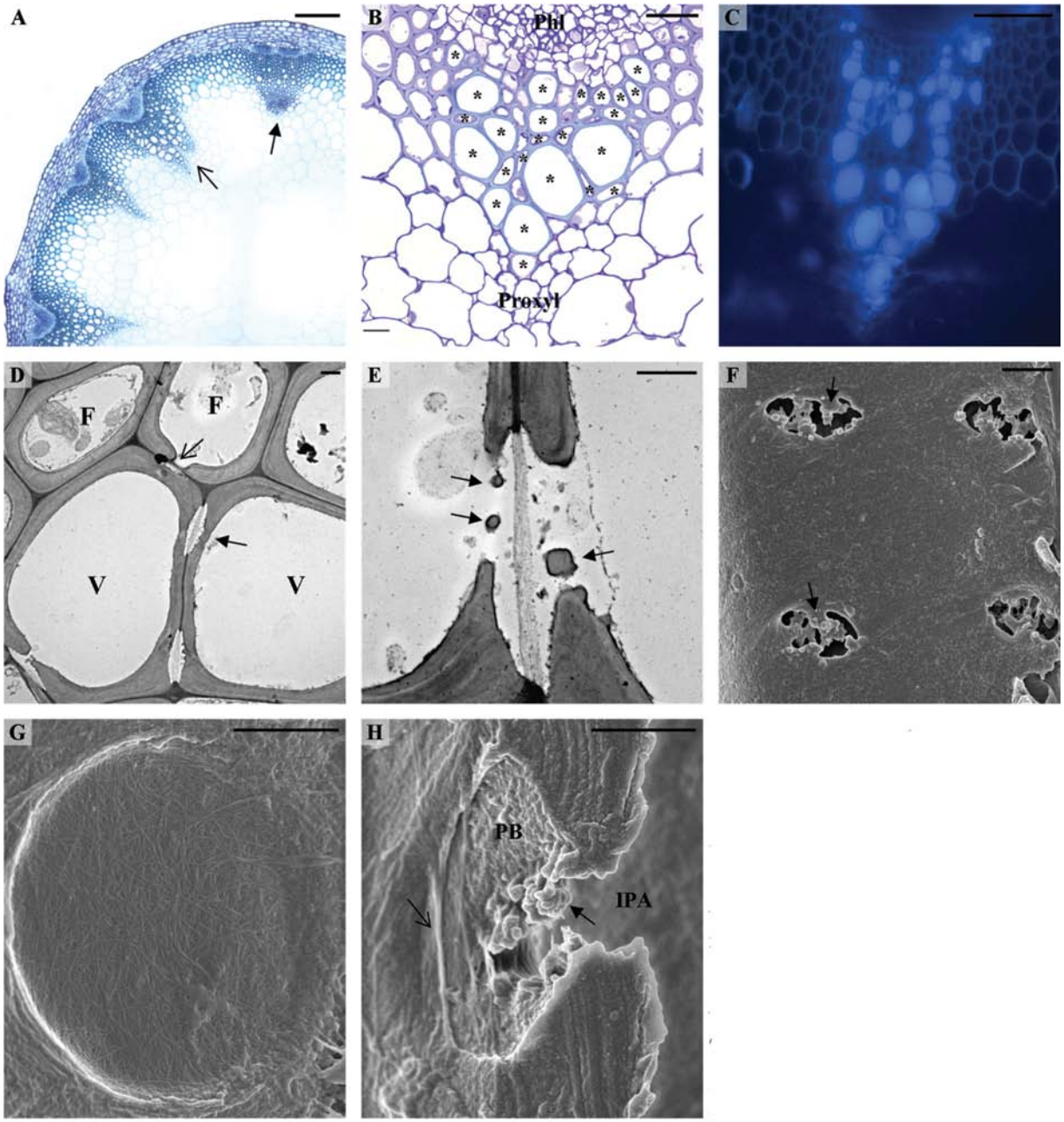

Fig. 1. Anatomical features of the xylem in the inflorescence stem of *A. thaliana*. (A) Transverse sections showing xylem organization in vascular bundles: the closed arrow shows a vascular bundle with secondary xylem dominance and the open arrow shows a vascular bundle with metaxylem dominance. (B) Detail of a vascular bundle with metaxylem and vessels marked by asterisks: toluidine blue stains vessels in blue and fibres and parenchyma cells in purple. (C) Transverse section of a vascular bundle from a stem injected with silicone: conductive vessels are filled with silicone mixed with the fluorescent dye Uvitex. (D) Transverse section of xylem vessels observed by TEM: the closed arrow shows an intervessel pit and the open arrow shows a vessel-fibre pit. (E) Intervessel pit observed with TEM: arrows show vestures. (F–H) SEM images of inner vessel wall with four vestured pit apertures (F), pit membrane (G), and pit chamber bearing vestures near the outer aperture (H): closed arrows show vestures and the open arrow shows a small part of the aspirated pit membrane. F, fibre; IPA, inner pit aperture; PB, pit border; Phl, phloem; Proxyl, protoxylem; V, vessel. Bars, 100 μm (A), 10 μm (B), 50 μm (C), 1 μm (D–H).

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

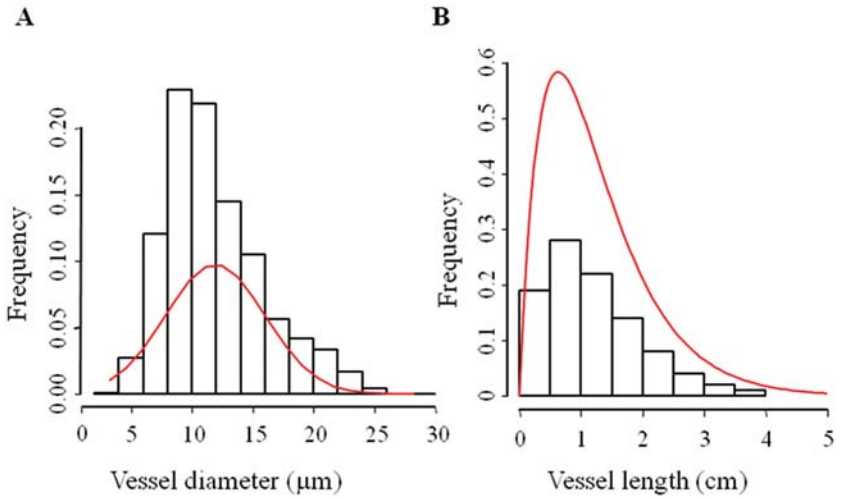

Fig. 2. Vessel diameter and vessel length distribution for inflorescence stem of *A. thaliana*. (A) Vessel diameter distribution analysed from eight plants. (B) Vessel length distribution analysed from three plants. Data are means ± SE. Solid line indicates the average frequency of vessel length (Sperry *et al.*, 2005).

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 ± 0.053 MPa and –2.98 ± 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 ± 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 ± 0.137 MPa and –2.44 ± 0.108 MPa, respectively (Fig. 4C, D). On the contrary, *PG* mutants showed a P_{50} value of –2.25 MPa ± 0.033, significantly different from WT plants (Student's t-test, $P < 0.05$, Fig. 4B).

Table 1. Intervessel pit morphology of *A. thaliana* infloresence 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 ± SE for *n* pits observed.

Pit character	*n*	Size (μm)
Aperture diameter (D_a)	19	0.69 ± 0.23
Membrane diameter (D_p)	17	3.55 ± 0.53
Membrane thickness (T_m)	20	0.17 ± 0.01
Chamber depth (D_c)	18	0.93 ± 0.66
Double intervessel wall thickness (T_w)	20	1.67 ± 0.69

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

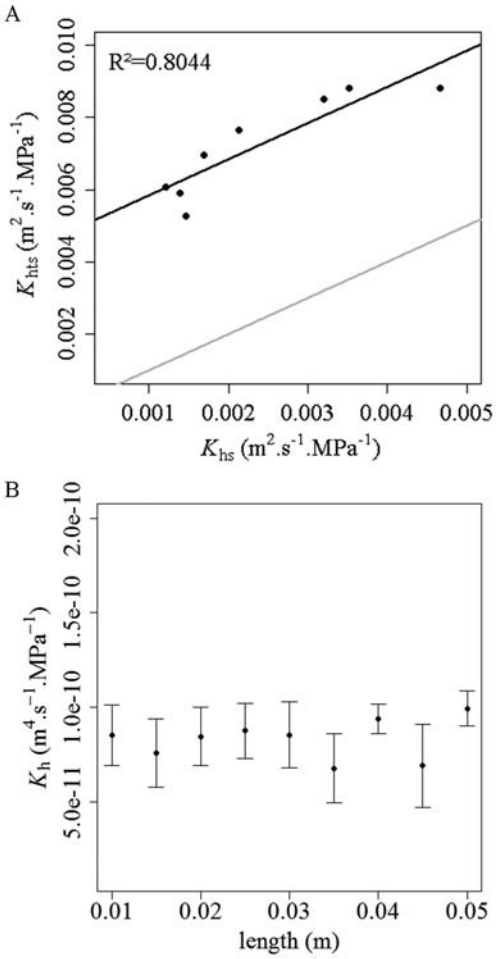

Fig. 3. Hydraulic conductivity of the inflorescence stem of *A. thaliana*. (A) Plot of the experimental (K_{hs}) versus theoretical (K_{hts}) hydraulic specific conductivity. K_{hs} was measured on 1-cm-long basal inflorescence stems from eight plants, then K_{hts} was calculated by measuring vessel diameters and applying the Hagen–Poiseuille law. Values are for each plant. The black line represents a linear correlation between K_{hts} and K_{hs} ($K_{hts} = 1.0043K_{hs} + 0.0048$; $R^2 = 0.8044$) and the grey line represents the 1:1 line. (B) Hydraulic conductivity (K_h) versus segment length. K_h was measured on 5-cm-long basal inflorescence stems that were perfused basipetally and shortened every 0.5 cm. Data are means ± SE from six plants. There was no significant difference between means (ANOVA, $P < 0.05$).

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 inflorescent stems 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

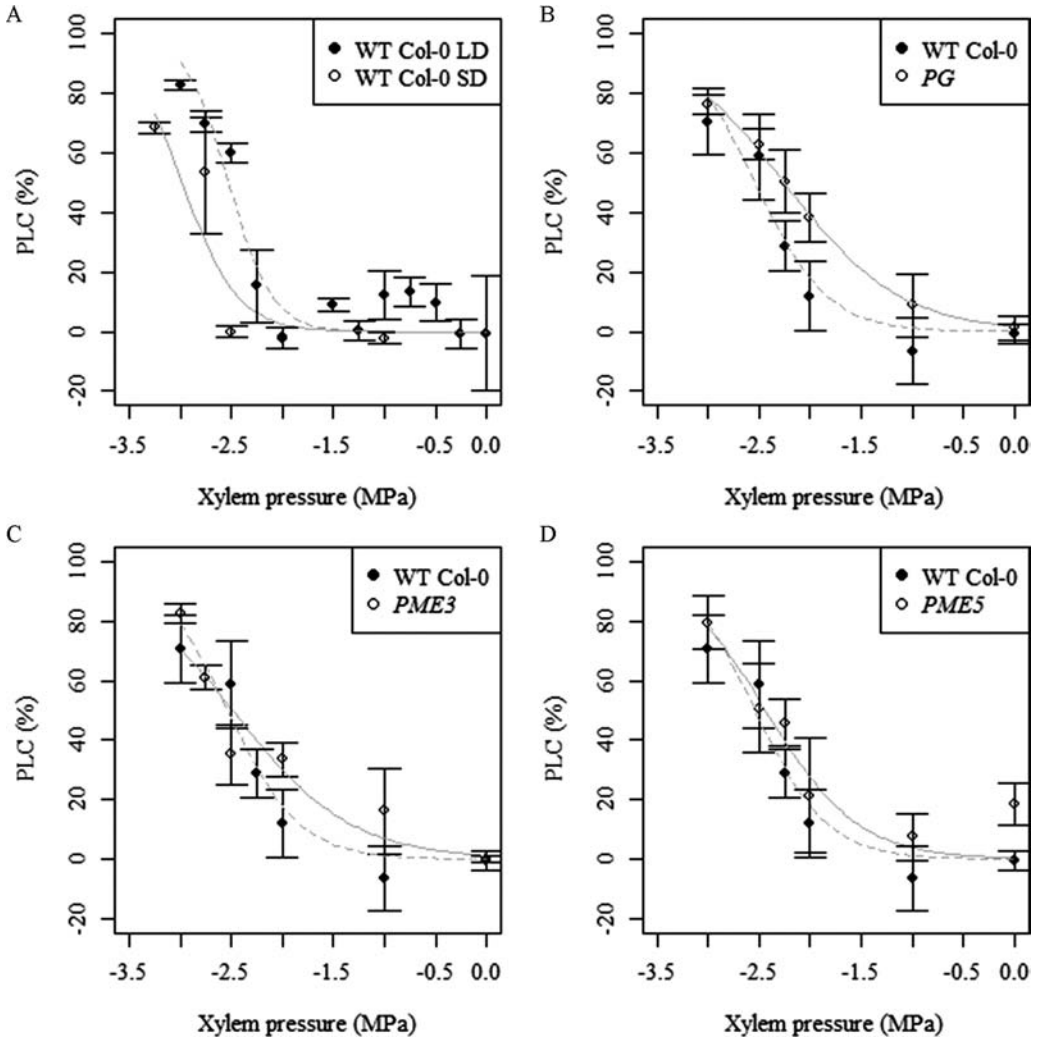

Fig. 4. Xylem vulnerability to cavitation of *A. thaliana* plants depending of the photoperiod or the genetic background. (A) Vulnerability curves of WT Col-0 plants grown under short days (SD, open circles) show a significant difference (Student's t-test, $P < 0.05$) with that of plants cultivated under long days (LD, closed circles). (B–D) Vulnerability curves of WT plants and mutants grown under long days. Vulnerability curves of WT (closed circles) show a significant difference (Student's t-test, $P < 0.05$) with *PG* mutants (open circles) (B), but no significant differences were found with *PME3* (C) and *PME5* (D) mutants (open circles). Values are means ± SE from 2–8 stem samples, and 15–24 plants were required for each curve. Grey lines represent regression curves according to Pammenter and Vander Willigen (1998). WT, wild type.

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; Choat *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 *A. thaliana*. Vessel dimensions of *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 *A. thaliana* could then be explained by the narrow diameter of the inflorescence stem.

Other structural similarities between *A. thaliana* and woody plants include the presence of simple perforation plates and bordered pits. Simple perforation plates are characteristic of *Brassicaceae* and very common in eudicots (Schweingruber, 2006; Wheeler *et al.*, 2007). The mean pit-membrane thickness of *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 *A. thaliana* and woody angiosperms at the pit-membrane level (Choat *et al.*, 2008).

The xylem of *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 micromorphology and silicon injection data, this study assumes that the fibres in *A. thaliana* are hydraulically non-functional (Sano *et al.*, 2011). Moreover, the lack of rays in xylem of *A. thaliana* confirms observations by Chaffey *et al.* (2002), although ray cells have been detected in *A. thaliana* plants in which secondary growth was induced (Mazur *et al.*, 2012). Hence, *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 paedomophic wood features in woody double mutants of *A. thaliana* such as a continuously decreasing length-on-age curve of vessel elements and delayed periderm formation.

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 *A. thaliana* allow the screening a large number of genes putatively involved in xylem hydraulics. The irregular xylem (*irx*) *A. thaliana* mutants have already brought various insights into the biochemical and genetic aspects of xylem structure (e.g. Turner *et al.*, 1997). The *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 *A. thaliana* and they mainly belong to multigene families (Farrokhi *et al.*, 2006). Thus, *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 *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 *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 *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 *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 *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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