

Using the Zip-Lignin Strategy to Build the Optimal Sorghum Biofuel Crop

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Project Goals: Increase the levels of zip-lignin in *Sorghum bicolor* plants to improve the cell wall digestibility.

Plants have large BAHD acyltransferase families that perform a wide range of enzymatic tasks in primary and secondary metabolism. Acyl-CoA monolignol transferases, which couple a CoA substrate to a monolignol through an ester linkage, represent a newer class of such acyltransferases. The resulting conjugates may be used for plant defense, but are, importantly, also used as ‘monomers’ for lignification, in which they are incorporated into the growing lignin polymer chain. These conjugates can add value to the lignin in the form of ‘clip-off’ phenolic acids. *p*-Coumaroyl-CoA monolignol transferases (PMT) increase the production of monolignol *p*-coumarates, thereby increasing the value of lignin with *p*-coumarate and its byproducts. Other conjugates can improve cell wall digestibility by incorporating mild-alkali-cleavable ester bonds into the lignin polymer backbone. Feruloyl-CoA monolignol transferases (FMT) improve cell wall saccharification, after mild pretreatments, by catalyzing the production of monolignol ferulate conjugates; their incorporation into the lignin generates so-called “zip-lignins”. Our previous work in *Brachypodium distachyon* and *Zea mays* has demonstrated that there is competition between different monolignol transferase enzymes for substrates, and accumulating pools of substrates for the enzymes is important for maximizing monolignol transferase activity. In *Brachypodium*, knocking out the native *PMT* gene and introducing an *FMT* gene resulted in the highest detectable levels of monolignol ferulates that we have observed to date. The level of monolignol ferulates was significantly higher than in the plants that only had increased FMT activity. In maize, accumulation of the FMT substrate feruloyl-CoA through knock-down of a lignin biosynthetic gene, *CINNAMOYL-CoA REDUCTASE (CCR)*, also significantly increased the production of zip-lignin and improved the cell wall digestibility (Smith et al., 2017). We hypothesize that the combination of knocking out the native *PMT* gene and overexpressing the native *FMT* in *Sorghum bicolor* in the *CCR* down-regulated background will yield *Sorghum* lines with the highest potential as bioenergy crops.

The *Sorghum bicolor* FMT and PMT enzymes were unknown, and therefore we used phylogenetics to discover potential FMT and PMT enzymes from *Sorghum* based on their similarity to previously identified rice FMT and PMT enzymes. The enzymes were synthesized using the wheatgerm cell-free translation system and tested for monolignol transferase activity. Based on these results, we have identified putative FMT and PMT enzymes in *Sorghum* and have compared their activities to those of known monolignol transferases. These putative *FMT*

and *PMT* genes encoding the enzymes were transformed into *Arabidopsis thaliana* to test their activities and abilities to biosynthesize monolignol conjugates for lignification *in planta*. *Arabidopsis* does not naturally produce monolignol conjugates, which simplifies the detection of the novel compounds. The presence of monolignol ferulates and monolignol *p*-coumarates on the lignin of these transformants indicated that the targeted FMTs and PMTs are acting as functional, and efficient, feruloyl-CoA and *p*-coumaroyl-CoA monolignol transferases within plants. Constructs have been developed and transformed into *Sorghum bicolor* to overexpress the native *FMT*, knock-down the native *PMT* using CRISPR-Cas9 technology, and knock-down the native lignin *CCR* gene using CRISPR-Cas9. When the best lines for each of these transformants have been established, the lines will be crossed to generate an *FMT* overexpression/*PMT* CRISPR/*CCR* CRISPR triple-transgenic line.

References

Smith, R.A., Cass, C.L., Mazaheri, M., Sekhon, R.S., Heckwolf, M., Kaeppler, H., de Leon, N., Mansfield, S.D., Kaeppler, S.M., Sedbrook, J.C., Karlen, S.D., and Ralph, J. (2017). Suppression of CINNAMOYL-CoA REDUCTASE increases the level of monolignol-ferulates incorporated into maize lignins. *Biotechnol Biofuels* **10**, 109: 101-110.

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