204. Dual Effect of Tubulin Manipulation on Populus Wood Formation and Stomatal Behavior

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Project Goals

Cortical microtubules play important roles in the regulation of plant morphogenesis, cell wall biogenesis and stomatal behavior, presumably by governing cellulose microfibril orientation. However, genetic manipulation of tubulins often leads to abnormal plant development, making it difficult to investigate the underlying structural bases. This project developed multiple suites of transgenic Populus with altered alpha- (TUA) and beta-tubulin (TUB) expression and/or post-translational modifications (PTMs), either singly or in combination, in a plant-wide, guard cell-specific or xylem-specific manner. We showed that it is feasible to obtain morphologically normal transgenic Populus with altered tubulin expression and/or PTMs. However, biased expression between TUA and TUB transgenes, as well as between leaves and xylem was evident, regardless of the promoter used. While transgene over-expression was consistently achieved in leaves, transgene expression was low in xylem, suggesting that high levels of tubulin transgene expression were not tolerated in vascular tissues during regeneration of transformants.

Counterintuitive to the proposed role of microtubules in directing microfibril deposition, cellulose content and microfibril angle were not changed in transgenic wood, nor were hemicelluloses and lignin. However, glycome profiling revealed increased cell wall glycan extractability, especially for lignin-bound pectin and xylan polysaccharides. This was corroborated by increased expression of cell wall-modifying enzymes in xylem based on RNA-Seq analysis, and by altered syringyl-to-guaiacyl monolignol ratio in the transgensics. A subset of transgenic plants exhibited prolonged epidermal cell expansion and reduced structural pectin and xyloglucan extractability in mature leaves. The results suggest that growth-compatible, mild perturbations of tubulins affected primarily noncellulosic polysaccharides, especially pectins, in transgenic Populus. Guard cell responses to drought and light were slower in the transgenic than wild-type plants. The findings were less consistent with an effect of tubulin perturbations on microtubule stability, but more consistent with an effect of altered pectin networks on cell wall flexibility critical for reversible stomatal movement. Our work so far suggests that pectin networks are more sensitive than cellulose to subtle tubulin perturbation, and that the tubulin effects on pectin incorporation may be conditioned by the degree of lignification in xylem and leaves during cell wall biogenesis.

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