Forage quality improvement in alfalfa by down-regulation of cytochrome P450 enzymes related to the lignin biosynthesis pathway

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Lignin is a structural material found in all plants. It is a major structural component of secondarily thickened plant cell walls. The presence of excessive lignin in plant feedstock reduces animal digestibility and negatively affects animal productivity. The reduction in natural lignin content in important forage crops, like alfalfa, will increase profitability for agricultural producers, enhance animal productivity and reduce the environmental impacts of large-scale dairy operations. Manipulation of lignin content requires a better understanding of the various enzymatic and regulatory processes involved in monolignol biosynthesis and lignin polymerization and deposition. An antisense-mediated gene down-regulation approach, coupled with lignin compositional analysis, metabolite profiling and forage digestibility analysis, was used to decipher the regulatory architecture of the monolignol pathway. Transgenic alfalfa lines down-regulated for "cinnamate 4-hydroxylase" (C4H), "4-coumarate 3-hydroxylase" (C3H), "ferulate 5-hydroxylase" (F5H), phenylalanine ammonia-lyase (PAL) and hydroxycinnamoyl transferase (HCT) were obtained. These lines showed significant differences in lignin content and composition. The *p*-hydroxyphenyl (H) lignin content was increased by ten-fold in few of the C3H and HCT down-regulated lines and the H/total lignin ratio increased by more than twenty-fold in many of these lines. Some of the C3H and HCT lines were phenotypically shorter than the controls. A loss of purple-blue flower pigmentation was seen in all the C4H lines. One of the C4H lines showed a five-fold decrease in total lignin and more than three-fold decrease in syringyl/guaiacyl (S/G) ratio. Similarly, there was a decreased S/G ratio in the F5H down-regulated alfalfa lines**.** Furthermore, lignin composition in plants with reduced C4H enzyme activity was different from that in plants with reduced PAL activity. Metabolic profiling of C3H transgenic lines showed an increase in wall-bound *p*-hydroxybenzaldehyde and a decrease in vanillin. *In situ* and *in vitro* digestibility and forage quality parameters such as ADF, NDF and ADL, were analyzed in several of the C3H, C4H and F5H lines. Forage digestibility was strikingly increased in C3H and C4H lines but not in F5H lines. There was a strong negative relationship between *in situ* digestibility and ADL level across all transgenic lines, irrespective of lignin composition. Segregating F1 progeny from selected C3H and C4H down-regulated lines were evaluated in the field (2005, West Salem, WI). Near Infrared Spectroscopy (NIRS) analysis of the lower portion of the stem from the progeny of these lines consistently showed lower lignin content and higher digestibility. Analysis of these lines showed that the key to improving digestibility in alfalfa is the reduction in lignin level rather than changes in lignin composition. Data from the analysis of these lines will be presented.