Engineering naringenin into the lignins of transgenic poplar

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Project Goals: Modifying the content and composition of lignin in poplar by diverting carbon away from the phenylpropanoid pathway and towards the production of naringenin, a valuable flavonoid, in lignifying tissues.

Lignin, a major chemical constituent of lignocellulosic biomass, is an important strategic target for genetic modification as this polyphenolic polymer poses a significant barrier to efficient industrial processing. Gain of function approaches that improve processing efficiency as well as add value to biomass can play a key role in development of cost-effective biomass processing methods. Lignin is typically composed of three canonical monolignols that undergo oxidative radical coupling to form lignin in the cell walls of developing xylem tissue. Efforts to genetically engineer the monolignol biosynthetic pathway have led to significant changes in both content and composition of lignin, highlighting the remarkable metabolic plasticity of lignin biosynthesis. Moreover, compounds found beyond the monolignol pathway, such as flavonoids and stilbenes, have been found naturally incorporated into lignins of various plant species.[1,2] Chalcone synthase (CHS) catalyzes the first committed reaction in the production of flavonoid compounds by combining *p*-coumaroyl-CoA, a precursor in the monolignol biosynthetic pathway, with three malonyl-CoA units to produce naringenin chalcone which is then cyclized to naringenin. Using a xylem-specific promoter, we have genetically engineered hybrid poplar (*Populus alba* × *grandidentata*) to express a previously characterized chalcone synthase (*CHS*) gene (*MdCHS3*) derived from apple (*Malus* x *domestica*).[3] *MdCHS3*-poplar displayed an accumulation of naringenin in xylem methanolic extracts, not inherently observed in wild-type trees. NMR analysis revealed the presence of naringenin in the extractive-free enzyme lignin (EL), residue of cellulase-treated xylem tissue, of *MdCHS3-poplar*, indicating the novel incorporation of this flavonoid into poplar secondary cell wall lignins. The transgenic trees also displayed lower total lignin content, increased cell wall carbohydrate content, and performed significantly better in saccharification assays than their wild-type counterparts. Moving forward, *MdCHS3*-poplars represent a useful genetic background into which new flavonoid biosynthetic enzymes may be introduced in order to produce other valuable lignin-compatible flavonoids.

References

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