Laccases and Lignification: Not so simple after all

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Lignin (lat. *lignum*: wood) is a hydrophobic polymer present in the cell walls of plant cells and the second most abundant bio-polymer on earth. It provides structural support, waterproofs cell walls and protects against herbivores and pathogens. It is a very heterogeneous material made up of a wide range of different monomers. Lignification of cell walls is directed by special enzymes resident within the cell wall called laccases and peroxidases. These enzymes oxidise the different lignin monomers in the cell wall to permit their polymerisation. This process was long believed to be under simple chemical control, assuming that the involved enzymes functioned redundantly, *i.e*. would be completely interchangeable. This view however is at odds with the fact that lignin composition has been shown to vary drastically in different - even adjacent - cell types.

To better understand how lignin formation permits for such differences in composition, we investigated whether the 17 different laccase variants - called isoforms - in the model plant *Arabidopsis thaliana* might have distinct roles within this process. Gene expression analysis pinpointed 6 of the 17 laccases as associated with the formation of the secondary cell wall in developing woody cells. These 6 isoforms thus were the candidates for important roles in lignification. That the remaining 11 laccases did not show this transcriptional regulatory association to secondary cell wall formation, on the other hand, already suggested distinct roles for different laccase isoforms. To detect the functions of our 6 candidate laccases, we grew *A. thaliana* plants mutated in single or multiple of these laccase isoforms. We then performed detailed morphological, biochemical and histochemical (*i.e.* stainings on the cellular level) analysis as well as enzyme activity assays of the mutant plants, defining the effects of the loss of different isoforms. These experiments revealed little effect of single mutations. Multiple mutations, however, in some case resulted in reduced growth, complex lignin composition changes, and reduced laccase activity. These deficiencies however were strongly dependent on the specific isoforms that were lost. This suggested functional redundancy for some, but not all candidate laccase isoforms. Furthermore, the loss of specific isoforms affected the capacity to oxidise different substrates distinctly in the analysed cell types.

Taken together, these results strongly suggested that the lignification of the different woody cell types in plants was directed by different sets of laccase isoforms with distinct substrate specificities. Linking the incorporation of different lignin monomers to the presence of specific laccase isoforms in the cell wall thus provides clear evidence for a lignification process which is not as random as is widely thought. Understanding the precise mode of action of laccases opens the door not only for the biotechnological engineering of wood to fit special requirements, but also for the use of laccases in bioremediation, where they are emerging as versatile scavengers of phenolic pollutants.

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