https://doi.org/10.1093/plphys/kiac414 PLANT PHYSIOLOGY 2022: 190: 2090–2093

Plant Physiology®

News and Views

A feast of consequences: Transcriptional and metabolic responses to lignin pathway perturbations

Yajin Ye [1] and Igor Cesarino [2,3,*]

1 Key Laboratory of Forest Genetics and Biotechnology, Ministry of Education of China, Co-Innovation Center for the Sustainable Forestry in Southern China, Nanjing Forestry University, Nanjing 210037, China
2 Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, 277, 05508-090 São Paulo, Brazil
3 Synthetic and Systems Biology Center, InovaUSP, Avenida Professor Lucio Martins Rodrigues, 370, 05508-020 São Paulo, Brazil

*Author for correspondence: icesarino@usp.br

Lignin is a phenolic biopolymer mainly found in secondary walls of specialized cells of supportive and water-conductive tissues. Lignin provides mechanical strength and hydrophobicity to such cells, allowing them to withstand the force of gravity and transport water and nutrients along the plant (Bonawitz and Chapple, 2010).

As products of the phenylpropanoid pathway, lignin monomers are synthesized via the deamination of phenylalanine, followed by the reduction of the carboxylic moiety of the propane tail and by the sequential methoxylation of the aromatic ring. The combined activities of more than 10 enzymes yield three hydroxycinnamyl alcohols (i.e. monolignols), *p*-coumaryl, coniferyl, and sinapyl alcohols, differing in the degree of methoxylation. Once incorporated into the polymer, these monolignols form the *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) lignin units, respectively (Ralph et al., 2019). Lignin content, composition, and the frequency of its linkage types vary according to the cell type, developmental stage, and plant species (Barros et al., 2015). Given that the investment of carbon and energy into lignin is irreversible, developmental lignification is tightly controlled at the transcriptional, post-transcriptional, and post-translational levels. Additionally, lignin deposition is also triggered in response to various biotic and abiotic stresses (Cesarino, 2019).

Several studies have shown that lignin content and composition can be altered via misregulation of lignin biosynthetic genes. However, these pathway perturbations not only result in altered lignification but also cause shifts in both primary and secondary metabolism and in global transcriptional reprogramming (Rohde et al., 2004; Dauwe et al., 2007; Vanholme et al., 2012; Bonawitz et al., 2014; Saluja et al., 2021; Ferreira et al., 2022). Moreover, lignin pathway perturbations often have far-reaching effects on other biochemical routes, affecting diverse biological processes that are unrelated to lignin metabolism. However, given that most of the studies have focused on a few individual genes in different species, the major regulatory mechanisms underlying the molecular responses to altered lignification remain largely unresolved. For instance, it is unclear whether blocking particular steps in monolignol biosynthesis redirects the carbon flux through the phenylpropanoid pathway in a systematic way and to what extent the pathway is regulated by feedback systems. A systems biology approach based on metabolomics and transcriptomics was previously employed for single mutants in ten lignin biosynthetic steps in Arabidopsis (*Arabidopsis thaliana*; Vanholme et al., 2012). Mutants that produced less lignin upregulated shikimate, methyl-donor, and phenylpropanoid pathways (i.e. the pathways supplying the monolignols), whereas mutants with altered lignin compositions downregulated the very same pathways. Additionally, reductions in the carbon flux towards lignin were associated with the accumulation of various classes of hexosylated phenylpropanoids (Vanholme et al., 2012). Despite this valuable information, our understanding of the systemic responses to defects in lignification remains fragmented and disconnected.

In this issue of *Plant Physiology*, Wang et al. (2022) employed a systemic approach based on large-scale

Received August 05, 2022. Accepted August 14, 2022. Advance access publication September 5, 2022

transcriptomics and phenylpropanoid profiling to study the transcriptional and metabolic responses to lignin pathway perturbations in a set of 13 Arabidopsis mutants (Figure 1A). Complementary to the previous work of Vanholme et al. (2012), which employed only single mutants for different steps of the monolignol pathway, here the authors selected double mutants with a stronger deficiency in some lignin biosynthetic steps, mutants with different syringyl/guaiacyl ratios in the lignin polymer, mutants of the transcriptional regulator Mediator subunits *MED5a* and *MED5b* (*MED5a/b*), and a mutant in the glucosinolate biosynthetic pathway that has reduced levels of phenylpropanoids.

RNA-seq experiments were conducted using the basal part of inflorescence stems, a tissue undergoing active lignification, from the 13 mutants and wild-type plants. First, the authors examined the effect of the mutations on transcript levels of genes involved in phenylpropanoid metabolism. Transcript abundance of phenylpropanoid biosynthetic genes and lignin-related transcription factors increased or remained unchanged in most of the mutants, except for *cse2* (*caffeoyl shikimate esterase2*), *f5h* (*ferulate 5-hydroxylase*), and *ref2* (*reduced epidermal fluorescence 2*), in which the same genes were downregulated. In line with the repressive role of *MED5a/b* in pathway homeostasis (Bonawitz et al., 2014), phenylpropanoid biosynthetic genes showed increased expression in genotypes containing *med5* mutations. Interestingly, genes encoding laccases and peroxidases involved in lignin polymerization were downregulated in these genotypes, suggesting that Mediator activates their expression, distinct from its role in repressing monolignol biosynthetic genes. The authors suggest that repression of lignin biosynthetic genes and activation of monolignol oxidases by MED5a/b may together suppress the accumulation of potentially toxic/bioactive soluble phenylpropanoids.

Next, the genes involved in the biosynthesis of aromatic amino acids were evaluated to determine whether phenylalanine production was affected at the transcriptional level in any of the mutants. Hierarchical clustering of the expression of the shikimate and monolignol pathway genes showed that genes from the two pathways were mixed in the clusters and were similarly upregulated in most of the mutants but downregulated in *cse2*, *f5h*, and *ref2*. The similar transcriptional responses found for shikimate and lignin biosynthetic genes in the same groups of mutants suggest that the transcription of genes involved in phenylalanine biosynthesis and in subsequent lignification is coordinated in rapidly lignifying Arabidopsis stem tissue.

Are there any cross-talks between phenylpropanoid metabolism and other biological processes? To answer this, the global impact of the 13 mutations on the transcriptome was analyzed by identifying the differentially expressed genes (DEGs) between the mutants and wild-type plants. In total, 5,581 genes were misregulated in all mutants, and these DEGs were quantitatively examined among the genotypes using Pearson's correlation analysis. Interestingly, groups of mutants with a similar number of DEGs also showed positively correlated DEG profiles, suggesting that common DEGs were similarly misexpressed. Hierarchical clustering analysis used to identify the DEGs that contributed to these correlations clustered the mutants and DEGs into five subgroups. The five mutants with fewer DEGs clustered together and were designated subgroups A and B, showing decreased transcripts for genes involved in defense responses, responses to ethylene and abscisic acid (ABA), and cold acclimation and increased transcripts for genes related to glucosinolate biosynthesis, auxin-related processes, cell wall-related processes, and photosynthesis (Figure 1B). The other eight mutants with more DEGs clustered into subgroups C, D, and E, showing downregulation of genes responsible for photosynthesis, lipid catabolism, and auxin signaling and upregulation of genes involved in various defense responses and salicylic acid, jasmonic acid, and ABA signaling (Figure 1B). These data indicate that mutations in genes of the phenylpropanoid metabolism affect other biological processes unrelated to lignin or another branch of the phenylpropanoid pathway.

To assess how the perturbations in the lignin pathway affect levels of phenylpropanoids, the authors first quantified 18 selected metabolites in the same stem tissues used for the transcriptomic analysis from all genotypes. Subsequently, they created a system-wide Pearson correlation matrix of pairwise comparisons between gene expression and metabolite content across all 14 genotypes to identify genes and metabolites with strong correlation, which would suggest the genes and metabolites are potentially functionally related in vivo. A total of 2,987 genes were identified with a significant correlation with at least one of the 18 metabolites. When hierarchical clustering analysis of the Pearson correlation coefficients was performed, these 2,987 genes were grouped into six clusters and the 18 metabolites into two major subgroups. The first subgroup comprised metabolites mostly upstream of the shikimate shunt in the lignin biosynthetic pathway, such as phenylalanine, *p*-coumarate, and *p*-coumaroyl CoA, among others. These metabolites generally showed a negative correlation with genes involved in the biosynthesis of methionine and glucosinolates and a positive correlation with genes related to cell cycle and cell division. The other subgroup included downstream pathway intermediates, together with free CoA, shikimate, and *p*-coumaraldehyde. These compounds strongly and positively correlated with genes related to the cytosolic ribosome and glucosinolate biosynthesis and negatively correlated with genes involved in cell cycle and acetyl CoA metabolism. These results indicate that the accumulation of certain phenylpropanoids upon lignin pathway perturbation correlates with genes involved in biological processes with no obvious connection with lignification.

The work of Wang et al. (2022) not only reveals the molecular responses to lignin pathway perturbations in a unique set of Arabidopsis mutants but also provides targets for the determination of functional relationships between the correlated genes and metabolites. Future analyses will

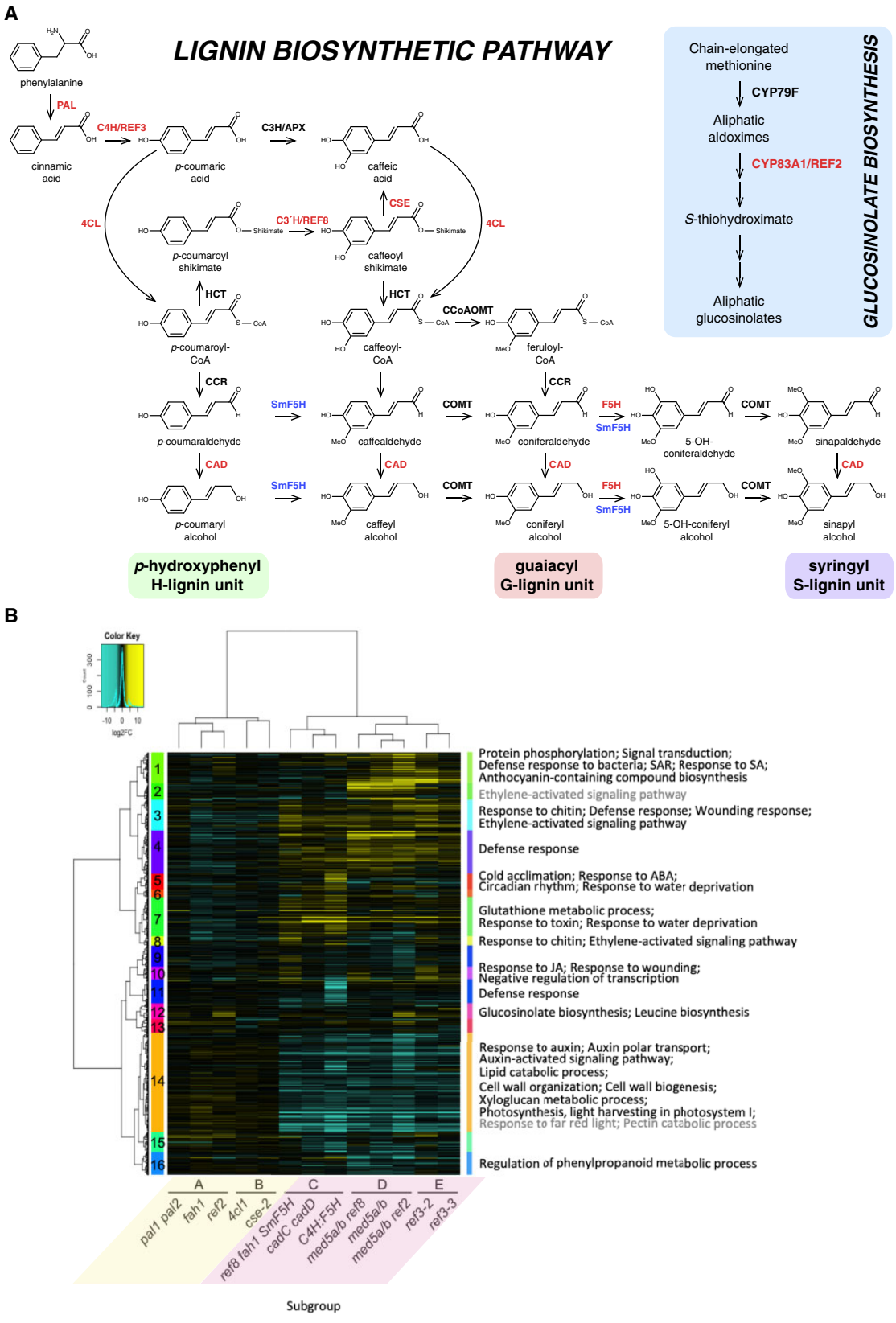

Figure 1 Transcriptional and metabolic responses to lignin pathway perturbations. A, Scheme of the lignin biosynthetic pathway. The enzymes for which genes were targeted in this study are highlighted in red. The enzyme corresponding with the *F5H* gene from *Selaginella moellendorffii* is highlighted in blue. The blue panel in the top right shows a simplified scheme of the aliphatic glucosinolate biosynthetic pathway. B, Hierarchical clustering of DEGs from 13 lignin mutants compared with wild type. Gene ontology terms of biological processes for 16 clusters are shown on the right. Terms written in black are statistically significant (false discovery rate, FDR < 0.01) and those in gray indicate FDR between 0.01 and 0.1. This figure was modified from figure 7 from Wang et al. (2022). Subgroups highlighted in yellow: mutants with fewer DEGs; subgroups highlighted in red: mutants with more DEGs.

help to determine whether and how phenylpropanoids might act as bioactive signaling molecules that regulate transcriptional reprogramming upon pathway perturbations.

Conflict of interest statement. None declared.

References

Barros J, Serk H, Granlund I, Pesquet E (2015) The cell biology of lignification in higher plants. Ann Bot **115**: 1053–1074

Bonawitz ND, Chapple C (2010) The genetics of lignin biosynthesis: connecting genotype to phenotype. Annu Rev Genet **44**: 337–363

Bonawitz ND, Kim JI, Tobimatsu Y, Ciesielski PN, Anderson NA, Ximenes E, Maeda J, Ralph J, Donohoe BS, Ladisch M, et al. (2014) Disruption of Mediator rescues the stunted growth of a lignin-deficient Arabidopsis mutant. Nature **509**: 376

Cesarino I (2019) Structural features and regulation of lignin deposited upon biotic and abiotic stresses. Curr Opin Biotechnol **56**: 209–214

Dauwe R, Morreel K, Goeminne G, Gielen B, Rohde A, Van Beeumen J, Ralph J, Boudet A-M, Kopka J, Rochange SF, et al. (2007) Molecular phenotyping of lignin-modified tobacco reveals associated changes in cell-wall metabolism, primary metabolism, stress metabolism and photorespiration. Plant J **52**: 263–285

Ferreira SS, Goeminne G, Simões MS, de Almeida Pina AV, Alves de Lima LG, Pezard J, Gutiérrez A, Rencoret J, Mortimer JC, del Río JC, et al. (2022) Transcriptional and metabolic changes associated with internode development and reduced cinnamyl alcohol dehydrogenase activity in sorghum. J Exp Bot http://dx.doi.org/10.1093/jxb/erac300

Ralph J, Lapierre C, Boerjan W (2019) Lignin structure and its engineering. Curr Opin Biotechnol **56**: 240–249

Rohde A, Morreel K, Ralph J, Goeminne G, Hostyn V, Rycke RD, Kushnir S, Doorsselaere JV, Joseleau J-P, Vuylsteke M, et al. (2004) Molecular phenotyping of the pal1 and pal2 mutants of Arabidopsis thaliana reveals far-reaching consequences on phenylpropanoid, amino acid, and carbohydrate metabolism. Plant Cell **16**: 2749–2771

Saluja M, Zhu F, Yu H, Walia H, Sattler SE (2021) Loss of COMT activity reduces lateral root formation and alters the response to water limitation in sorghum brown midrib (bmr) 12 mutant. New Phytol **229**: 2780–2794

Vanholme R, Storme V, Vanholme B, Sundin L, Christensen JH, Goeminne G, Halpin C, Rohde A, Morreel K, Boerjan W (2012) A systems biology view of responses to lignin biosynthesis perturbations in Arabidopsis. Plant Cell **24**: 3506–3529

Wang P, Guo L, Morgan J, Dudareva N, Chapple C (2022) Transcript and metabolite network perturbations in lignin biosynthetic mutants of Arabidopsis. Plant Physiol **190**: 2828–2846