

## Letter

## Stem anatomy supports *Arabidopsis thaliana* as a model for insular woodiness

### Introduction

*Arabidopsis thaliana* (L.) Heynh. (Brassicaceae) is a small annual distributed in open and disturbed habitats of temperate Eurasia (O’Kane & Al-Shehbaz, 1997). It has generally been accepted that the major part of its aboveground inflorescence stem does not show secondary growth under normal growth conditions. However, treatments that stop or delay flower development, such as manual clipping of flowers (Lev-Yadun, 1994) and changing of the photoperiod from long to short days (Chaffey *et al.*, 2002), stimulate and prolong to a limited extent cambial activity in roots, hypocotyls, rosette-level short stems and/or the basal part of inflorescence stems in the Columbia (Col) wild-type accession. Likewise, other perturbations such as applying weights to the inflorescence stem (Ko *et al.*, 2004) or intra-tissue tensions (Sehr *et al.*, 2010) have a similar stimulating effect on the cambium. There is also extensive literature on plant hormones functioning as an essential stimulus in cambium initiation and activity, especially auxin (Aloni, 1987; Little *et al.*, 2002; Agusti *et al.*, 2011) and cytokinin (Matsumoto-Kitano *et al.*, 2008; Hejatko *et al.*, 2009). In addition, other hormones such as ethylene (Love *et al.*, 2009), gibberellins (Bjorklund *et al.*, 2007) and jasmonate (Sehr *et al.*, 2010) can also stimulate secondary growth. Consequently, modulated expression of wood-forming genes still present in *A. thaliana* can cause a significant increase in wood formation (Oh *et al.*, 2003; Ko *et al.*, 2004; Groover, 2005). Interestingly, at least some key genes associated with the shoot apical meristem are also expressed in the cambial zone during secondary growth (Schrader *et al.*, 2004), which might explain why there is a strong evolutionary pressure for these wood-inducing genes to be maintained in herbaceous plants (Spicer & Groover, 2010).

Compared with trees and even normal-sized shrubs, the amount of secondary growth in the inflorescence stems of the Col accession remains limited to only a few cell layers, and this hampers our understanding of the regulatory process involved in wood formation when *A. thaliana* is used as a model plant (Li *et al.*, 2006). Consequently, the search for woodier *A. thaliana* mutants, such as *cov1* (continuous vascular ring) (Parker *et al.*, 2003) and *hca* (high cambial activity) (Pineau *et al.*, 2005), and woodier transgenic plants over-expressing *AtHB8* (*Arabidopsis thaliana* homeobox) (Baima *et al.*, 2001) has had the aim of unraveling the genetic mechanism for wood formation. We recently described a double mutant (in the Col background) showing wood development throughout all stems and to a much

larger extent than any *A. thaliana* mutant described to date (Melzer *et al.*, 2008). In this double mutant, two MADS box genes, *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) and *FRUITFULL* (*FUL*), which both play a role in flowering time control, were knocked out. *SOC1* integrates signals derived from photoperiod (Abe *et al.*, 2005; Wigge *et al.*, 2005), temperature (Searle *et al.*, 2006), gibberellins and age-related signals (Wang *et al.*, 2009; Lee & Lee, 2010), while *FUL* shows only a mild flowering time effect and plays additional roles in floral meristem identity, and carpel and leaf development (Ferrandiz *et al.*, 2000; Teper-Bamnolker & Samach, 2005). In *soc1 ful* double mutants, flowering time is synergistically delayed compared with single mutants, indicating that both genes have redundant roles in flowering time control. Besides the shrub-like habit, the double mutants show a combination of perennial-like features, such as a prolonged plant lifetime of up to 18 months, the co-occurrence of active vegetative and reproductive meristems, and recurrent flowering cycles (Melzer *et al.*, 2008).

Woody *soc1 ful* double mutants have been created in a Col background, implying that the double mutant is derived from an herbaceous Col accession and must therefore be called secondarily woody. There is ample molecular phylogenetic evidence for a herbaceous ancestry of secondarily woody taxa in many flowering plant families occurring on islands (therefore often referred to as insular woodiness; for example, Bohle *et al.*, 1996; Lee *et al.*, 2005) or island-like regions such as tropical mountain peaks (Jordon-Thaden *et al.*, 2010). Furthermore, there are also wood anatomical characters – so-called pedomorphic features – that can be used to identify secondary or insular woodiness (Carlquist, 1962, 2009; Lens *et al.*, 2009; Dulin & Kirchoff, 2010). Pedomorphic or juvenile wood features resemble characters of the primary xylem that are protracted into the more mature secondary xylem (= wood) of secondarily woody species. Likewise, delayed periderm formation is sometimes considered a pedomorphic bark feature (e.g. Cumbie, 1983), although cortical photosynthesis in primarily woody trees can also lead to delayed periderm formation.

The objectives of this study were: to describe the stem anatomy of the woody *A. thaliana* mutant (Supporting Information Notes S1); to look for characters in the woody mutant stem that point to a herbaceous descent; and to compare the wood anatomy of *A. thaliana* with that of other members of Brassicaceae that develop into woody shrubs in their natural environment (Carlquist, 1971; Kowal & Cutler, 1974; Schweingruber, 2006). A strong wood anatomical similarity between the *soc1 ful* double mutant and other woody Brassicaceae would provide evidence that the induced wood formation in the mutant is triggered by the ‘normal’ genetic pathway, and this would reinforce the idea that *A. thaliana* is a valid model organism with which to study the genetic mechanism behind wood formation in insular eudicots.

## The boundary between herbaceousness and woodiness is vague

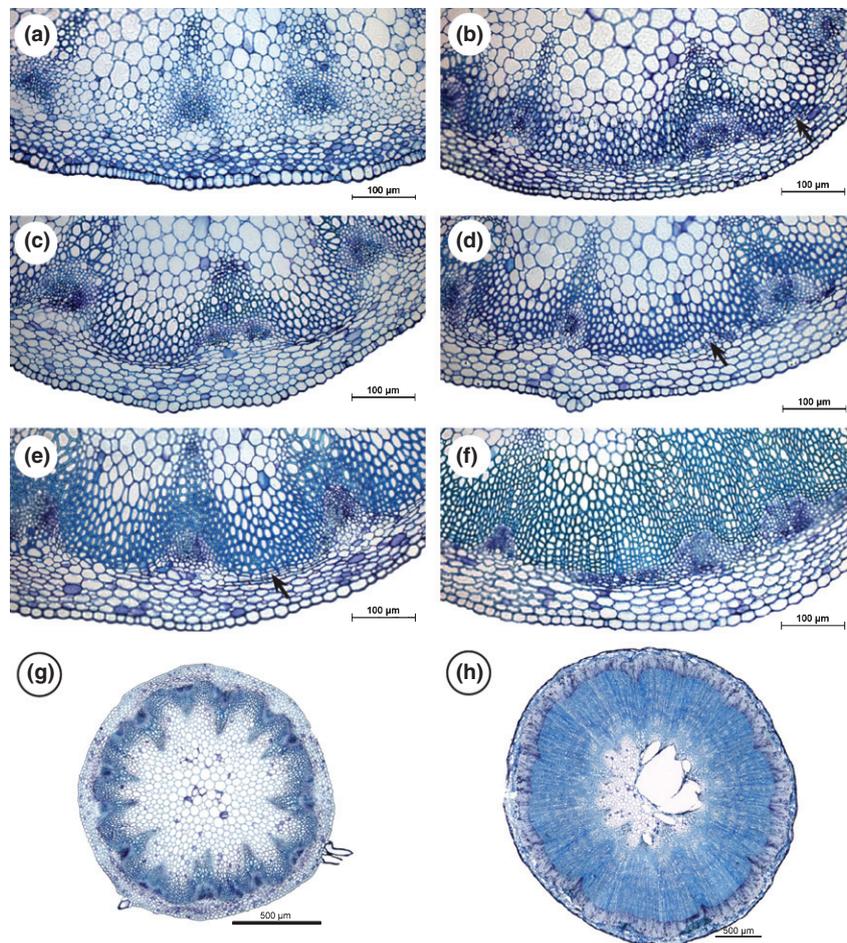
We found that basal inflorescence stems of Col plants expressed a limited amount of cambial activity in the intrafascicular regions (Fig. 1a,c,e,g), and started to develop an incomplete cambium ring in the interfascicular regions when inflorescence stems were between 15 and 20 cm in length (Fig. 1e). This limited secondary growth has been described before, although intraspecific variation exists: some studies have shown a whole ring of vascular cambium producing a few cell layers of wood in the basal stem parts, and cambial activity may reach up to 7 mm above the basal rosette in 30-cm-long inflorescence stems of the Col accession (Sehr *et al.*, 2010). Nonetheless, this increased cambial activity remains limited compared with the woodier *soc1 ful* mutant (Fig. 1f,h) and can be linked to the more juvenile stages (Fig. 1b,d). The presence of secondary tissues in the Col accession agrees with numerous anatomical studies on herbaceous plants showing a continuous range of limited wood formation in

basal stem parts (cf. Dulin & Kirchoff, 2010; references cited therein). Therefore, the boundary between the terms ‘herbaceousness’ and ‘woodiness’ is fuzzy from an anatomical point of view.

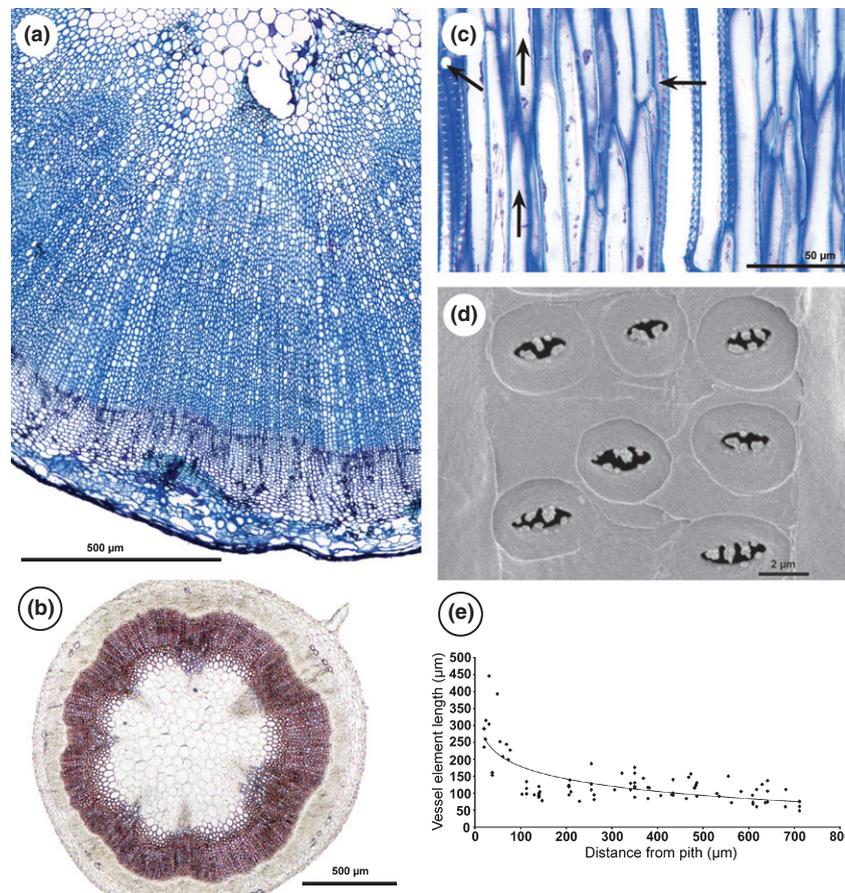
While the Col accession showed negligible wood development in the inflorescence stems, the double mutant can be considered a truly woody shrub (Melzer *et al.*, 2008). However, roots of the double mutant did not show an increase in secondary growth to the same extent as in stems, suggesting that other key regulatory genes are involved in wood formation within roots. The increasing difference in wood development with age between the aboveground and underground tissues of the double mutants does probably inhibit the growth of these plants for several years.

## Striking wood anatomical similarity between *A. thaliana* and other members of Brassicaceae

The microscopic wood structure of the *soc1 ful* double mutant was found to be remarkably similar to that of the other (secondarily) woody Brassicaceae investigated (Figs 2, S1 and Notes S1),



**Fig. 1** Transverse sections of a kinetic series representing the *Arabidopsis thaliana* Columbia (Col) accession (a, c, e, g) and the *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1/FRUITFULL* (*soc1 ful*) double mutant (b, d, f, h); plants were grown on soil in growth chambers in long-day conditions (16 h light : 8 h dark) at 22°C, and sectioned using LR White embedding (hard grade; London Resin, Reading, UK). (a, b) Sections made at 5 cm height and at the base (c, d) of a 6-cm-long inflorescence stem; (e, f) sections made at the base of a 20-cm-long inflorescence stem; (g, h) sections made at the base of fully mature stems. (a) No secondary growth visible. (b) Interfascicular cambium is forming (arrow). (c) No secondary growth visible. (d) Interfascicular cambium (arrow) producing a few layers of new cells; the wood cylinder is still incomplete. (e) Interfascicular cambium is forming (arrow). (f) The complete wood cylinder has been formed. (g) Overview of a mature Col stem with negligible secondary growth. (h) Overview of a fully mature *soc1 ful* stem illustrating extensive secondary growth.



**Fig. 2** Selected anatomical characters in mature wood of the *Arabidopsis thaliana* SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1/FRUITFULL (*soc1 ful*) double mutant: (a, b) transverse sections, (c) a radial section, and (d) a tangential section. (a) Detail showing the absence of rays and limited periderm formation. (b) A relatively young stem stained with phloroglucinol showing lignified wood. (c) Wood composed of narrow vessels with simple perforations (oblique arrow), fibres (vertical arrows), and axial parenchyma (horizontal arrow). (d) Vestured intervessel pits. (e) Flat length-on-age curve of vessel elements.

showing notably short vessel elements (usually < 200 µm), exclusively simple vessel perforations (Figs 2c, S1b), solitary vessels in combination with vessels arranged in (often radial) multiples, vestured intervessel pitting (Figs 2d, S1d), axial parenchyma adjacent to vessels, and fibres with simple pits or with reduced pit borders (Carlquist, 1971; Kowal & Cutler, 1974; Schweingruber, 2006). Noteworthy was the presence of vestured pits in the double mutant and other woody Brassicaceae genera. Within the order Brassicales, vestures are present in only a few families, including Brassicaceae and its two most closely related families, Capparaceae and Cleomaceae, which re-emphasizes the informative phylogenetic value of vestured pits at high taxonomic levels (Jansen *et al.*, 2001, 2004). One of the minor anatomical differences in wood structure between *soc1 ful* mutants and other woody Brassicaceae was the absence of rays (Fig. 2a) vs the presence of multiseriate rays, including predominantly square to erect ray cells (Fig. S1a,c). This distinction is only a minor one, however, and has been observed in many secondarily woody taxa (Carlquist, 1962, 2009; Lens *et al.*, 2007, 2009). Furthermore, it is known that rayless species with relatively thin stems, such as *A. thaliana*, may develop rays at a later stage when the stem reaches larger diameters (Carlquist, 1970).

The fact that the wood anatomical characters of the *A. thaliana* double mutant were nearly identical to the anatomy of other woody Brassicaceae strengthens our assumption that inactivation of both the *SOC1* and *FUL* genes triggers the 'normal' pathway leading to increased wood formation. The observed anatomical similarity supports the hypothesis that the genetic mechanisms underlying the developmental processes of wood development may potentially be highly conserved between woody and herbaceous plants (Kirst *et al.*, 2003; Schrader *et al.*, 2004; Groover, 2005; Spicer & Groover, 2010), implying that knock-out mutants would be perfect models for secondary growth and wood developmental research (Chaffey *et al.*, 2002; Nieminen *et al.*, 2004).

### Paedomorphic wood features in the *soc1 ful* mutant and other woody Brassicaceae indicate herbaceous descent

A second strong anatomical argument for the activation of the 'normal' genetic pathway leading to extensive wood formation in the double mutant is the expected occurrence of two paedomorphic wood features, that is, the absence of rays (Figs 1h, 2a) and

the continuously decreasing length-on-age curve of vessel elements (Fig. 2e); the delayed periderm formation in the double mutant (Fig. 2a) might be considered a third pedomorphic bark feature, and is also present in some woody Brassicaceae (Fig. S1a). According to Carlquist (1962, 2009), the most convincing wood character that identifies secondarily woody species is the flat or continuously decreasing length-on-age-curve of vessel elements: after a strong decrease in vessel element length in the primary xylem and first formed secondary xylem from 450 to 150  $\mu\text{m}$ , the length stabilizes and shows an extremely low value of *c.* 100  $\mu\text{m}$  (Fig. 2e). A similar situation was observed in the three other woody Brassicaceae species studied (Fig. S1e). Although we are aware that the maximal stem diameter in our double mutant might not be large enough (5 mm) to reach full maturity in its wood structure, we consider Fig. 2(e) a representative length-on-age curve. This is based on Bailey (1920), who demonstrated that the range of vessel elements remains almost constant in the secondary xylem in species that are characterized by so-called derived vessel elements, that is, very short vessel elements (< 300  $\mu\text{m}$  on average) combined with exclusively simple perforations (cf. Carlquist, 1962). A hydraulic explanation of the variation in vessel element length during secondary growth has not been forthcoming: it is assumed that the length of entire vessels is more important in the water transport mechanism of plants, and greatly overwhelms the importance of vessel element length (e.g. Lens *et al.*, 2011).

Although Brassicaceae are a predominantly herbaceous family, truly woody genera – representing shrubs and even lianas – are scattered over at least 12 tribes of all major lineages in the latest molecular phylogenies (Beilstein *et al.*, 2006; Franzke *et al.*, 2009, 2011; Couvreur *et al.*, 2010; Warwick *et al.*, 2010). This supports our observation of pedomorphic wood features indicating secondary woodiness in Brassicaceae (Appel & Al-Shehbaz, 2003). Also the more fine-scale molecular phylogenies at the genus level demonstrate that the (secondarily) woody species are derived from their herbaceous relatives in, for instance, *Crambe* (Francisco-Ortega *et al.*, 1999), *Lepidium* (Mummenhoff *et al.*, 2001) and *Draba* (Jordan-Thaden *et al.*, 2010).

## Conclusions

The *soc1 ful* double mutant of *A. thaliana* produced substantial secondary growth throughout all aboveground stems, whereas in the Col accession only a few cell layers of wood were produced at the base of old stems. This increased wood formation may be linked to inactivation of the flowering time genes *SOC1* and *FUL* (Melzer *et al.*, 2008). These two genes could play a crucial regulatory role in triggering genetic mechanisms that lead to insular woodiness outside *A. thaliana*, and this ‘simple’ two-gene loss could explain why many nonrelated herbaceous families have independently evolved into woody insular lineages throughout the world. However, *SOC1* and *FUL* might not be the only suppressors of cambium formation; other upstream, downstream or parallel-acting (positive or negative) regulators could be even more important. Therefore, it is evident that many missing pieces of this intriguing genetic puzzle leading to insular woodiness in

eudicots need to be found before we can better understand one of the most fascinating developmental aspects of plants (cf. Lev-Yadun, 1994; Chaffey *et al.*, 2002; Nieminen *et al.*, 2004).

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## Supporting Information

Additional supporting information may be found in the online version of this article.

**Fig. S1** Selected anatomical characters in mature wood of Brassicaceae that become shrubby in their natural environment.

**Notes S1** Description of mature wood in the *soc1 ful* double mutant of *Arabidopsis thaliana* (Figs 1h, 2).

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**Key words:** *Arabidopsis thaliana*, Brassicaceae, cambium formation, insular woodiness, model plant, secondary woodiness, wood anatomy, wood formation.



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