New research reveals insight into lignin biosynthesis

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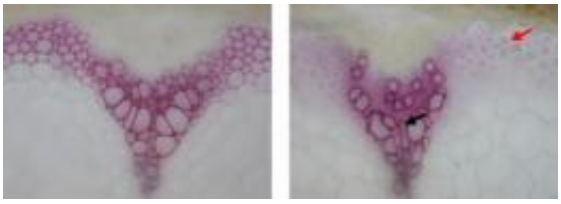

The amount of lignin (stained pink) in the stems of mutants that lack both LACCASE4 and 17 (right) is much lower than in normal plants (left). Fiber cells (red arrow), which have a supportive role, are dramatically hypolignified (no staining), while vascular cells (black arrow) have an irregular shape or are collapsed. Credit: Catherine Lapierre

Lignin is the durable biopolymer that gives carrots their fiber and crunch and meat grilled over a campfire its characteristic smoky flavor. Acting as the glue that holds the plant cell wall together, lignin imparts tremendous mechanical strength to the plant. Present in all land plants except mosses, lignin performs three important functions: it allows plants to grow upright as they compete for sunlight, it facilitates the upward movement of water and minerals through the plant's vascular tissue, and it protects plants from pathogens and foraging animals. Lignin also sequesters atmospheric carbon in its tissues and thereby plays an important role in the carbon cycle. Approximately 30% of non-fossil organic carbon is stored in lignin, and, after cellulose, lignin is the most abundant biological polymer on Earth.

Lignin consists of three phenylpropanoid subunits, G (guaiacyl), S (syringyl), and H (p-hydroxyphenyl). The precursors of these subunits are generated inside the cell and transported to the cell wall, where they are oxidized by enzymes and then join together to form lignin's highly complex and heterogeneous three-dimensional structure. Biologists have long since wondered how this process of lignification is regulated. Two families of enzymes, the peroxidases and laccases, occur in plant cell walls and have been proposed to catalyze the oxidation of lignin precursors. Whereas the involvement of peroxidases in lignification has been confirmed, that of laccases had not.

Now, a team of researchers at the Institut Jean Pierre Bourgin INRA, France, provide compelling evidence that laccases do indeed contribute to lignification in the model plant Arabidopsis (a member of the mustard and cabbage family). Seventeen laccase genes are present in Arabidopsis. Since genes involved in lignification would most likely be expressed in the stem, the researchers examined the expression of all 17 laccase genes. Two of these genes, *LACCASE4* and *LACCASE17*, were found to be strongly expressed in stems and were selected for further analysis.

The researchers then identified mutant Arabidopsis plants in which *LACCASE4* and *17* were silenced. They crossed these mutants to generate double mutants that lacked both LACCASE4 and 17 enzymes. Whereas both the single and double mutants grew normally in the greenhouse, the lignin content was slightly reduced in the single mutants and reduced by up to 40% in the double mutants. Interestingly, the reduction of lignin in the double mutant appeared to have a positive effect on saccharification, the process whereby sugars are liberated from plant biomass. Given that resistant cell walls represent a major obstacle in the production of biofuels, this finding may have useful applications in the biofuel industry.

The scientists went on to demonstrate that disruption of *LACCASE17* specifically reduced the incorporation of G subunits into the lignin of fiber cells and that introducing an intact version of the *LACCASE17* gene into lines that contained a mutated version of this gene corrected this mistake. Thus, LACCASE17 appears to contribute to the fiber-specific deposition of G subunits into lignin. Disruption of *LACCASE4* did not affect the ratio of phenylpropanoid subunits in the stem, suggesting that this gene catalyzes the deposition of all lignin subunits equally.

This work provides strong evidence that laccases play a central role in lignification. According to Catherine Lapierre, "The genetic engineering of lignin-specific laccases is a potentially innovative and promising tool for increasing the saccharification of plant cell walls when used for the production of biofuels."

More information: Berthet, S., Demont-Caulet, N., Pollet, B., Bidzinski, P., Cézard, L., Le Bris, P., Borrega, N., Hervé, J., Eddy Blondet, Balzergue, S., Lapierre, C., and Jouanin, L. (2011). Disruption of LACCASE4 and 17 results in tissue-specific alterations to lignification of Arabidopsis thaliana stems. Plant Cell doi:10.1105/tpc.110.082792 . First Published on March 29, 2011.

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