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IDENTIFICATION AND GENETIC CHARACTERIZATION OF MAIZE CELL WALL VARIATION FOR IMPROVED BIOREFINERY FEEDSTOCK CHARACTERISITCS

Final technical report

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Project Title: Identification and genetic characterization of maize cell wall variation for improved biorefinery feedstock characteristics

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Objectives and Accomplishments: The objectives of this program are to 1) characterize novel maize mutants with altered cell walls for enhanced biorefinery characteristics and 2) find quantitative trait loci (QTLs) related to biorefinery characteristics by taking advantage of the genetic diversity of maize.

1) The project emphasized the abundance of hemicellulosic sugars in maize plants. These are glucose (representing amorphous cellulose) and xylose and arabinose (representing the dominant hemicellulose arabinoxylan). Maize lines with a higher abundance of these sugars in corn stover (leaves and stalk; not kernels) can be considered superior feedstock for a lignocellulosic biorefinery leading to higher fuel yields. A high throughput assay has been developed in the Pauly lab to assess the abundance of these sugars. In brief, plant material is dried, destarched, and subjected to a weak acid hydrolysis. The released monosaccharides are then quantified by HPLC.

Several chemically mutagenized maize lines have been identified with altered hemicellulose compositions. One of the lines, *candy leaf1* (*cal1*), has been further characterized. *cal1* mutants exhibit a 240% increase of hemicellulosic glucan. This glucan was identified to be a non-crystalline β-1,3-1-4-mixed-linkage glucan that leads to 35% increase in saccharification yield when the corn stalk and leaf material were subjected to a standard digestion assay. The maize genome contains 2.5 billion base-pairs, and we were able to find the single point mutation that is responsible for the observed high glucan content. The mutation destroys the active site of a lichenenase, a plant enzyme that usually degrades this mixed-linked glucan.

We have identified a Mu-tagged line from Pioneer-Hibreed (Dupont) that confirms the role of the licheninase CAL1 in increased glucan content in leaf tissue as this line also contains a higher glucan content and increased saccharification yield. In addition, we have overexpressed wildtype CAL1 under the control of the ubiquitin promotor in B73 corn as well as the *Cal-1* maize mutant. The transformation was performed by the Iowa State University transformation facility. In both cases overexpression of the licheninase lead to a reduced glucan content in the stover material and reduced saccahrification yields, hence confirming the role of CAL-1 in lignocellulosic glucan metabolism.

CAL1 has 77 homologous genes in maize. We anticipate that most of those represent β-glucanases involved in warding off plant pathogens. However, a few of those could represent additional licheninases. We tested licheninase activity of CAL1 and its 5 closest homologs by heterologous expression in tobacco and in vitro activity assays. Surprisingly, only CAL-1 showed lichninase activity. Hence, no additional maize licheninases were identified that could have represented targets for increasing mixed linkage glucan levels in corn even further.

cal1 has been crossed with well-characterized brown-midrib maize mutants, which exhibit low/altered lignin. Stacking of these traits (high glucan, low lignin) resulted in non-transgenic maize lines with an even higher saccharification yield (+67% compared to wildtype) for the conversion to biofuels or other commodity chemicals.
Cal1 has been introgressed into maize elite varieties B73 and Mo17 and a small field trial was performed to evaluate yields. So far, neither a change in growth habit nor plant morphology has been observed; kernel yield and dry biomass yields are also not impacted by the *CAL1* mutation.

2) Twenty three maize founder lines encompassing the whole spectra of genetic diversity of maize (4%) were grown and subjected to the above mentioned procedure. Based on the initial analysis two of the lines (B73 and Mo18W) displayed the strongest difference in the abundance of these sugars. Wall glucose content is increased by 57.4% in Mo18W compared to B73 in mature leaf tissue grown in the field. In addition, a 17.4% and 20.1% decrease in wall xylose and arabinose was observed in Mo18W. A more detailed wall analysis indicated that the crystalline cellulose and lignin content was the same in both lines with a slight S/G monolignol ratio decrease in Mo18W.
Due to difference in the hemicellulosic composition the corresponding Nested Assocation Mapping (NAM) population lines of the B73/Mo18W cross were subjected to a QTL analysis. Several QTLs were identified for hemicellulosic glucan content (4 loci, encompassing genomic regions between 4-27 Mbp), saccharification yield (enzymatic sugar release of plant material with a standard lignocellulosic enzyme mix and condition; 3 loci, genomic regions between 5-38 Mbps) and arabinose to xylose ratio (4 loci; 4-31 Mbp). Interestingly, none of the identified loci overlapped i.e., there is no correlation with glucan content and saccharification yield. Unfortunately, the LOD scores of these QTLs were very low hindering the fine-mapping of the genes responsible for the observed traits.

Broad Impacts: A novel non-transgenic maize plant (*cal1*) has been identified, whose stover (leaves and stalk) contain more glucan in their walls leading to a higher saccharification yield, when subjected to a standard enzymatic digestion cocktail. Stacking this trait with altered lignin mutants yielded evene higher saccharification yields. Cal-1 mutants do not show a loss of kernel and or biomass yield when grown in the field . Hence, *cal1* biomass provides an excellent feedstock for the biofuel industry.

Public Oral/ Poster Presentations:
- Pauly M, Plant cell walls: Biosynthesis, structure, function, and resource for biofuels Max-Planck Institute for terrestrial microbiology, Marburg, Germany, June 14, 2013 **(invited speaker)**
- Pauly M, Plant cell walls: Biosynthesis, structure, function, and resource for biofuels ETH Zurich, Switzerland, June 11, 2013 **(invited speaker)**
- Pauly M, Methods to elucidate the structure of plant cell walls for the production of biofuels, University of California, Davis, Oct 3, 2012 **(invited speaker)**
- Pauly M, Plant biotechnology for biofuels, University of Rome, Italy, July 16, 2012
- Pauly M, Plant biotechnology for biofuels Max Planck Institute for molecular Plant Physiology, Golm, Germany, June 7, 2012 **(invited speaker)**

- Pauly M, Plant biotechnology for biofuels, FU Berlin, Germany, June 6, 2012 **(invited speaker)**
- Pauly M, Biofuel crop research, Novozymes, Bagsvaerd, Denmark, June 4, 2012 **(invited speaker)**
- Pauly M, Biofuel Crop research, Arcadia Bioscience, April 9, 2012 **(invited speaker)**
- Pauly M, Challenges and opportunities for Glycoscience in Energy, National Academy of Sciences, Washington DC, January 12, 2012 **(invited speaker)**
- Pauly M, Improving plant cell wall properties for biofuels applications, 3rd international Symposium on Bioenergy and Biotechnology, Wuhan, China, Oct 14-17 **(invited speaker)**
- Kraemer F, Kun B, Hake S, Pauly M, Identification and characterization of Cal-1, a high glucan maize mutant, 34th Symposium on Biotechnology for Fuels and Chemicals, New Orleans, April 30 – May 3, 2012 **(invited speaker)**
- Pauly M, How Biotechnology enhances crop properties, 5th Berkeley Bio-economy conference, Berkeley, March 26-28, 2012 **(invited speaker)**
- Kuhn B, Kraemer T, Thomik T, Hake S, Pauly M, Characterization of maize candy-leaf mutants for improved biorefinary feedstock characteristics, USDA-DOE Plant Feedstock Genomics for Bioenergy Program meeting, San Diego, Jan 13, 2012
- Kraemer F, Hake S, **Pauly M**, Characterization of novel cell wall mutants in maize, 2011 Plant and Animal Genome Conference, San Diego, January 15-19, 2011 **(invited speaker)**
- Kraemer F, Thomas T, Hake S, **Pauly M**, Characterization of novel cell wall mutants in maize, 2011 USDA-DOE Plant Feedstock Genomics for Bioenergy Program meeting, Crystal City, April 10-13, 2011 (**Poster**)
- Kraemer F, Hake S, **Pauly M**, Characterization of novel maize cell wall mutants, 2nd Pan American Congress on Plants and BioEnergy, Sao Pedro, Brazil, August 8-11, 2010 (**oral presentation**)

Other products/ outcomes:

Training:

- Ben Kuhn (postdoc): Characterization of *cal1/bm* double mutants
- Moritz Koch (undergraduate student): expression of Cal1 homologs and their activity
- **Florian Kraemer** (graduate student): Identification and characterization of *cal1*
- **Thomas Thomik** (graduate student): QTL analysis of glucan and saccharification in maize
- **Grace Kayser** (undergraduate student): mapping of *cal1*
- **China Lunde** (Lab manager): Mapping of *cal1* and allele identification

Collaborations:

The specific expertises and interaction between the labs of the two PDs also involved collaborations in other areas such as represented by the following publication: Chuck G, Tobias C, Kraemer F, Sun L, Li C, Arora R, Singh S, Dibble D, Vogel J, Simmons B, **Pauly M**, **Hake S**, 2011, Overexpression of the maize conrgrass1 microRNA gene prevents flowering, improves digestability and increases starch content of biofuel crop plants, **Proceedings of the National Academy of the USA** 108 (42) 17550-17555