Unlocking plant power: gene editing for bioenergy and bioproducts

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Summary:

As an early-career scientist, I've always been eager to share my research projects with everyone, showcasing the amazing potential of science and its transformative impact on our lives. Programs like the Wisconsin Initiative for Science Literacy are a unique opportunity to show people what we are doing in the lab and how our work can help society. I want to express my gratitude to Elizabeth Reynolds, Cayce Osborne, and Professor Bassam Shakhashiri, for the opportunity and for reviewing this text. The PMT project has been developed in the Smith lab group, led by Professor Rebecca Smith, at the University of Wisconsin-Madison and the Wisconsin Energy Institute, which, together with other institutions, is part of the Great Lakes Bioenergy Research Center. Our main goal is to improve plant biomass using gene editing, thereby enhancing bioenergy production. I'm delighted to share with you our discoveries and reinforce the idea of the plant's potential to help us change the way we use energy and ultimately contribute to a better future for the environment.

Climate change and fossil fuels: How big is the problem?

Sometimes, talking to friends or my family, I notice that different people have the same feeling: every winter is becoming colder than the last one, while the summer is hotter compared to the previous one. As much as I wish it were just a feeling, the data points to an alarming scenario. Global temperatures recently passed the critical 1.5°C increase, which results in extreme weather, severe storms, increased drought, loss of animal and plant species, and health risks. The carbon-based organisms that decomposed millions of years ago formed the fossil fuels that we have used since the Industrial Revolution, with an extremely heavy and uncontrolled burning since then. There are many potential causes of global climate change, but the biggest consensus among scientists is that the continuous burning of fossil fuels is primarily responsible.

The big problem with fossil fuels is that the burning of their products, like gasoline, kerosene, and diesel that we use in our cars, planes, and trucks, results in the emission of carbon dioxide or CO2, which acts as a barrier in our atmosphere, retaining solar heat. Cutting forests, generating power, manufacturing goods, producing food, and powering buildings are all very common activities that also involve greenhouse gas emissions.

But there is one more piece of bad news about fossil fuels; they are limited. Even though the reserves we still have are considered abundant, they will not last forever, and they will not be enough to fuel our growing society at some point. Because of these challenges, scientists around the world started to look for different options for energy, either to reduce greenhouse gas emissions or to provide renewable options of energy resources.

Plant power: the best resource for clean and renewable energy?

Plants are a valuable source of renewable materials, with the capacity to be converted into transportation fuels, chemical intermediates, and bulk chemicals. It can also be used for heat and power generation. Plant biomass, therefore, represents an abundant, low-cost, non-food material option to replace or reduce dependence on fossil fuels. The term biomass includes all organic material from plants, both above ground and below ground. It can include wood, agricultural crops, and waste from a variety of industrial processes, such as sugar production, for example. And the big deal, as you may know, is that plants are a renewable source, as they can be grown indefinitely for different purposes. To substitute fossil fuels for transportation, plant biomass can be converted into liquid biofuels, such as ethanol for cars, biodiesel for heavy trucks, or biokerosene and biogasoline, designed for aviation. Chemically, opportunities are wide open; pharmaceuticals, cosmetics, and a variety of products can be made using chemical molecules from plants. To generate power, it's simple; biomass is a great source for heat, leading to space heating, residential hot water, and industrial settings.

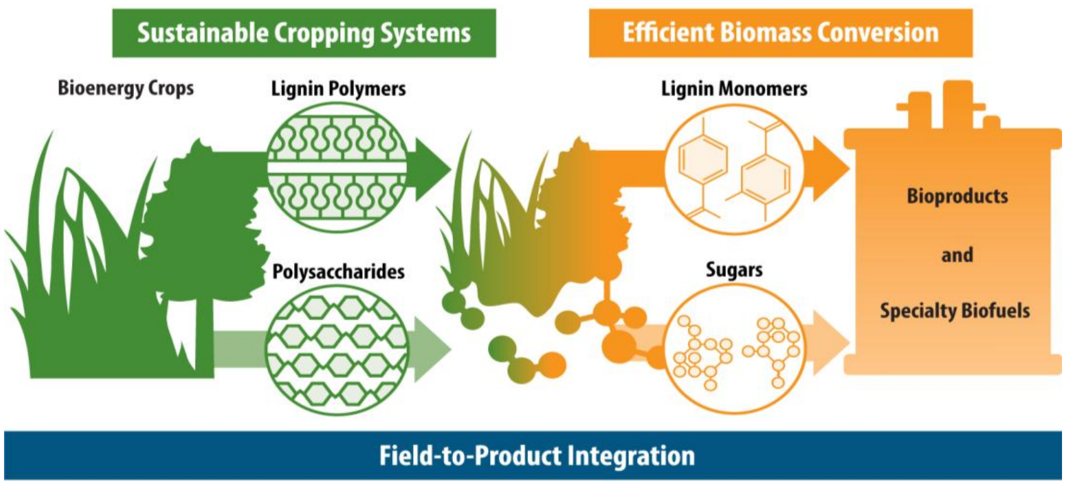

***Figure 1**: Integrated pipeline from biomass to bioproducts. The figure shows the summary of main research areas at the Great Lakes Bioenergy Research Center (GLBRC). Available at glbrc.org.*

To make all these products, it's necessary to know the composition of the plant biomass. Biomass includes cellulose, hemicelluloses, lignin, extractives, lipids, proteins, simple sugars, starches, water, hydrocarbons, ash, and other compounds. Plant biomass can be divided into three groups: 1º generation – plants that can provide sugar or starch, for example maize and sugar beet; 2º generation – plants, or materials (agricultural waste, for example) that are mainly composed of lignocellulosic biomass, such as wheat straw, sugarcane residue, sorghum stalks; and 3º generation: plants that are triglyceride-based, or, in other words, can produce oils, such as canola and sunflower, and also micro-algae. In this text, we are going to discuss one important type of plant biomass, lignocellulosic biomass, which is the most abundant organic substance on Earth.

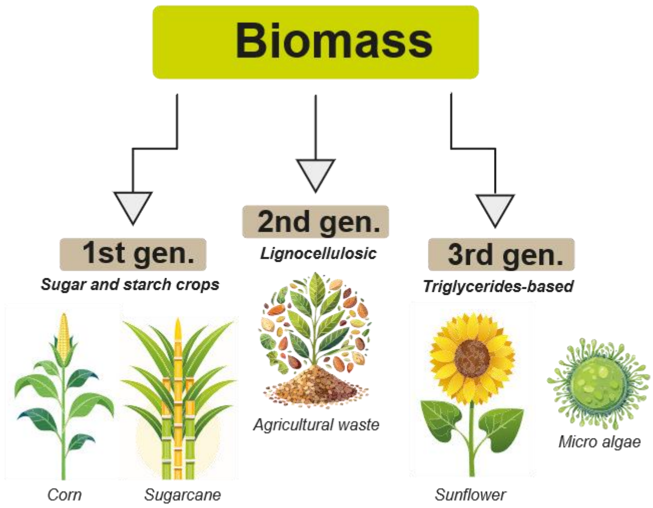

Figure 2: *Examples of types of biomass. The classification separates the biomass based on different compositions. 1st gen refers to plants rich in sugar and starch; 2nd gen refers to lignocellulosic material from agricultural waste, also rich in sugars; and 3rd gen refers to plants or microalgae rich in triglycerides, a type of fat that can be converted into biodiesel.*

The challenge and the solution: biomass digestibility and gene editing

The composition of all lignocellulosic biomass is based on three main components: **cellulose**, which contains glucose sugars; **hemicellulose**, which is important to facilitate plant growth and structure; and **lignin**, which is extremely important for development and support, as it is responsible for keeping plants standing. These three components are present in what we call the plant cell wall, a specialized form of matrix that surrounds all plant cells and is responsible for mechanical and structural support. Some plant cells have additional cell wall layers, a secondary cell wall, that provides further support but also serves as defense against pathogens and weather conditions, and aids the distribution of water and nutrients.

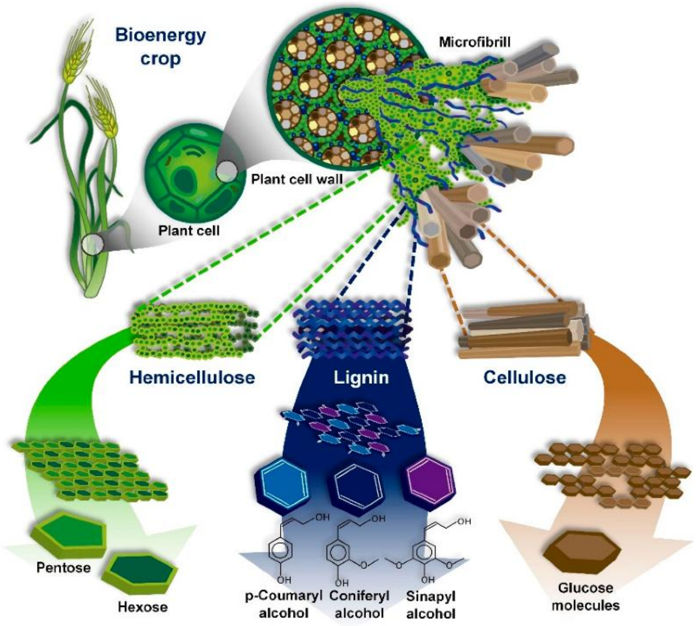

Figure 3: *Representation of the composition of the plant cell wall, showing the complexity of the cellulose-hemicellulose-lignin structure. Figure taken from Balagurusamy et al. (2019), available at https://www.mdpi.com/2076-3417/9/18/372.*

As we can see in Figure 3, the three components are forming a complex structure, making it hard to access the glucose sugars present in the cellulose, and the other sugars present in hemicellulose (pentose and hexose), all of which are valuable compounds for bioenergy production. The inaccessibility of these sugars is called recalcitrance: that is, the difficulty in deconstructing the cell wall, and consequently accessing the sugars, caused by the complexity of cell wall structure and the presence of lignin. The lignin itself can also be converted into valuable products. As such, the process of breaking down the cell wall to separate the components is important because it enables the access of the sugars from cellulose and hemicellulose, which can then be used to produce bioenergy, such as ethanol, and it also allows lignin to be separated and converted into other products, like adhesives, polymers, and plastics.

The separation of the cell wall components is currently achieved by using pretreatments. But the process is not ideal because such pretreatments often require high temperatures, high pressures, or high concentrations of acid or alkaline products, resulting in high energy consumption and the production of contaminated waste. One strategy to sidestep this challenge is to use gene editing techniques that allow us to change the composition and structure of the plant cell wall, making it more digestible or less recalcitrant, which means that the products can be accessed more easily and with less energy consumption.

Gene editing is present in our lives in a variety of forms. GMOs – genetically modified organisms - are present in all supermarkets. An example of this is the genetically modified maize and wheat present in vegetable oil, animal feed, snacks, refrigerants, etc. In the medical field, gene editing can be seen in some vaccines, as well as in insulin production and cancer therapy with genetically improved immune cells. Cleaning products can also contain modified enzymes. This technology can even be found in your clothes, where the cotton may be improved to be resistant to pathogens and pests.

In 2020, two amazing scientists were awarded the chemistry Nobel prize for their discovery related to gene editing. Jennifer Doudna and Emmanuelle Charpentier showed the development of the CRISPR-Cas9 technique as a genome editing tool. This discovery changed the way scientists do gene editing because it allows us to alter DNA sequences very precisely, opening the doors for different applications in gene therapy, disease treatment, and agricultural advances. The discovery was so remarkable because now we can design the system to act like genetic scissors, enabling us to “cut” the DNA at specific regions, making the process cheaper, faster, and accurate compared to other techniques.

Considering all this background, the main goal of our work at the PMT project is to improve biomass digestibility by using the CRISPR/Cas9 technique to change the sequence of the *PMT* gene, and make it dysfunctional. The hypothesis behind this work is that the *p*-coumarates, the ultimate product of the *PMT* gene, play a key role in lignin composition, so shutting off the gene, and consequently the protein, could lead to fewer *p*-coumarates present in lignin. This would allow scientists to alter lignin structure and composition and subsequent organization in the cell wall, resulting in an improvement in biomass digestibility.

The PMT gene: why does it play such a key role in digestibility?

PMT is the abbreviation for *p-COUMAROYL-CoA: MONOLIGNOL TRANSFERASE*. As the name suggests, the PMT is a transferase active during lignin biosynthesis. A transferase is responsible for transferring chemical functional groups from one molecule to another. Different transferases act in the plant cell wall, playing a crucial role in modifying and building the complex structure of the cell wall. Consider the transferases as a bricklayer, always fitting pieces together and reinforcing the cell wall structure. They will take a piece from one place - some sugar, for example - and put it in the correct spot during the construction of the cell wall. Without the action of these constructors, the wall would be fragile and not strong enough to give the necessary resistance to protect the cells against pathogens and environmental conditions.

In grasses like Sorghum bicolor, or Sorghum, the PMT acts in the lignin, transferring the *p*-coumarates (also called *p*CA) from a molecule called pCA-CoA to the lignin, specifically into the lignin building blocks. Lignin is mainly composed of three units: S – syringyl, H - p-hydroxyphenyl, and G – guaiacyl. During the developmental stage of the plant, these units are decorated, or complemented, by the PMT, adding the pCA in the correct locations for each lignin block.

What is interesting about the PMT is that the enzyme shares the same substrate, with another important transferase, FMT, or feruloyl-CoA monolignol transferase, and this one is particularly interesting for cell wall digestibility. The substrate is the initial product where the transferase takes one functional group to transfer to a different molecule; in other words, it is where the bricklayer takes the bricks; and PMT and FMT take bricks from the same place. The FMT is responsible for producing a lignin precursor, called monolignol ferulate, which contains ester bonds that are weaker and easily broken. When integrated into the lignin, these ester bonds create the so-called “zip-lignin”, a type of lignin that could be “zipped”, or easily opened, because of the higher presence of weak ester bonds. Because of these weaker bonds, the plant’s cell walls can be broken down with weaker pretreatments, making the deconstruction process much more energy efficient.

Now, you may already get the idea: what if we extinguish the activity of the PMT enzyme so it no longer competes for substrate with the FMT? In other words, what if we fire the PMT from our company and leave the FMT with all the bricks available? This could lead to the FMT producing more ferulate, making more ester bonds in the zip-lignin, which could make the biomass deconstruction process more efficient.

You may wonder about other strategies at this point, and I want to address these concerns. First, why don't we simply reduce the lignin amount in plants? The explanation for that is that reducing too much lignin may cause negative effects, such as impacting plant growth and support, making them not strong enough to stay upright, as shown in the next figure.

Figure 3: *In this strategy, Professor Rebecca Smith knocked out a lignin biosynthesis gene, resulting in reduced lignin content. We can see that the mutant plants, on the right side, are very different from the wild type, on the left. Figure taken from Smith et al., (2013), available at* *https://pmc.ncbi.nlm.nih.gov/articles/PMC3877792/*.

The second strategy that you may be thinking about is: why haven’t you overexpressed the *FMT* gene to improve ferulate production? We are doing it! We are growing FMT overexpression plants, and the plan is to cross those plants with the PMT plants, to get the best of both worlds: no *p*-coumarates being formed by the disrupted PMT enzyme, and more ferulates being formed by the *FMT* gene overexpression. But for now, let's get back to the PMT project to look at our exciting findings.

Improving digestibility with the PMT disruption

Our transgenic CRISPR/Cas9 *Sorghum bicolor* plants grew in the UW-Madison greenhouses during 2024. After harvesting, I started to analyze different aspects of the plant biomass, focusing on how the disruption of the *PMT* gene could impact the plant cell wall.

The first step was to check the sequence of the *PMT* gene in our mutants generated by the genetic transformation. Using sequencing tools, I found one mutant with a single base deletion; in other words, it was possible to remove one base of the gene sequence, resulting in the disruption of the respective PMT protein. With that deletion, we cause a mutation, resulting in a change in the protein structure. Each group of three bases codifies one amino acid, so when we remove one base, it changes the "reading" of the protein, with a totally different amino acid sequence.

The second step was checking if the plants were still producing *p*-coumarates and where these *p*CAs were located. I found that our mutant has no *p*CA in the lignin composition, with a small amount still present in the cell wall, attached to hemicellulose, but much less than the wild type. To test the digestibility of the biomass, I did a sugar release experiment, testing different concentrations of pretreatment, where I measured the amount of sugar released from the mutant and the wild type over time. I found that the mutant has more sugars being released from the biomass at all times tested, at very low concentrations of NaOH, which is what I used for the pretreatment. I tested different concentrations of NaOH, and found that low concentrations are enough to remove the lignin. The result indicates that with more sugar being released from these genetically modified plants, we can produce more biofuels, using less energy for the biomass deconstruction process.

The third step was to check if this new, modified biomass could be converted into biofuels. We are currently testing fermentation experiments with a collaborator group from our research center. Using the biomass from our plants, we want to check if it's possible to produce more ethanol by the fermentation process, where yeast is used to break down the plant sugars and convert them into ethanol. Initially, we were able to see that yes, our mutant plants are more efficient in producing ethanol than the wild type, but we still need to confirm these findings.

It's also important to highlight that our plants have no negative growth effects and have grown beautifully, very similar to the wild-type sorghum. These results are aligned with our expectations that the *PMT* gene has a significant role in biomass digestibility.

Final takeaways

Climate change is a big challenge that we must deal with. Finding solutions that can help us improve our lives and protect the environment is more than necessary; it is mandatory. Science has helped us understand our environment and find solutions since the beginning of our civilization. Taking advantage of all the technologies that we have available for scientific research to help us against climate change is the best way to make our society less harmful to our planet.

Gene editing tools are unique and extremely powerful tools that we can use for different purposes, including producing bioenergy and bioproducts, to create renewable energy options that can help us reduce our dependence on fossil fuels.

In conclusion, I really hope that my research can lead to a better understanding of the cell wall in plant biomass, create new opportunities for producing bioenergy, and, finally, help us improve our world for future generations.