

Cell Wall Remodeling during Wood Development

Wood formation occurs via radial proliferation of vascular cambium cells and the deposition of secondary cell wall layers (Déjardin et al., 2010). This is crucially important for the mechanical stability of the plant and also affects the chemical and mechanical properties of wood, paper, and other ligno-cellulosic products.

An important focus area for cell wall research is to understand better how cell wall composition relates to cell expansion and cell wall mechanical properties. The hemicellulose xyloglucan is one of the major structural components of cell walls and noncovalently bonds with cellulose within cell walls. However, the role of xyloglucans in primary cell walls has been particularly hotly debated, as double mutants of the only two xyloglucan synthesis enzymes in *Arabidopsis* (*Arabidopsis thaliana*) show fairly mild phenotypes (Cavalier et al., 2008). As a result, some have proposed alternative models for primary cell wall composition, suggesting that xyloglucan forms more limited connections with cellulose (Cosgrove 2014). This raises the question as to the function of xyloglucan within cell walls.

In this issue of *Plant Physiology*, Kushwah et al. (2020) investigate the role of xyloglucans in secondary cell wall formation by examining two *Arabidopsis* xyloglucan remodeling enzymes that are expressed in vascular cambium tissue. Intriguingly, they find that these genes act noncell-autonomously in xylem fiber development.

When cell walls are remodeled, for example during cell expansion or in response to stress, linkages between cell wall polysaccharides must be undone and reformed. XYLOGLUCAN ENDOTRANSGLYCOSYLASE/HYDROLASES (XTHs) are a class of enzymes that perform this function, i.e. they cleave xyloglucan chains and reattach the cleaved end to a new xyloglucan poly- or oligosaccharide (Rose et al., 2002).

In aspen (*Populus tremula*), several XTHs have been previously identified that are expressed in vascular cambium (Sundell et al., 2017). To study their function in greater detail, the authors selected two *Arabidopsis* XTHs (*XTH4* and *XTH9*) based on sequence similarity to the aspen genes and based on expression pattern (Fig. 1).

Using histology, spectroscopy, and enzymatic assays, the authors investigated a range of phenotypes in *Arabidopsis* lines with mutations in the two XTH genes and in a line overexpressing an aspen XTH. They found that the two *xth* mutants exhibited reduced xyloglucan endotransglycosylase activity, while the overexpressor line showed an increase. Furthermore, the mutants had increased hypocotyl diameters, but the overexpressor had narrower hypocotyls.

Not all phenotypes showed the straightforward opposite effects in mutants versus overexpressor. For example, plant height was greater than wild type in all mutants as well as the overexpressor and the number of cell wall layers showed interesting tissue-dependent effects that varied with the single *xth* mutants, the double mutant, and the overexpressor (Fig. 1). The authors neatly demonstrated that the number of cell wall layers increased with increasing cellulose content but not with lignin content. Furthermore, changes to lignin content in the *xth* mutants compared to wild type were clearly observed in interfascicular fibers. Given that XTHs are not expressed in these tissues, it appears that XTHs act noncell autonomously.

A particularly interesting finding was that *XTH4* and *XTH9* appear to regulate vessel fiber cell length, primarily by affecting intrusive growth. XTHs have not previously been associated with intrusive growth, so this represents an important area for future research.

The impact of altered xyloglucan endotransglycosylase activity on intrusive growth and the noncell-autonomous phenotypes indicated that structural or mechanical features may be altered compared to wild-type plants. For other *Arabidopsis* XTHs, overexpression has led to altered mechanical properties and thicker cell walls that were thought to be an indirect result of increased cellulose deposition (Miedes et al., 2013). To gain further insight

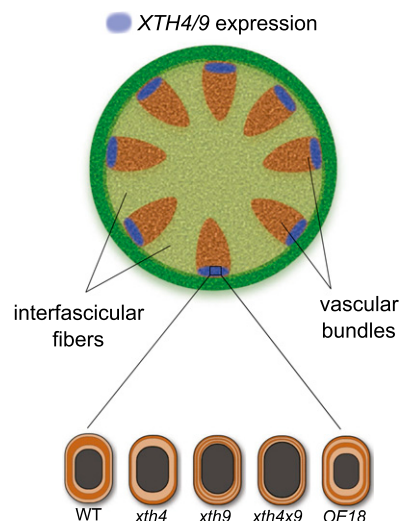


Figure 1. Schematic illustrating a radial stem section and cell wall characteristics of different transgenic lines. Blue shading indicates regions where *XTH4* and *XTH9* are expressed. Cells illustrated below indicate approximate numbers of cell wall layers and cell wall thickness. Wild-type (WT) and single mutants are as indicated; *xth4x9* refers to a double mutant of *xth4* and *xth9*; and *OE18* refers to an *Arabidopsis* line expressing a hybrid aspen (*P. tremula* × *Populus tremuloides*) XTH under the 35S promoter. Figure produced by M.S.

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www.plantphysiol.org/cgi/doi/10.1104/pp.20.00260

into possible indirect effects of modified *XTH* expression on cell wall properties, Kushwah et al. (2020) investigated the expression of genes involved in cell wall integrity sensing. They found that genes encoding *THESEUS*, *FERONIA*, and *WALL-ASSOCIATED KINASES* all exhibited altered expression in either the *xth* mutants or the *XTH* overexpressor. This indicates that cell wall integrity signaling may mediate some of the phenotypes observed.

The authors have identified some key functions of *XTH4* and *XTH9* and established their role in regulating xyloglucan within vascular cambium. Additionally, they have investigated more wide-reaching implications of altering xyloglucan linkages and highlighted the complex relationships between *XTHs* and cell walls, potentially reflecting feedback among cell wall deposition, composition, and remodeling within and between tissue types. It will be interesting in the future to understand further how intrusive fiber growth is regulated by *XTH* activity and the mechanism behind the noncell-autonomous effects on cell walls. Other recent work in the shoot apical meristem found that functional microtubule dynamics were particularly important when xyloglucans were perturbed (Zhao et al., 2019). This suggests that examination of the cytoskeleton in vascular cambium may be fruitful for understanding the interactions among *XTHs*, xyloglucans, and other cell wall components.

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