Characterization of a bifunctional C3H enzyme revises the currently accepted model of lignin biosynthesis in model plants

Background

Lignin is an abundant plant cell wall polymer with great importance for bioproduct production. The biosynthesis of lignin is considered to be highly conserved among higher plant species. However, increasing evidence questions the established enzymatic pathway to lignin formation in vascular plants.

Approach

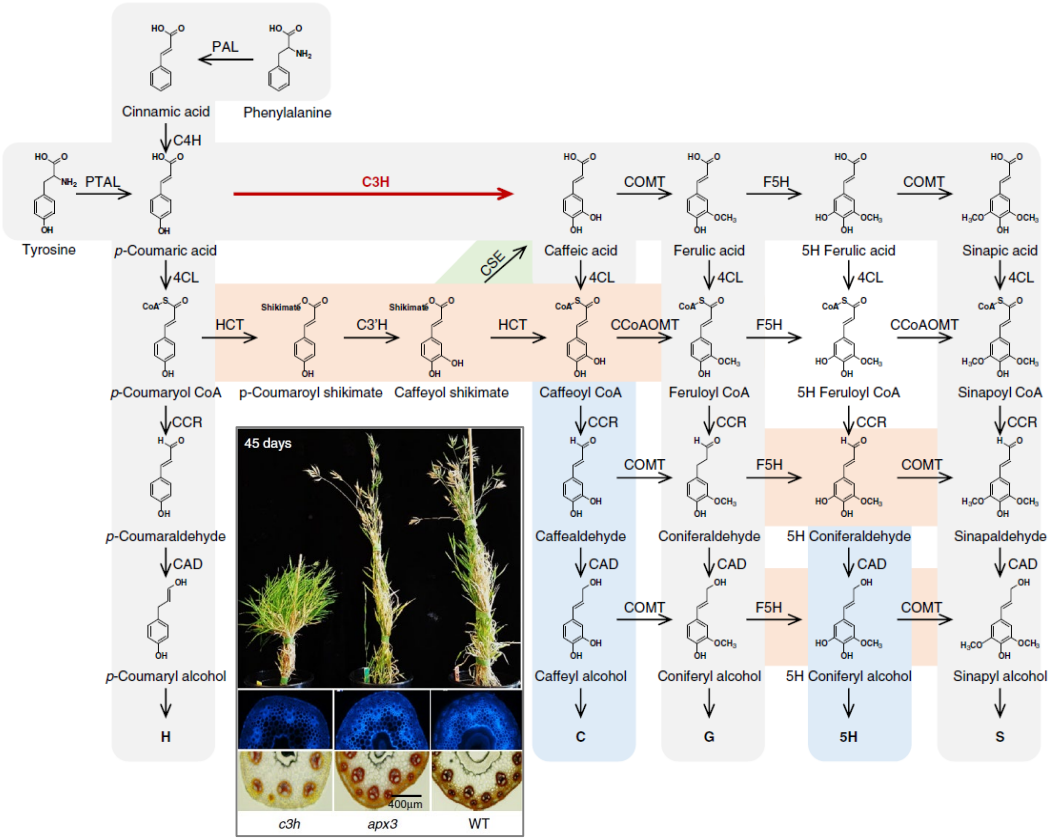
- We used biochemical and genetic approaches in model plant species, to prove a that a **coumarate 3-hydroxylase** (**C3H**) enzyme provides an alternate route to lignin monomer production from the phenolic precursor **4-coumarate**

Outcome

- The enzyme catalyzing the direct 3-hydroxylation of 4-coumarate to caffeate is a bifunctional peroxidase that oxidizes ascorbate and 4-coumarate at comparable rates.
- Plants with mutant C3H showed impaired growth and lignin accumulation phenotypes (see inset)
- Biochemical assays support the subsequent efficient *O*-methylation (COMT) of caffeate to ferulate in grasses.

Significance

- We have finally uncovered the missing enzyme in a lignin pathway proposed over 40 years ago. This conclusive evidence for C3H's role and existence, suggests the previously characterized pathways may be less important.
- The bifunctional nature of this enzyme suggests close links between lignin synthesis and abiotic stress (ROS) — which may be important for engineering lignin in the face of a more variable climate.



The lignin biosynthetic pathway highlighting the 4-coumarate 3-hydroxylase (**C3H**) reaction (in red) characterized in this study, compared to current knowledge shaded in grey, brown and green. Inset: impaired growth phenotype and lignin phenotype of *c3h* mutants versus *apx3* and wild-type controls in *Brachypodium*.

Barros, J.; Escamilla, L; Song, L.; Rao, X.; Serrani, J.C.; Palacios, M.D.; Engle, N.; Choudhury, F.K.; Tschaplinski, T.J.; Venables, B.J.; Mittler, R.; Dixon, R.A. (2019) 4-Coumarate 3-hydroxylase in the lignin biosynthesis pathway is a cytosolic ascorbate peroxidase. *Nature Communications* 10(1):1994

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