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## CRISPR/Cas9-mediated P-CR domain-specific engineering of CESA4 heterodimerization capacity alters cell wall architecture and improves saccharification efficiency in poplar

编号	040034504
推送时间	20220530
研究领域	<a href="#">森林培育</a>
年份	2022
类型	期刊
语种	英语
标题	CRISPR/Cas9-mediated P-CR domain-specific engineering of CESA4 heterodimerization capacity alters cell wall architecture and improves saccharification efficiency in poplar
来源期刊	Plant Biotechnology Journal
期	第345期
发表时间	20220309
关键词	<a href="#">cellulose</a> ; <a href="#">CESA4 homo/ heterodimerization</a> ; <a href="#">CRISPR/Cas9</a> ; <a href="#">Populus</a> ; <a href="#">saccharification</a> ; <a href="#">secondary</a> ; <a href="#">cell wall</a> ;
摘要	<p>Cellulose is the most abundant unique biopolymer in nature with widespread applications in bioenergy and high-value bioproducts. The large transmembrane-localized cellulose synthase (CESA) complexes (CSCs) play a pivotal role in the biosynthesis and orientation of the para-crystalline cellulose microfibrils during secondary cell wall (SCW) deposition. However, the hub CESA subunit with high potential homo/heterodimerization capacity and its functional effects on cell wall architecture, cellulose crystallinity, and saccharification efficiency remains unclear. Here, we reported the highly potent binding site containing four residues of Pro435, Trp436, Pro437, and Gly438 in the plant-conserved region (P-CR) of PaCESA4 subunit, which are involved in the CESA4-CESA8 heterodimerization. The CRISPR/Cas9-knockout mutagenesis in the predicted binding site results in physiological abnormalities, stunt growth, and deficient roots. The homozygous double substitution of W436Q and P437S and heterozygous double deletions of W436 and P437 residues potentially reduced CESA4-binding affinity resulting in normal roots, 1.5–2-fold higher plant growth and cell wall regeneration rates, 1.7-fold thinner cell wall, high hemicellulose content, 37%–67% decrease in cellulose content, high cellulose DP, 25%–37% decrease in cellulose crystallinity, and 50% increase in saccharification efficiency. The heterozygous deletion of W436 increases about 2-fold CESA4 homo/heterodimerization capacity led to the 50% decrease in plant growth and increase in cell walls thickness, cellulose content (33%), cellulose DP (20%), and CrI (8%). Our findings provide a strategy for introducing commercial CRISPR/Cas9-mediated bioengineered poplars with promising cellulose applications. We anticipate our results could create an engineering revolution in bioenergy and cellulose-based nanomaterial technologies.</p>
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