TUBULINS, RHYTHMS AND CELL WALLS IN POPLAR LEAVES: TIMING MIGHT BE EVERYTHING

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We have recently reported on transgenic poplars in which the cell wall structure of xylem was changed due to tubulin manipulation (Swamy et al., 2015). While pleiotropic effects on plant development or morphology were not observed, stomatal closure in response to drought, and stomatal opening in response to light were impaired. The present study was undertaken to measure the impact of tubulin manipulation on leaf primary cell wall composition. A model is discussed in which constitutive over-expression of tubulins interfered with diurnal control of pectin and hemicellulose accrual, thereby altering primary cell wall composition and flexibility.

Alcohol-insoluble cell wall residue of source leaf tissue was sequentially extracted with different solvents to remove pectins, hemicellulose-linked pectins, hemicellulose xylans and other more tightly bound hemicelluloses. The yield of oxalate-extractable pectins was highest in the transgenic line with the highest accumulation of ectopic tubulin protein. The apparent pectin increase in transgenic leaves occurred at the expense of xylose. RNA-Seq analysis revealed that the expression of key genes controlling the partitioning of UDP-glucuronic acid for UDP-xylose (hemicellulose) and UDP-galacturonic acid (pectin) was slightly reduced in the transgenic lines. Ratios of their transcript levels were significantly perturbed compared to the ratios in WT plants in a way that would favor UDP-galacturonic acid formation. The normally oscillatory pattern of changes in the expression ratio of these genes in *Populus* (Filichkin et al., 2011) suggests sensitivity of UDP-glucuronic acid utilization to circadian control. We hypothesize, and there is literature support, that tubulin protein polymers (microtubules) facilitate the delivery of pectic polysaccharides from the golgi network to the cell wall. We suggest that constitutive overexpression of tubulin subunits in the transgenic lines favored utilization of UDP-glucuronic acid for pectic polysaccharides during the day when hemicellulose biosynthesis is less active. As a result, the availability of UDP-glucuronic acid for UDP-xylose synthesis at night was reduced.

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