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Unraveling the regulatory network of bamboo lignification

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The ability to deposit the complex phenolic polymer lignin in the cell walls of specialized cell types was a key evolutionary step toward the dominance of the terrestrial ecosystem by plants (Renault et al., 2019). Lignin confers mechanical strength to supportive tissues and hydrophobicity to xylem cells, allowing the efficient transport of water and nutrients throughout the plant body. Lignin also plays a major role in the response of plants to various biotic and abiotic stresses (Cesarino, 2019). Although essential to plant growth and development, lignin constitutes a major hurdle in the conversion of plant biomass into downstream products in biorefineries (Liu et al., 2021). In a bio-based economy, plant lignocellulosic biomass emerges as a sustainable and renewable resource that can be deconstructed into simple sugars and aromatics, which are further converted into several high-value products, including fuels, chemicals, and advanced materials (Ning et al., 2021).

Among potential feedstocks, bamboo has been suggested as a prominent resource due to its abundance and rapid growth, in addition to the excellent mechanical properties of its fibers (Qu et al., 2020). Because lignin is recognized as a limiting factor to biomass-to-products conversion, the efficient exploitation of bamboo as a sustainable feedstock for biorefineries requires a deeper understanding of the molecular mechanisms underlying lignin deposition. This knowledge will enable biotechnological strategies for the development of improved bamboo genotypes for the bioeconomy.

In this issue of *Plant Physiology*, Yang et al. (2021) applied an integrative approach based on large-scale transcriptomics, small RNAseq, and degradome sequencing (i.e. a technique to identify miRNA cleavage sites) to study the lignification process in moso bamboo (*Phyllostachys edulis*). This giant bamboo species grows rapidly, reaching heights of up to 28 m, and is economically exploited in China as timber and in the textile industry. To study the developmental progression of lignin deposition, the 13th internode was sampled from plants of varying size (i.e. developmental stage) growing in a wild bamboo forest (Figure 1A). Each internode was also divided into three portions, lower, middle and top, to investigate the gradient of secondary wall deposition as a consequence of developmental program within each internode (Zhang et al., 2014; Martin et al., 2016).

Histochemical and quantitative analyses showed that lignin levels increased with internode maturity and from bottom to top in each internode, except for the completely mature internode which showed higher lignin levels in the lower portion. Similar results were observed when the activity of two key enzymes involved in lignification (phenylalanine ammonia-lyase and laccase) was determined in the same samples.

RNAseq analysis was employed to study the expression pattern of lignin-related genes during internode development in moso bamboo. Genes encoding transcription factors homologous to known regulators of lignin deposition in Arabidopsis (*Arabidopsis thaliana*), as well as genes encoding lignin biosynthetic enzymes and monolignol oxidases (i.e. peroxidases and laccases), showed a similar developmental expression pattern of initial upregulation in less mature tissue followed by decreased expression in more mature tissue. Notably, peak expression of regulatory genes occurred earlier and was stronger than that of downstream genes.

Small RNAseq and degradome sequencing allowed the identification of miRNAs with similar expression patterns to those of lignin-related genes and of miRNA–mRNA pairs potentially involved in the regulation of shoot lignification. A weighted gene co-expression network analysis including all differentially expressed genes further revealed modules of co-expressed genes highly correlated with lignification, suggesting these genes might play a role in lignin deposition in bamboo shoots.

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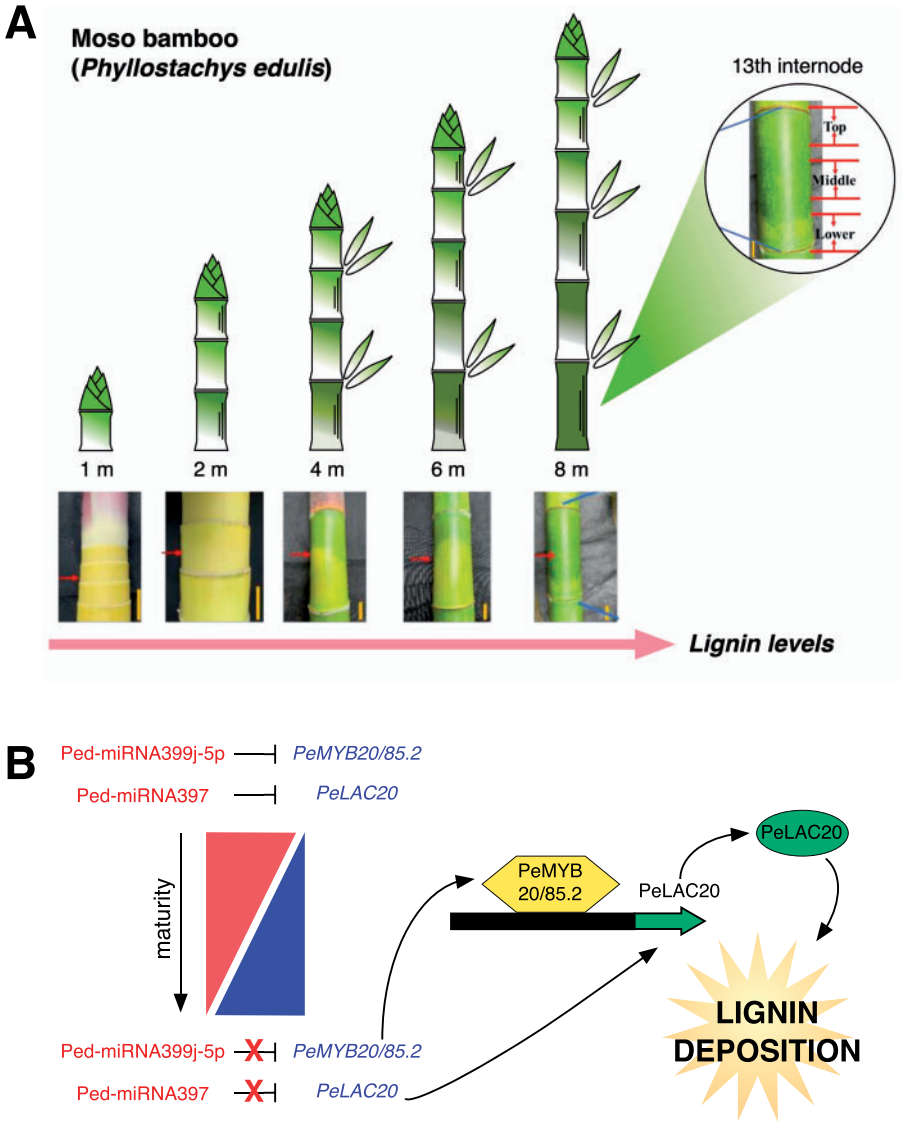

Figure 1 An integrative approach to study the lignification process of moso bamboo (*P. edulis*) shoots. A, Lignin deposition was evaluated in the 13th internode from bamboo shoots of different developmental stages (plant height of 1–8 m); internodes were also divided into three portions, named lower, middle, and top. Lignin levels increased with internode maturity and from bottom to top in each internode, except for the mature internode (8 m) which showed higher lignin levels in the lower portion. B, A miRNA-mediated "MYB-*PeLAC20*" regulatory module for lignin polymerization during lignification of bamboo shoots was proposed: (1) in young internodes, higher expression of *Ped-miRNA399j-5p* and *Ped-miRNA397* leads to repression of *PeMYB20/85.2* and *PeLAC20*; (2) with increased maturity, the expression levels of *Ped-miRNA399j-5p*, and *Ped-miRNA397* decrease, resulting in the upregulation of *PeMYB20/85.2* and *PeLAC20*; (3) PeMYB20/85.2 binds to the promoter of *PeLAC20*, boosting its expression and inducing lignin deposition. Figure *adapted from Figures 8 and S15* of Yang et al. (2021).

By integrating the co-expression data with miRNA–mRNA pair analysis, in addition to the identification of lignin-related regulatory *cis*-elements in the promoter of candidate biosynthetic genes, the authors constructed a regulatory network of the genetic elements involved in lignin deposition in moso bamboo, which included 22 transcription factors, 11 miRNAs, and 36 downstream genes (i.e. lignin biosynthetic genes and monolignol oxidases).

Some of the genetic elements and their interactions within this proposed network were further validated. For instance, some miRNA–mRNA pairs examined by RT-qPCR showed opposite expression trends of miRNA and mRNA along bamboo internodes, similar to the results found with large-scale transcriptomics. Among these miRNA–mRNA pairs, the authors found that *Ped-miR397* targets *LACCASE20* (*PeLAC20*), the putative ortholog of the lignin-related *AtLAC4* from Arabidopsis (Berthet et al., 2011). The function of this bamboo laccase gene was further investigated via constitutive overexpression in Arabidopsis, which resulted in higher lignin levels in inflorescence stems when compared to those of control plants.

The regulatory network also suggested a positive interaction between *PeLAC20* and the transcription factors PeMYB4.1 and PeMYB20/85.2, which are putative orthologs of lignin-related MYBs from Arabidopsis (Behr et al., 2019). Yeast one-hybrid assay and electrophoretic mobility shift assay showed

both transcription factors can bind to the promoter of *PeLAC20*, validating the interaction. Altogether, these results, in addition to the identification of target sites of *Ped-miR399j-5p* in *PeMYB20/85.2*, led the authors to propose a miRNA-mediated "MYB-*PeLAC20*" regulatory module for lignin polymerization during lignification of bamboo shoots (**Fig. 1B**).

The multiple-level regulatory network unraveled by Yang and colleagues represents a major advance in our knowledge regarding lignin deposition in bamboo. Importantly, only a few genetic elements of this network were characterized in the present study, so we can expect that the data provided here will serve as the basis for the identification of additional mechanisms underlying bamboo lignification in the future. For instance, it will be interesting to evaluate whether a similar miRNA-mediated module for lignin polymerization also occurs involving peroxidase genes. Additionally, similar miRNA-mediate modules in which lignification is regulated post-transcriptionally by targeting transcription factors or biosynthetic genes have been rarely characterized. Ultimately, a better understanding of lignin metabolism in bamboo will allow its efficient exploitation as a sustainable resource for biorefineries in our way toward a bio-based economy.

References

Behr M, Guerriero G, Grima-Pettenati J, Baucher M (2019) A molecular blueprint of lignin repression. Trends Plant Sci **24**: 1052–1064

Berthet S, Demont-Caulet N, Pollet B, Bidzinski P, Cézard L, Le Bris P, Borrega N, Hervé J, Blondet E, Balzergue S, et al. (2011) Disruption of *LACCASE4* and *17* results in tissue-specific alterations to lignification of *Arabidopsis thaliana* stems. Plant Cell **23**: 1124–1137

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

Liu Y, Cruz-Morales P, Zargar A, Belcher MS, Pang B, Englund E, Dan Q, Yin K, Keasling JD (2021) Biofuels for a sustainable future. Cell **184**: 1636–1647

Martin AP, Palmer WM, Brown C, Abel C, Lunn JE, Furbank RT, Grof CPL (2016) A developing *Setaria viridis* internode: an experimental system for the study of biomass generation in a C4 model species. Biotechnol Biofuels **9**: 45

Ning P, Yang G, Hu L, Sun J, Shi L, Zhou Y, Wang Z, Yang J (2021) Recent advances in the valorization of plant biomass. Biotechnol Biofuels **14**: 102

Qu C, Ogita S, Kishimoto T (2020) Characterization of immature bamboo (*Phyllostachys nigra*) component changes with its growth via heteronuclear single-quantum coherence nuclear magnetic resonance spectroscopy. J Agric Food Chem **68**: 9896–9905

Renault H, Werck-Reichhart D, Weng J-K (2019) Harnessing lignin evolution for biotechnological applications. Curr Opin Biotechnol **56**: 105–111

Yang K, Li L, Lou Y, Zhu C, Li X, Gao Z (2021) A regulatory network driving shoot lignification in rapidly growing bamboo. Plant Physiol. **187**: 900–916

Zhang Q, Cheetamun R, Dhugga KS, Rafalski JA, Tingey SV, Shirley NJ, Taylor J, Hayes K, Beatty M, Bacic A, et al. (2014) Spatial gradients in cell wall composition and transcriptional profiles along elongating maize internodes. Bmc Plant Biol **14**: 27