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Spotlight

Genome editing of wood for sustainable pulping

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Wood is an abundant and renewable feedstock for pulping and biorefining, but the aromatic polymer lignin greatly limits its efficient use. Sulis *et al.* recently reported a multiplex CRISPR editing strategy targeting multiple lignin biosynthetic genes to achieve combined lignin modifications, improve wood properties, and make pulping more sustainable.

Designing wood for the bioeconomy

Increasing demand for natural resources, driven by rapid population growth, has contributed to our current global environmental crisis. Our production of food, fuels, and materials has been largely based on the exploitation of fossil resources whose consumption releases carbon dioxide into the atmosphere, a key cause of climate change. This scenario has motivated our transition to a sustainable bioeconomy in which fossil resources are replaced by biomass feedstocks that are converted to a plethora of downstream products [1]. Lignocellulosic biomass is enriched in structural polysaccharides and aromatics that can be processed and converted to fuels, chemicals, and polymers. In this context, wood is the most abundant plant biomass on the planet and a major source of fibers, and >170 million metric tons of wood are produced annually [2]. However, the efficient isolation of cellulosic fibers from wood is hampered by the presence of lignin in the secondary cell walls – a complex phenolic biopolymer that is highly resistant to chemical and enzymatic degradation [3]. Therefore, lignin bioengineering holds promise for tailoring plant biomass to allow efficient conversion to bioproducts, thus empowering the bioeconomy. Genetic engineering strategies designed to alter lignin quantity, composition, and structure often result in increased biomass processability [4] (see Figure 1).

Lignin is predominantly formed by the polymerization of three monolignols, *p*-coumaryl, coniferyl, and sinapyl alcohols, which give rise to the lignin monomer units *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) [5]. Extensive characterization of the molecular mechanisms underlying different aspects of lignification, including regulation, biosynthesis, and polymerization, has provided fundamental insights into the development of diverse strategies for lignin bioengineering in different crops. Despite the complexity of lignin metabolism and the availability of a vast number of targets, most lignin bioengineering approaches have predominantly focused on targeting a single gene or gene family, whereas the combinatorial effects of multigenic perturbations have remained largely unexplored. In a recent study, Sulis *et al.* [6] employed multiplex CRISPR editing for combinatorial improvement of lignin composition and wood properties in poplar (*Populus trichocarpa*), a fast-growing tree.

Lignin biosynthesis is a complex process that is governed by at least 11 gene families and hundreds of genetic and metabolic regulatory elements [7]. The starting point for the work of Sulis and colleagues to select the best candidate gene targets was a previously proposed computational predictive model for monolignol biosynthesis in poplar [8,9], which had been developed on the basis of integrative transcriptomic, proteomic, fluxomic, and phenomic data from ~2000 transgenic poplar lines. Because the model predicts the transduction of quantitative relationships from gene transcript abundances to absolute enzyme abundances, pathway metabolic fluxes, and 25 wood physicochemical properties, the model revealed how individual wood properties could be modified through multiplex editing of monolignol genes. The ideal wood design included (i) low lignin content, (ii) a high syringyl-to-guaiacyl lignin (S/G) ratio, (iii) an increased carbohydrate-to-lignin (C/L) ratio, and (iv) good growth (comparable with or higher than the control). By exploring the 69 123 possible combinations, the best seven strategies each involving the modification of three to six genes were selected for gene editing in poplar, based on the extent and robustness of the predicted improvements in fiber traits.

To evaluate the effectiveness of these predicted strategies in modulating wood properties for fiber production, the corresponding multiplex CRISPR-edited poplars were generated, and these exhibited varying extents of loss-of-function mutations in the target genes. Phenotypic characterization of these lines showed that multiplex targeting of monolignol biosynthetic genes substantially altered the physicochemical properties of the wood, as predicted by the model. Lignin content reduced by up to 49% and the C/L ratio increased by up to 228% compared with control trees. In addition, the lignin monomer S/G ratio increased from 2.7 in control plants to as high as 4.0 in edited lines. As expected, the most significant alterations in wood properties were observed in CRISPR-edited lines for four to six genes, although some strategies targeting three genes also showed positive wood trait modifications. Despite these promising results, most of the edited low-lignin lines showed an undesirable yield penalty, including reduced tree growth and stem volume, which would potentially offset any gains in biomass processability. These data suggest that multiplex CRISPR editing can enable the efficient improvement of

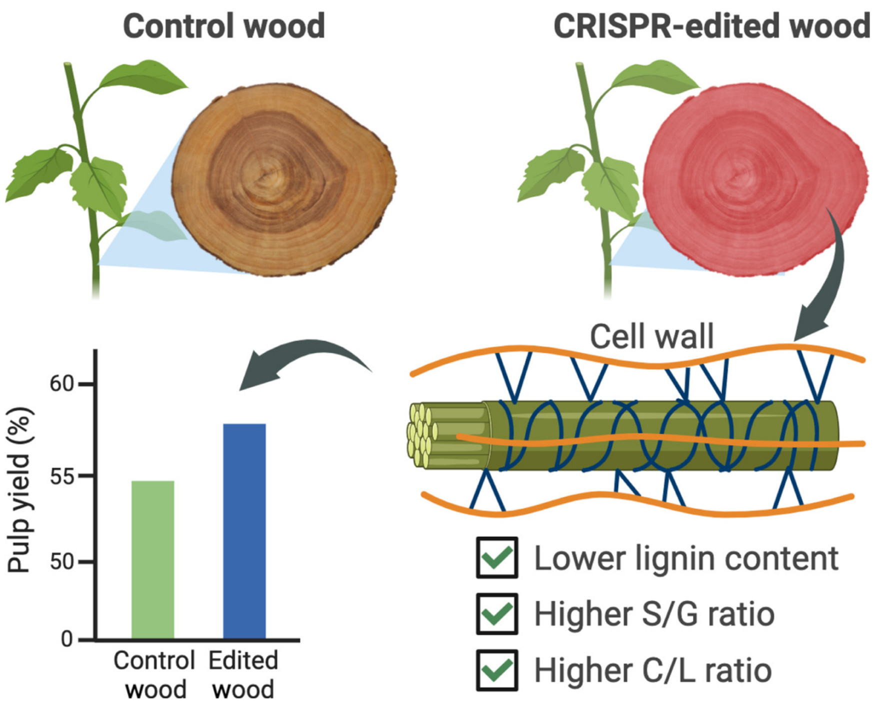

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Figure 1. Multiplex CRISPR editing targeting multiple biosynthetic genes leads to combined lignin modifications and improved wood properties. Edited wood shows a distinct red coloration of the xylem as a consequence of alterations in lignin metabolism. As predicted by the model, edited wood shows reduced lignin content, an increased lignin monomer syringyl-to-guaiacyl (S/G) ratio, and an increased carbohydrate-to-lignin (C/L) ratio, which ultimately result in higher pulp yield in micropulping experiments.

wood properties via combinatorial lignin modifications, but potential negative effects on plant growth should still be considered, even if not predicted by integrative models.

Given that kraft pulping is a dominant method for industrial wood fiber production, the authors performed micropulping experiments with wood from control and edited plants to understand the impacts of combinatorial wood modifications on paper pulping. As expected, lignin modifications achieved via multiplex CRISPR editing increased pulp yield, a feature that could reduce both the usage of pulping chemicals and the solid content of black liquor – a waste solution containing lignin. Because the lignin content of black liquor correlates negatively with the operation capacity of the recovery boiler in pulp mills – arguably the most crucial and rate-limiting energy component of the process – it was suggested that the low-lignin wood could debottleneck the recovery boiler and increase the production potential of the mill by up to 40%. Another benefit from the edited wood was the increased C/L ratio, which means that less biomass is necessary to provide the same amount of cellulose. Finally, the authors showed that the observed higher pulp yield gained with edited wood could reduce the estimated global warming potential of the industrial process by up to 20%, thus providing environmental benefits and helping to mitigate climate change.

Concluding remarks and future perspectives

The study of Sulis *et al.* illustrates how biomass feedstocks can be rationally engineered via multiplex CRISPR editing to enable more sustainable production of biomaterials. This work also provides several interesting perspectives. First, their predictive model was developed based on years of fundamental research on lignin biosynthesis in poplar. Cell-wall composition differs considerably among plants, especially between eudicots and monocots, suggesting that group- or species-specific underlying molecular mechanisms will need to be properly characterized to allow the development of similar predictive models for the desired output in terms of biomass composition and processability in other bioeconomy crops such as herbaceous eudicots (e.g., alfalfa) and grasses (e.g., maize and sugarcane). Second, the manufacture of different types of biomass traits might dictate fundamentally different strategies for feedstock development. In the work of Sulis *et al.*, wood with a low lignin content and high S/G ratio was the expected outcome that would allow more efficient fiber production, given that the strategy was focused on the accessibility and processability of cell-wall polysaccharides, especially cellulose. However, lignin itself is considered to be a renewable and sustainable aromatic resource that can be converted into various chemicals, fuels, and materials [1,10]. Therefore, strategies based on lignin valorization will demand completely different feedstock designs to achieve precise control of lignin content and structure. Third, although the results by Sulis *et al.* are promising in terms of sustainable fiber production, field trial experimentation is a 'must-do' step to ensure that the engineered trees can perform efficiently under environmental conditions, thus bringing them closer to industrial applications [3]. Moreover, CRISPR-engineered trees are still considered to be genetically modified organisms (GMOs) in several countries [11]. However, genome editing is able to accelerate tree domestication and improve wood traits in shorter times than conventional breeding, and regulatory organizations should therefore not delay updates to biosafety regulations

[12]. A long but exciting way towards sustainability is ahead of us, but innovative efforts will certainly help to address key environmental challenges and empower the bioeconomy.

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Declaration of interests

The authors declare no conflicts of interest.

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References

1. Li, J. *et al.* (2022) Feedstock design for quality biomaterials. *Trends Biotechnol.* 40, 1535–1549
2. RISI (2022) *RISI Annual Review of Global Pulp and Paper Statistics*, RISI
3. De Meester, B. *et al.* (2022) Lignin engineering in forest trees: from gene discovery to field trials. *Plant Commun.* 3, 100465
4. Mottiar, Y. *et al.* (2016) Designer lignins: harnessing the plasticity of lignification. *Curr. Opin. Biotechnol.* 37, 190–200
5. Vanholme, R. *et al.* (2019) Lignin biosynthesis and its integration into metabolism. *Curr. Opin. Biotechnol.* 56, 230–239
6. Sulis, D.B. *et al.* (2023) Multiplex CRISPR editing of wood for sustainable fiber production. *Science* 381, 216–221
7. Yao, T. *et al.* (2021) Phylogenetic occurrence of the phenylpropanoid pathway and lignin biosynthesis in plants. *Front. Plant Sci.* 12, 704697
8. Wang, J.P. *et al.* (2018) Improving wood properties for wood utilization through multi-omics integration in lignin biosynthesis. *Nat. Commun.* 9, 1579
9. Matthews, M.L. *et al.* (2020) Modeling cross-regulatory influences on monolignol transcripts and proteins under single and combinatorial gene knockdowns in *Populus trichocarpa*. *PLoS Computat. Biol.* 16, e1007197
10. Liu, Z.-H. *et al.* (2022) Creative biological lignin conversion routes toward lignin valorization. *Trends Biotechnol.* 40, 1550–1566
11. Editorial (2023) EU rethinks genome editing. *Nature Plants* 9, 1169–1170
12. Anders, C. *et al.* (2023) Accelerating wood domestication in forest trees through genome editing: advances and prospects. *Curr. Opin. Plant Biol.* 71, 102329