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Seasonal development of cambial activity in relation to xylem formation in Chinese fir

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ABSTRACT

The vascular cambium is a lateral meristem which can differentiate into secondary phloem and xylem. The secondary growth of woody plants resulting from vascular cambium activity has been a focus of considerable attention, but the quantitative relationships between cambial activity and secondary xylem formation have been little studied. Our analysis of cytological changes in the cambium of Chinese fir (*Cunninghamia lanceolata*), revealed a significant positive correlation between vascular cambium cell numbers and cambium zone width through the seasonal cycle. Cambium cell numbers and the cambium cell radial diameter were closely related to xylem formation. Immuno-labeling showed that de-esterified homogalacturonan and (1–4)-β-D-galactan epitopes were highly abundant in cell walls of dormant-stage cambium, whereas high methylesterified homogalacturonan was strongly labeled in the active stage. Raman spectroscopy detected significant changes in the chemical composition of cell walls during the active-dormant stage transition. More pectin and less monolignols occurred in radial cell walls than in tangential walls during the dormant stage, but no significant changes were found in other stages, indicating that pectin accumulation facilitates cell wall expansion, with cambium activity transition. Our quantitative analysis of the relationship between cambial activity and xylem formation, as well as the cell wall modification during the active stage provides useful information about cambial characteristics and xylogenesis.

1. Introduction

Cumulative increase in height and girth proceed through the entire ontogenetic process of trees. Increases in tree girth are generated by the activities of the vascular cambium, which produces secondary xylem and phloem (Rossi et al., 2012; Zhang et al., 2014). In northern temperate regions, the vascular cambium of woody plants has a seasonal cycle of activity and dormancy (Catesson, 1994; Gričar et al., 2014). The initiation, duration, and cessation of vascular cambium activity are usually decisive factors in determining the quantity and quality of wood. As the site of secondary growth in trees, the vascular cambium has been a focus of considerable attention in recent decades (Aref et al., 2014; Arend and Fromm, 2013; Farrar and Evert, 1997a).

Vascular cambium activity is strongly seasonal at high latitudes (Deslauriers et al., 2009; Rossi et al., 2012; Savidge, 2001), and many studies have examined the regulation of temporal activity in this meristem. For example, the effects of hormones, photoperiod, moisture, soil nutrients, and temperature have been largely investigated (Begum et al., 2013; Druart et al., 2007; Ursache et al., 2013). Multiple investigations have examined the influence of these factors on variations in cell morphology in the secondary xylem (Patel et al., 2014; Prislan et al., 2013). Transmission electron microscopy (Catesson, 1994; Farrar and Evert, 1997b), biochemical procedures (Chu-Bâ et al., 1994; Prislan et al., 2013), and fluorescence microscopy (Chen et al., 2010) have been used to track the dynamics of vascular cambium cells and their walls during the ontogenetic process. However, little information is available on the quantitative relationship between cambial activity and xylem formation. We studied this relationship through the seasonal cycle.

Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook.) is one of the fastest growing tree species within its primary distribution range across southern China and northern Vietnam (Qiu et al., 2013),

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where it is used for timber and eco-environmental protection purposes. Previous studies have largely focused on the regulation of cambial activity in this species at the molecular level (Qiu et al., 2013; Wang et al., 2013) to provide detailed information on cambial differentiation and wood-forming candidate genes.

As a continuation of our previous studies on Chinese fir (Qiu et al., 2015, 2013), the present work investigated cytological changes in cambium cells, the modifications of cell wall architecture and composition, and xylem formation using light microscope, immuno-labeling, and Raman spectroscopy. Our overall aim was to determine the relationships between cambial activity and xylogenesis, which is useful for further investigations into the developmental control of wood formation.

2. Materials and methods

2.1. Plant material

We selected healthy 13-year-old Chinese fir trees with straight boles and similar diameters at breast height within a stand at the Forestry Station in the Minhou District of Fujian Province (26°12′N, 119°04′E; Fujian, China). Samples were collected from the trees twice a month from March through August, and once a month from August through December 2014. We divided seasonal cambial activity anatomically into three developmental stages: reactivating (March) and active (April–November) stages, dormant (December–February), following previous concepts (Qiu et al., 2013; Yu, 1988).

On each sampling occasion, we collected specimens each measuring 10 × 5 × (10–20) mm (length × width × depth) and containing phloem, cambium, and the outermost xylem rings from four tree trunks (1.3 m above ground level). To reduce wounding effects, neighboring samples were spaced at intervals of ≥10 cm; thus, each new sample contained no tissue with trauma damage or wound-wood, which can form as a response to mechanical damage of the cambium (Gričar et al., 2007). The samples from the trees were immediately fixed in 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer (PBS, pH 7.2). After vacuum treatment, the blocks were fixed in fresh fixative and preserved at 4 °C until further analysis.

2.2. Preparation of samples for light microscopy

The chemically-fixed blocks were washed in 0.1 M PBS (pH 7.2) four times, dehydrated in a graded ethanol series (30, 50, 60, 70, 80, 90, 95, and 100%) and acetone, after which they were embedded in Spurr's resin (Sigma, USA) for light microscopy. Transverse radial sections (2 μm thick) were cut using a rotary microtome (Leica-RM2265, Germany), stained with an aqueous solution of 1% toluidine blue (Sigma, USA) for light microscope observation (Feder and O'Brien, 1968), and viewed under a Zeiss Axioskop 2 Plus microscope equipped with a computer-assisted digital camera.

2.3. Immuno-labeling of pectin epitopes

Specimens collected on each sampling occasion were embedded in LR White resin (Sigma, USA) after a standard dehydration procedure. Transverse sections 2 μm thick were cut with Leica microtome. After washing thrice in PBS, the sections were blocked for 45 min with 1% (w/v) bovine serum albumin in 0.1 M PBS at room temperature. The primary monoclonal antibodies were diluted (1:10 v/v for JIM5 and JIM7; 1:4 v/v for LM5 and LM6) in PBS and applied to the sections overnight at 4 °C. Subsequently, we washed the sections thrice for 10 min in PBS, and incubated them with the secondary antibody for 2 h at room temperature. The secondary antibody, fluorescein isothiocyanate- (FITC)-conjugated goat-anti-mouse IgG (Sigma-F6258, USA), was diluted (1:100) in PBS. After rinsing the sections thrice in PBS and once in double-distilled water (ddH_2O), we mounted them in a mixture (1:1) of PBS and glycerol, and examined them by epifluorescence microscopy (Olympus-CX31, Japan) using standard filter combinations for FITC (excitation filter: 488 nm; barrier filter: 522 nm).

We applied the four primary antibodies as described in previous studies. JIM5 labels the low relatively de-esterified homogalacturonan epitope (Clausen et al., 2003). JIM7 indicates the presence of relatively high methylesterified homogalacturonan epitope (Clausen et al., 2003). LM5 and LM6 were raised against short-chain linear oligomers of (1–4)-β-D-galactan (Jones et al., 1997) and (1–5)-α-L-arabinan (Willats et al., 1998), respectively.

2.4. Raman spectra and Raman mapping

Raman spectra and Raman maps were acquired with an XploRA confocal Raman system (Horiba Jobin Yvon, USA) equipped with an Olympus BX51 inverted confocal microscope. Using a Leica microtome (Leica-RM2265, Germany), we cut 2 μm thick transverse sections of active (May 30) and dormant stages (December 17) without further sample preparation. Each cut section was immediately placed on a glass slide and immersed in a drop of distilled water beneath a coverslip sealed with nail polish to avoid evaporation of water during measurements. A 100× oil immersion microscope objective (Nikon, NA = 1.25) and a laser in the visible wavelength spectrum (k = 532 nm) were used for the measurements.

We used the LabSpec5 software package (Horiba Jobin Yvon SAS, France) for measurements and data processing. The spectra were baseline-corrected and smoothed with the Savitsky–Golay algorithm for spectroscopic analysis to reveal the fine structures of the tissues. We selected eight points randomly in both radial and tangential walls of each cell. The chemical images were obtained by integrating over-defined wave numbers of a series of spectra. The raw data were baseline-corrected and de-spiked. Smoothing filtration was undertaken with the Savitsky–Golay algorithm set at a moderate level and Fourier smoothing coupled with a cosine apodization function. Image contrast was further improved using the histogram contrast routine in Origin Pro 8 software (Microsoft, USA).

2.5. Anatomical observations and statistical analysis

Three tree trunks were sectioned for anatomical observations and statistical analysis at each time point. Two sections of every tree trunk were observed and 15 radial cell files were measured on each section. We used a light microscope (Leica-DM2500B, Germany) and Image J software (National Institutes of Health, MD, USA) to measure cambium cell numbers, radial and tangential diameters of cambial cells, cambial zone width, and numbers of immature and mature xylem cells, as described by Cui et al. (1992) and Mwange et al. (2003). Cambial cells and immature xylem cells were identified by their primary walls; mature xylem cells were identified by their secondary walls, which had strong birefringence under polarized light (Riding and Little, 1984).

Pearson's correlation (R) coefficients were calculated using SPSS 18.0 software to detect significant relationships in the data. Duncan's new multiple range test was used for pairwise comparisons between means of samples from six trees in order to detect statistically significant differences ($P < 0.05$).