A novel laccase enzyme facilitates engineering of C-lignin

Background

- Catechyl lignin (C-lignin) is a linear homopolymer of caffeyl alcohol found in the seed coats of diverse plant species. Its properties make it a natural source of carbon fibers and high-value chemicals, but the mechanism of *in planta* polymerization of caffeyl alcohol remains unclear.
- Cleome hassleriana is an excellent model system for studying C-lignin biosynthesis, as the lignin produced in the seed coat switches abruptly from G- to C-lignin at around 12 days after pollination.

Conclusions

- RNA sequencing analysis identified ChLAC8 as a seed-coat specific laccase that is expressed at the onset of C-lignin biosynthesis.
- ChLAC8 has the unique property of oxidizing caffeyl alcohol.
- The protein structure of ChLAC8 possesses a unique glutamine residue (Q289) in the active site that stabilizes caffeyl alcohol binding.
- ChLAC8 is necessary and sufficient for the synthesis of C-lignin from supplied caffeyl alcohol in transgenic *Arabidopsis thaliana*.

Significance

- LACCASE8 plays a critical role in initiating C-lignin polymerization.
- Given a successful strategy for engineering sufficient levels of caffeyl alcohol, ChLAC8 can be an important component of a gene toolkit for engineering of C-lignin into vegetative tissues of commercial biomass crops such as switchgrass and poplar.
- This work also demonstrates that individual laccase specificity, in addition to availability of the required monolignol building blocks, can control the composition of lignin.



The predicted residues and hydrogen bonds (dashed lines) involved in caffeyl-alcohol positioning are indicated.

Wang, X et al. (2020). Substrate-specificity of LACCASE 8 facilitates polymerization of caffeyl alcohol for C-lignin biosynthesis in the seed coat of *Cleome hassleriana*. *Plant Cell*.

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