

## **Development of Plants with Multiple Traits for High Yield of Fermentable Sugars**

Aude Aznar<sup>1</sup>, Camille Chalvin<sup>1,2</sup>, Aymerick Eudes<sup>1</sup>, Khanh Vuu<sup>1</sup>, Mi Yeon Lee<sup>1</sup>, Patrick Shih<sup>1</sup>, Vibe Marie Gondolf<sup>1,3</sup>, Berit Ebert<sup>1,3</sup>, Devon Birdseye<sup>1</sup>, Dominique Loqué<sup>1</sup>, **Henrik Vibe Scheller**<sup>1,4</sup> \*([hscheller@lbl.gov](mailto:hscheller@lbl.gov))

<sup>1</sup>Feedstocks Division, Joint BioEnergy Institute, Lawrence Berkeley National Laboratory, Berkeley, California 94720; <sup>2</sup>Ecole Normale Supérieure de Cachan, 94230 Cachan, France; <sup>3</sup>University of Copenhagen, Department of Plant and Environmental Sciences, Frederiksberg, DK-1871, Denmark; <sup>4</sup>University of California, Department of Plant and Microbial Biology, Berkeley, CA, 94720

<http://www.jbei.org>

### **Project Goals: Development of bioenergy crops with increased ratio between hexoses and pentoses, low lignin content and low recalcitrance.**

Second-generation biofuels produced from biomass can help to decrease dependency on fossil fuels, which would have many economical and environmental benefits. To make biomass more suitable for biorefinery use we need a better understanding of plant cell wall biosynthesis. Increasing the ratio of C6 to C5 sugars in the wall is an important target for engineering of plants that are more suitable for downstream processing for second-generation biofuel production. Likewise, decreasing the content of lignin is an important goal. We have studied the basic mechanisms of cell wall biosynthesis and identified genes involved in biosynthesis of pectic galactan including the GALS1 galactan synthase[1] and the URGT1 UDP-galactose transporter[2]. We have applied these findings to engineer plants that have a more suitable biomass composition and have developed synthetic biology and gene stacking tools to achieve this goal. Plants were engineered to have up to three-fold increased content of pectic galactan in stems by expressing GALS1, URGT1 and a UDP-glucose epimerase. Furthermore, the increased galactan was engineered into plants that were already engineered to have low xylan content by restricting xylan biosynthesis to vessels where this polysaccharide is essential[3]. Finally, the high galactan and low xylan traits were stacked with low lignin obtained by expressing the *QsuB* gene encoding dehydroshikimate dehydratase[4]. By targeting the transgene expression to specific cell types, we could substantially improve saccharification while avoiding adverse effects on plant growth and development.

## **References**

1. Liwanag, A.J., et al., Pectin biosynthesis: GALS1 in *Arabidopsis thaliana* is a beta-1,4-galactan beta-1,4-galactosyltransferase. *Plant Cell*, 2012. 24(12): p. 5024-36.
2. Rautengarten, C., et al., The Golgi localized bifunctional UDP-rhamnose/UDP-galactose transporter family of *Arabidopsis*. *Proc Natl Acad Sci U S A*, 2014. 111(31): p. 11563-8.

3. Petersen, P.D., et al., Engineering of plants with improved properties as biofuels feedstocks by vessel-specific complementation of xylan biosynthesis mutants. *Biotechnology for Biofuels*, 2012. 5(1): p. 84.
4. Eudes, A., et al., Expression of a bacterial 3-dehydroshikimate dehydratase reduces lignin content and improves biomass saccharification efficiency. *Plant Biotechnol J*, 2015.

*This work was part of the DOE Joint BioEnergy Institute (<http://www.jbei.org>) supported by the U. S. Department of Energy, Office of Science, Office of Biological and Environmental Research, through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U. S. Department of Energy.*