Suppression of CINNAMOYL-CoA REDUCTASE increases the level of monolignol-ferulates in maize lignins

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Project Goals: To determine how to maximize the utilization of monolignol ferulate conjugates in cell wall lignification, where they introduce readily cleavable ester linkages ('zips') into the lignin polymer backbone in ways that significantly improve biomass-processing energetics.

One of the most promising alternatives to fossil fuels involves generating ethanol (or second-generation biofuels) from plant cell wall polysaccharides (sugars), such as cellulose and hemicelluloses. The problem with this strategy is that the production of cellulosic ethanol is not yet as efficient or cost-effective as fossil fuels. The main reason for this is the harsh treatments required to remove the recalcitrant cell wall phenolic polymer lignin in order to access the cell wall sugars. For this reason, decreasing or altering lignin content in potential biofuel crops has received a lot of attention from the scientific community. Lignin, though detrimental to biofuel processing, is essential to the support and defense of the plant body and decreases in lignin content are therefore usually associated with pendant stems, increased susceptibility to pathogens and reduced biomass yield. The digestibility of the cell walls can be improved by introducing labile ester bonds, which are broken under weak base treatment at room temperature, into the lignin backbone. The FERULOYL-CoA MONOLIGNOL TRANSFERASE (FMT) enzyme, which is naturally found in many plants, uses feruloyl-CoA and monolignols to synthesize the ester-linked monolignol ferulate conjugates. A mutation in the first lignin-specific biosynthetic enzyme, CINNAMOYL-CoA REDUCTASE (CCR), results in an increase in the pool of feruloyl-CoA. Maize (*Zea mays*) has a native putative FMT enzyme, and its *ccr* mutants produce an increased pool of feruloyl-CoA that can be used for conversion to monolignol ferulate conjugates. The increase in conjugates correlates with an improvement in the digestibility of maize stem rind tissue.

Publications

1. “Monolignol ferulate transferase introduces chemically labile linkages into the lignin backbone” Wilkerson, C. G.; Mansfield, S. D.; Lu, Fachuang; Withers, S.; Park, J.; Karlen, S. D.; Gonzales-Vigil, E.; Padmakshan, D.; Unda, F.; Rencoret, J; Ralph, J *Science*, **2014**, *344*, 90−93.

2. “Monolignol-ferulate conjugates are naturally incorporated into plant lignins” Karlen S.D., Peck M.L., Zhang C., Smith R.A., Padmakshan D., Helmich K.E., Free H.C.A., Lee S., Smith B.G., Lu F., Sedbrook J.C., Sibout R., Grabber J.H., Runge T.M., Mysore K.S., Harris P.J., Bartley L.E., and J. Ralph. Science Advances, **2016**, 2, e1600393

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