The application of RNA interference technology to modulate the expression of key enzymes in the lignin biosynthetic pathway

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Lignification of secondary cell walls during plant development has been identified as the major factor limiting forage digestibility and thus animal performance. Lignins are complex phenolic polymers which are associated with the polysaccharides of the cell wall in specific plant cells primarily in mature stems. In alfalfa, the lignin polymer comprises guaiacyl (G) units and syringyl (S) units. Several recently published studies have demonstrated that transgenic plants with down regulated caffeic acid 3-*O*-methyltransferase (COMT) and caffeoyl CoA 3-*O*-methyltransferase (CCOMT) had reduced lignin content and altered lignin subunit composition. These studies all utilized antisense mediated technology to achieve gene suppression. While these studies have been successful in down regulating the target gene and producing transgenic alfalfa with significantly reduced lignin levels the efficiency is limited. Typically only 5-10% of the transgenic events in these studies exhibited significant levels of down regulation of the target enzyme and corresponding transcript. This limits our ability to identify plants that are suitable for commercial development.

RNA interference technology offers a new and powerful methodology for efficiently down regulating genes in the lignin biosynthetic pathway. RNAi constructs targeting COMT and CCOMT were introduced into alfalfa and the resulting populations of transgenic plants were evaluated for levels of the target enzymes and their effect of lignin content and composition. Enzyme activities were reduced to 10-15% of the corresponding wild type level in many events with the majority of events tested exhibiting at least 50% down regulation. Preliminary data on the lignin content and compositional analysis on these plants grown under greenhouse conditions will be discussed. In an accompanying paper (Riday et al 2006) data on the field evaluation of these plants will be presented

References

Riday, H., Whalen, D., Bouton, J., Temple S. J., McCaslin, M., and Dixon, R.A. (2006) Agronomic field evaluation of caffeic acid 3-*O*-methyltransferase and caffeoyl CoA 3-*O*-methyltransferase downregulated alfalfa. 39th North American Alfalfa Improvement Conference, St. Paul/Minneapolis, MN, July 16-19.