

Improving forage quality of tall fescue (*Festuca arundinacea*) by genetic manipulation of lignin biosynthesis

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Introduction Lignification of plant cell walls is a major factor limiting forage digestibility and concomitantly animal productivity. Improvement in forage grass cell wall digestibility has become an important goal of many plant-ruminant animal research programs. Lignins are complex phenolic heteropolymers associated with the polysaccharidic components of the wall in specific plant cells. Lignin in forage grasses comprises guaiacyl (G) units derived from coniferyl alcohol, syringyl (S) units derived from sinapyl alcohol, and *p*-hydroxyphenyl (H) units derived from *p*-coumaryl alcohol. Cinnamyl alcohol dehydrogenase (CAD) and caffeic acid *O*-methyltransferase (COMT) are key enzymes involved in lignin biosynthesis. Tall fescue is the predominant cool-season forage grass in the United States.

Materials and methods Lignification in stems of tall fescue (cv. Kentucky 31) were analyzed at three elongation and three reproductive stages. CAD and COMT cDNA sequences were cloned from tall fescue by screening a cDNA library. Transgenic tall fescue plants carrying either sense or antisense CAD and COMT gene constructs were obtained by microprojectile bombardment of single genotype-derived embryogenic suspension cells. Detailed molecular and biochemical analyses were carried out for the transgenic plants.

Results Anatomical comparisons of tall fescue stems at six different developmental stages revealed a gradient increase in lignification with progressive maturity. Digestibility is negatively correlated with lignin content, S lignin content, as well as S/G ratio. Relative *O*-methyltransferase activities increased during stem development, and in parallel with the lignification process of stem. The expression of COMT and CAD genes increased during the stem elongation stage and remained at high levels during the reproductive stages. The changes at anatomical, metabolic and molecular levels during plant development were closely associated with lignification and digestibility (Chen *et al.*, 2002).

Analysis of transgenic tall fescue plants by northern hybridization revealed that several plants had severely reduced mRNA levels. Enzyme activity analysis using different substrates showed that these transgenic plants had significantly decreased COMT or CAD enzymatic activities. These CAD and COMT down-regulated tall fescue plants had reduced total lignin content and altered lignin composition. No significant changes in cellulose, hemicellulose, neutral sugar composition, *p*-coumaric acid and ferulic acid levels were observed in the transgenic plants. In vitro dry matter digestibility increased by 7.2% to 10.5% in the transgenic lines, thus providing novel germplasm to be used for the development of grass cultivars with improved forage quality (Chen *et al.*, 2003, 2004).

Conclusions Consistent and closely related molecular and biochemical data demonstrated that the transgenic CAD and COMT lines were down-regulated in their lignin biosynthesis and had improved forage digestibility. Genetic manipulation of lignin biosynthesis is an effective approach to improve digestibility of grasses.

References

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