Gene Expression and Biochemical Analysis of a Bioenergy Sorghum Panel for Lignin Content

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Future production of renewable and sustainable transportation fuels will require a consistent supply of lignocellulosic biomass produced specifically for energy generation. However, due to technological limitations, which include the interfering effect of cell wall lignin in the biomass conversion process, large scale production of lignocellulosic biofuels has yet to become economically feasible. Therefore, the selection of dedicated crops presenting improved cell wall composition for biomass conversion is expected to make commercial biofuel production possible. Due to its high biomass yield, low water and fertilizer requirements and tolerance to mineral, drought and heat stress, sorghum has the potential to become one of these dedicated crops. In this work we applied gene expression and biochemical analysis to identify sorghum genotypes showing lower lignin content, which has been shown to increase biomass conversion efficiency. A genetically diverse sorghum panel comprising 100 accessions was screened for lignin content and other cell wall components, using standard biochemical methods. Lignin content varied from 2 to 11% on the basis of total dry matter and averaged 5.8%. Also, in order to better understand lignin synthesis in sorghum, we have used Real-Time PCR to study the expression of sorghum homologs of key genes involved in the lignin biosynthesis pathway. Five of these genes, C3H1, C3H2, HCT, COMT and F5H, appear to be co-regulated as suggested by highly correlated expression levels in a subset of 35 accessions of the diversity panel. In addition, expression levels of genes, which corresponding enzymes act early within the pathway (C3H and HCT), showed high and positive correlation coefficients. Among the panel of accessions were *bmr*-6 (*brown midrib*) mutants, which have lower levels of lignin. One of them (TX2784R *bmr*-6) and its corresponding non-mutant line (TX2784R) were also analyzed for their fiber and lignin content, as well as gene expression related to the lignin pathway. The *bmr*-6 gene encodes the CAD enzyme, which acts at a later step within the pathway. The mutant and non-mutant materials were significantly different for lignin and fiber content, with a 50% reduction in lignin content in the mutant, as previously reported. The gene expression results support the hypothesis that the genes are co-regulated since the mutation in the CAD gene is followed by a down-regulation of other genes in the pathway, such as C3H, HCT and COMT. Expression analysis of five other *bmr*-6 lines showing different genetic backgrounds supports these findings. In the future, the remaining genes of the lignin pathway will be evaluated for their expression and those showing differential expression among accessions that have contrasting lignin content will be validated by association analysis in order to identify superior alleles involved in lignin content.

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