Lignin manipulation via plant synthetic biology to produce a biodegradable polyester precursor with concurrent improvement in biomass quality for biofuel production

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Project Goals: Engineer bioenergy crops with improved biomass and sustainability traits

Lignin manipulation targeting the shikimate pathway can redirect the metabolic flux of lignin biosynthesis for bioproduct production and/or reduce biomass recalcitrance [(1)]. By expressing a bacterial 3-dehydroshikimate dehydratase (QsuB), we observed concomitant accumulation of protocatechuate (PCA), reductions of lignin content, and more than a twofold improvement in saccharification efficiency from the genetic-engineered biomass [(2)]. Dual expression of PCA decarboxylase with catechol 1,2-dioxygenase (CatA) can convert PCA to muconic acid (MA) in planta, which is a value-added bioproduct for adipic acid, terephthalic acid, and caprolactam [(3)]. 2-Pyrone-4,6-dicarboxylic acid (PDC) is a promising building block biomaterial that can serve as a starting monomer to manufacture performance-advantaged polymers and biodegradable plastics. The lack of a chemical synthesis method has hindered the large-scale PDC utilization, and metabolic engineering approaches for its biosynthesis have recently emerged. In this study, we first increased the carbon flux through the shikimate pathway for PCA production by overexpressing a feedback-insensitive 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase (AroG*). Co-expression of PCA 4,5-dioxygenase (PmdA and PmdB) with 4-carboxy-2-hydroxymuconate-6-semialdehyde dehydrogenase (PmdC) enabled the PDC biosynthesis for the first time in plant chassis while maintaining the low biomass recalcitrance under QsuB-overexpressing background. Using plant synthetic biology, we stacked all five genes (AroG*, QsuB, PmdA, PmdB, and PmdC) in a single T-DNA and successfully introduced them into the wildtype Arabidopsis. The expression of the five genes enabled PDC production at high titers (up to 3% dry weight), accompanied by a substantial reduction in lignin content and improvements in biomass saccharification efficiency. The lignin manipulation strategy for PDC biosynthesis is currently implementing in bioenergy crops to stack low-recalcitrance traits with value-added bioproduct. We hope these promising results can ultimately improve the biomass quality and value towards the sustainable development of biorefineries.

References

1. *Lin CY, Eudes A. Strategies for the production of biochemicals in bioenergy crops. Biotechnology for biofuels. 2020 Dec;13:1-25.*
2. *Eudes A, Sathitsuksanoh N, Baidoo EE, George A, Liang Y, Yang F, Singh S, Keasling JD, Simmons BA, Loqué D. Expression of a bacterial 3-dehydroshikimate dehydratase reduces lignin content and improves biomass saccharification efficiency. Plant Biotechnology Journal. 2015 Dec;13(9):1241-50.*
3. *Eudes A, Berthomieu R, Hao Z, Zhao N, Benites VT, Baidoo EE, Loqué D. Production of muconic acid in plants. Metabolic engineering. 2018 Mar 1;46:13-9.*

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