

Variation in Sequences and Expression Levels of Lignin Associated Genes in Alfalfa Stems

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Alfalfa is the most important forage legume in the world and the fourth most important crop in the United States. In addition to its value as a forage crop, alfalfa is a promising lignocellulosic feedstock due to its high biomass yield and established agricultural practices. Lignin is the major component of plant cell walls. Lignin provides mechanical support to the plant and provides a framework for the transport of water and nutrients. The lignin content and composition of the feedstock affect forage quality, animal digestibility and conversion efficiency of lignocellulosic biomass to biofuels. Reducing lignin content increases saccharification efficiency during cellulosic ethanol production. The objectives of this study were to identify genes and transcription factors involved in lignification and to evaluate variation in both sequence and expression levels of these genes in alfalfa genotypes contrasting for lignin content. Genes and transcription factors involved in lignin biosynthesis were identified in the annotated genomes of four plant species (Arabidopsis, corn, poplar and Medicago truncatula) and used to identify 342 unique alfalfa TCs and a total of 20,822 single nucleotide polymorphism (SNP) markers were identified in the stem transcriptome sequences of 27 alfalfa genotypes. High-resolution melting (HRM) analysis was used to validate over 94% of a subset of the *in silico* predicted SNPs located on multiple alfalfa chromosomes and their corresponding allelic dosages. Lignin content variation was identified in four alfalfa genotypes based on UV-microscopy and wet chemistry analysis. Lignin content was negatively correlated with forage quality, total sugar content and processing efficiency in the alfalfa samples evaluated. Quantitative RT-PCR was used to assess the levels of gene expression that were associated with differences in lignin content of the genotypes evaluated. Gene-derived SNP markers were integrated onto existing linkage maps in alfalfa and are being used to identify genomic regions relevant to lignin content in segregating populations. The identification of SNP markers in genes affecting lignin accumulation can be used to develop alfalfa cultivars with improved forage quality and processing efficiency by implementing molecular breeding approaches.