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Brief Communication

Genetic manipulation of a COBRA gene, *PtrCOB11*, substantially alters wood properties in poplar

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Wood is the most abundant terrestrial biomass, sequestering more than 300 billion tons of carbon annually. Wood-derived materials have potential applications in various fields, including green electronics, biological devices, and energy applications, due to their unique properties (Zhu *et al*., 2016). Approximately 50% of woody biomass is composed of cellulose, which consists of linear chains of β-1,4-linked glucose that aggregate to form cellulose microfibrils (CMFs). Plant CMFs are synthesized by the cellulose synthase (CesA) complex (CSC) in the plasma membrane. Genetic studies indicate that secondary cell wall (SCW) CesAs contribute to 90% of the cellulose in poplar wood (Xu *et al*., 2021). As a renewable and sustainable fibre resource, cellulose is utilized in papermaking, nanocellulose manufacturing, and biofuel conversion. Therefore, a deeper understanding of cellulose production in wood is essential for advancing these applications.

Because of the class specificity and complexity of CSC (Kumar *et al*., 2017), overexpression of an SCW CesA gene alone is unlikely to significantly enhance cellulose content in wood. Studies have revealed that COBRA (COB) proteins, a class of glycosyl phosphatidylinositol-anchored proteins containing a carbohydrate-binding module, play a critical role in cellulose deposition within herbaceous plant cell walls (Liu *et al*., 2013). COBL4 and COBL7 have been identified as critical modulators of cellulose organization in the SCW and stomatal development in *Arabidopsis* (Ge *et al*., 2024; Xue *et al*., 2024). It remains unclear whether a crucial COB protein executes cellulose deposition in wood and whether its genetic modification can improve wood quality.

Analysis of the AspWood RNAseq dataset for *Populus trichocarpa COB*s (*PtrCOB*s) revealed that *PtrCOB11* exhibited a dominant transcription level in wood SCW formation, with its promoter tissue expression activities primarily localized to vascular cambial cells and secondary xylem fibres of *proCOB11::GUS* transgenic trees (Figures S1 and S2). We generated Cas9/gRNA-induced *ptrcob11* mutants and *PtrCOB11*-overexpressing trees (Figure 1a,b, Figure S3). The mutants exhibited significantly reduced stem diameter and plant height, whereas *PtrCOB11*-overexpressing trees (OE-8 and OE-12), similar in height to the wild-type (WT), showed significantly larger stem diameters (Figure 1c,d), suggesting a role of *PtrCOB11* in stem growth. Additionally, mutant stems were extremely fragile and considerably easier to break by hand compared to WT and overexpression trees (Figure 1e). Furthermore, the modulus of rupture (MOR), a key indicator of mechanical strength, reduced to 63% of the WT level in mutant stems, whereas the overexpression trees showed an increase in stem MOR (Figure 1f). The cellulose concentration in mutant wood was merely 26% (compared to 42% in WT wood) but increased to approximately 48% in the overexpression lines (Figure 1f). As cellulose, lignin, and hemicellulose account for approximately 95% of wood composition, hemicellulose and lignin concentrations exhibited a corresponding increase in mutant wood (Figure S4a,b). In overexpression trees, lignin content increased slightly, whereas xylose content was marginally reduced, and glucose content increased significantly (Figure S4c,d). Increased cellulose deposition resulting from *PtrCOB11* overexpression is expected to influence SCW assembly, thereby impacting wood composition.

To investigate structural changes, we used scanning and transmission electron microscopy (SEM/TEM) to detect SCW thickness and layering in mutant wood. SEM observations showed a striking reduction in fibre SCW thickness in mutant trees compared to WT (Figure 1g, Figure S5). TEM images of the mutant stem cross-sections revealed poorly defined boundaries between the S1 and S2 layers, extremely thin SCWs at two wood cell edges, and the disorganized S-layer deposition in wood fibre corners (Figure 1h). Under a polarized microscope, the S1-layers of mutant wood SCWs exhibited weak and inconsistent light compared to those of the WT, indicating considerable structural impairment (Figure 1i). Additionally, field emission SEM (FE-SEM) revealed a disorganized orientation of CMFs in the S2-layers of mutant wood fibre walls (Figure 1j), in contrast to the well-ordered arrangement observed in WT. This structural disorganization resulted in a notable reduction in the mechanical strength of mutant stems. Overall, the loss of *PtrCOB11* disrupted the wood fibre SCW structure by impairing cellulose assembly.

In contrast, *PtrCOB11*-overexpressing trees exhibited significantly thicker SCWs in developing and mature xylem fibres, as evidenced by SEM analysis (Figure 1k). TEM pictures revealed a more than a one-fold increase in the thickness of wood fibre SCWs, as well as their S1 and S2 layers, in *PtrCOB11*-overexpressing trees (Figure 1l,m). Because of the increased stem diameter, the overexpression lines produced more wood than WT trees, and their wood density increased substantially (Figure 1n). Additionally, we examined long-term phenotypes of *PtrCOB11*-overexpressing trees undergoing 3, 6 and 10 months of growth

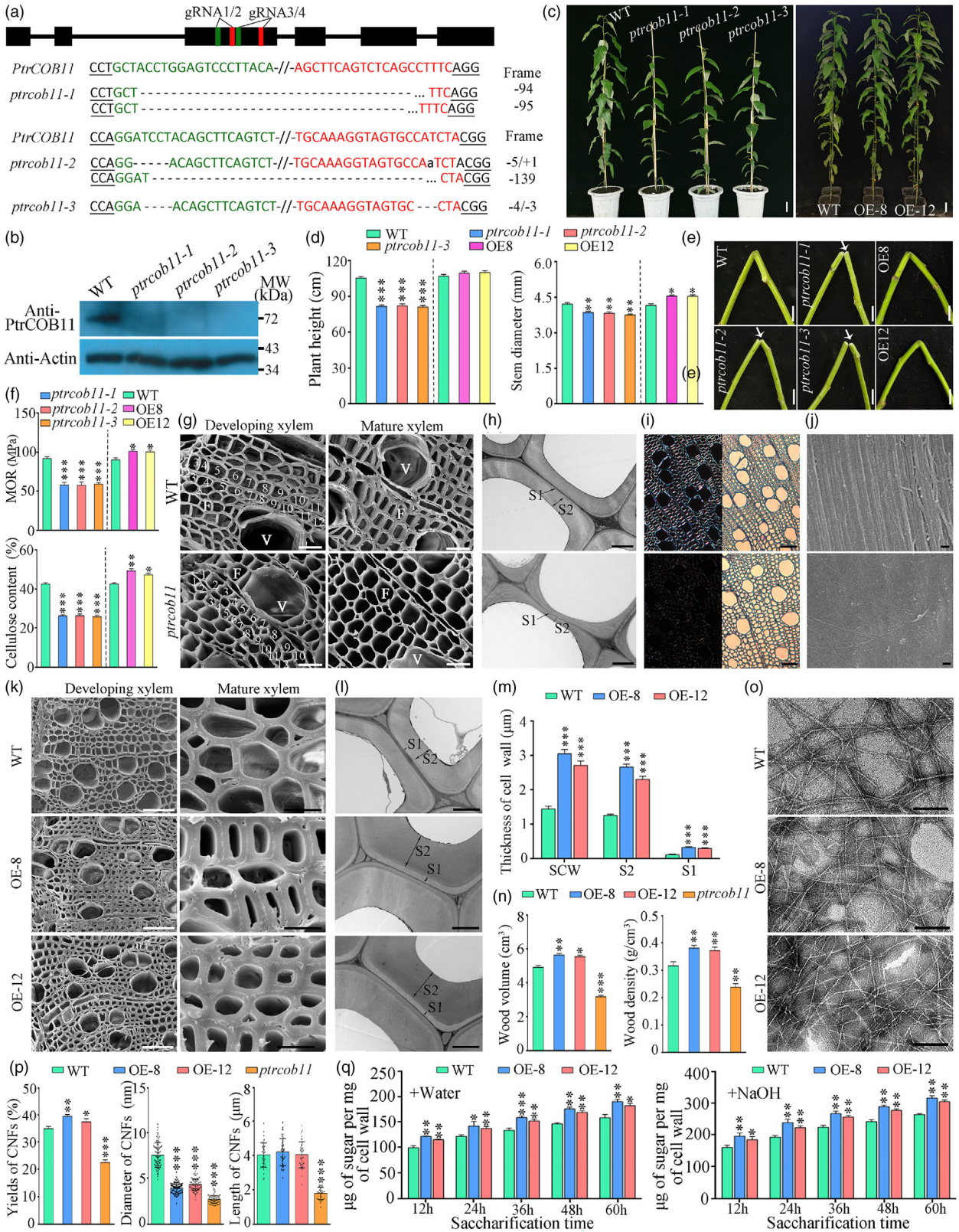

Figure 1 Genetic manipulation of *PtrCOB11* affects wood biomass and its properties in *Populus trichocarpa* (Details provided in Data S1).

in the greenhouse. The overexpression trees consistently exhibited substantial increases in fibre SCW thickness, cellulose content, and wood biomass compared to WT (Figure S6). WT and overexpression trees exhibited no significant differences in photosynthetic activity, stomatal aperture, leaf water loss, secondary xylem vessel wall thickness, and xylem-specific conductivity. In contrast, the mutants showed significant reductions in photosynthetic activity, stomatal function and xylem

water transport capacity (Figure S7). These data indicate that *PtrCOB11* overexpression does not negatively impact tree health and growth, supporting its potential for use in breeding programs.

A comparative transcriptome analysis identified genes and pathways influenced by *PtrCOB11* during wood formation. The top 20 enriched Gene Ontology (GO) terms between *ptrcob11* mutants and WT were primarily associated with biological processes related to cell wall synthesis (Figure S8a). Approximately 18.6% of differentially expressed genes (DEGs) in the mutants were associated with SCW formation (Figure S8c; Tables S2 and S3). In contrast, the expression of biosynthetic genes for cellulose, hemicellulose, and lignin remained largely unchanged. The mutants also exhibited lower expression levels of most DEGs involved in xylem cell differentiation. Between the overexpression line and WT, four GO clusters of the TOP 20 terms were related to cell wall synthesis, with many DEGs involved in wood formation being shared with the mutant dataset (Figure S8b,d; Tables S4 and S5). These findings provide evidence for the role of *PtrCOB11* in stem growth and SCW formation.

Given that the cellulose content and wall structure of the transgenic wood were altered, we investigated its potential applications in nanocellulose production. Among WT, mutant, and overexpression samples, *PtrCOB11*-overexpressing wood yielded the highest amounts of cellulose nanofibers (CNFs) when processed using a conventional chemical-ultrasonic approach (Figure 1p). The CNF diameters of overexpression wood (averaging 3.95 nm from OE-8 and 4.32 nm for OE-12) significantly reduced, whereas CNF length was comparable to that of WT (Figure 1o,p). In contrast, both CNF diameter and length in the mutants were noticeably reduced in the mutants (Figure 1p, Figure S9). FE-SEM analysis revealed that chemically purified cellulose fibres from overexpression wood, in contrast to those from WT, were broken down into smaller micron-sized fibres with a higher fragmentation degree and no coarse fibre aggregation (Figure S10). In the overexpression samples, hydrogen bonding between fibre bundles was likely reduced, weakening the inter-fibre bonding forces and enabling cellulose fibre dispersion. Consequently, ultrasonication treatment resulted in CNFs with smaller diameters. The considerable decrease in the cellulose crystallinity index of chemically purified cellulose fibres from overexpression wood (Figure S11) may be attributed to weaker hydrogen bonding than in the WT. As a result, the overexpression wood exhibits a high CNF aspect ratio, making it suitable for nanocellulose-based applications, such as tensile-strength-enhancing components in paper and materials for wood adhesives.

We also assessed the potential of overexpression wood for conversion to biofuel. Wood cell wall powder was gently pretreated with either hot water or a diluted NaOH alkaline solution. Following enzymatic saccharification, overexpression wood cell walls released considerably higher amounts of sugars than WT cells (Figure 1q). Overexpression of *PtrCOB11* enhanced cellulose digestibility in wood cell walls, likely due to reduced hydrogen bonding between fibre bundles, which facilitated enzymatic saccharification, as well as increased cellulose content, which contributed to an increased sugar release yield.

In conclusion, *PtrCOB11* plays a crucial role in determining wood biomass, cellulose content, and fibre wall structure in poplar. Furthermore, this study highlights the potential applications of poplar lignocellulosic wood with *PtrCOB11* overexpression in the nanocellulose and biofuel industries.

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Conflict of interest

The authors declare no conflicts of interest.

Author contributions

Y.C. designed the experiment and conception; W.X., H.C., S.Z., C.W., J.C., M.G. and N.E. performed the experiments; W.X., H.C., X.L. and Y.C. analysed the data; W.X. and Y.C. wrote the manuscript. All authors reviewed the manuscript.

Data Availability Statement

The data that supports the findings of this study are available in the supplementary material of this article.

References

Ge, S., Sun, P., Wu, W., Chen, X., Wang, Y., Zhang, M., Huang, J. *et al*. (2024) COBL7 is required for stomatal formation via regulation of cellulose deposition in *Arabidopsis*. *New Phytol.* **115**, 227–242.

Kumar, M., Atanassov, I. and Turner, S. (2017) Functional analysis of cellulose synthase (CESA) protein class specificity. *Plant Physiol.* **173**, 970–983.

Liu, L., Shang-Guan, K., Zhang, B., Liu, X., Yan, M., Zhang, L., Shi, Y. *et al*. (2013) Brittle Culm1, a COBRA-like protein, functions in cellulose assembly through binding cellulose microfibrils. *PLoS Genet.* **9**, e1003704.

Xu, W., Cheng, H., Zhu, S., Cheng, J., Ji, H., Zhang, B., Cao, S. *et al*. (2021) Functional understanding of secondary cell wall cellulose synthases in *Populus trichocarpa* via the Cas9/gRNA-induced gene knockouts. *New Phytol.* **231**, 1478–1495.

Xue, J.Y., McNair, G., Watanabe, Y., Kaplen, M.V., Guevara-Rozo, S., Schuetz, M., Schneider, R. *et al*. (2024) COBRA-LIKE4 modulates cellulose synthase velocity and facilitates cellulose deposition in the secondary cell wall. *Plant Physiol.* **196**, 2531–2548.

Zhu, H., Luo, W., Ciesielski, P.N., Fang, Z., Zhu, J.Y., Henriksson, G., Himmel, M.E. *et al*. (2016) Wood-derived materials for green electronics, biological devices, and energy applications. *Chem. Rev.* **116**, 9305–9374.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Data S1–S2 Figure legend and Materials and methods.
Figure S1–S11 Supplementary Figures.
Table S1–S5 Supplementary Tables.