

Identification and Characterization of a New Set of Monocot BAHD Monolignol Transferases

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Project Goal: Identify new FMT and PMT BAHD acyltransferases from bioenergy monocot species.

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 as the phenolate esters can be easily clipped off to provide a valuable aromatic commodity chemical stream. *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”. The prevalence of monolignol ferulate conjugates in cell walls throughout the angiosperm clade, particularly throughout the commelinids, suggests that there are more FMT enzymes yet to be uncovered. We used phylogenetics to find potential FMT and PMT enzymes from *Sorghum bicolor* and *Panicum virgatum* based on their similarity to previously identified rice FMT and PMT enzymes. The enzymes were synthesized using the wheat germ cell-free translation system and tested for monolignol transferase activity. Based on these results, we have identified putative FMT and PMT genes for sorghum and switchgrass and have compared their activities to those of known monolignol transferases. Enzyme kinetics of the new and previously identified FMT and PMT enzymes was performed to ascertain which enzyme(s) had the highest catalytic efficiency. These putative FMTs and PMTs were transformed into *Arabidopsis thaliana* to test their activities and to discern the plants’ ability to biosynthesize monolignol conjugates *in planta*. *Arabidopsis* does not naturally produce monolignol conjugates, which simplifies the detection of the novel compounds. The presence of ferulates and *p*-coumarates on the lignin of these transformants indicated that the putative FMTs and PMTs are acting as functional, and efficient, feruloyl-CoA and *p*-coumaroyl-CoA monolignol transferases within plants.

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