RE-DESIGNING ALFALFA FOR IMPROVED DAIRY PERFORMANCE

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For years plant scientists working on forage improvement and animal scientists working on ruminant nutrition tended to work independently, with an occasional interdisciplinary collaboration bridging scientific specialties and focused on a common goal. New tools in plant biology/biotechnology have greatly broadened the potential for the creation of new plant types with novel forage quality traits, offering a unique opportunity for a broader collaboration between plant and animal scientists. In 2002 three groups came together to start an inter-institutional, interdisciplinary research program to use plant biotechnology to improve key alfalfa characteristics that affect forage quality and efficiency of use by dairy cows. The Consortium for Alfalfa Improvement (CAI) was formed by the U.S. Dairy Forage Research Center, the Samuel Roberts Noble Foundation and Forage Genetics bringing together acclaimed experts in plant biochemistry, molecular biology, plant breeding, agronomy and ruminant nutrition.[2] This interface between animal and plant scientists; and public and private institutions is recognition of both the complexity of the tasks at hand, and the challenges of translating ideas into commercial products benefitting alfalfa and dairy producers.

The group has focused their collaborative research on two areas: increased fiber digestibility and improved efficiency of protein utilization. The starting point for both projects was basic research on manipulation of key plant metabolic pathways to create transgenic plants with unique characteristics. The ongoing plan is to analyze these transgenic plants for chemical composition, *in vitro* characteristics (e.g. Neutral Detergent Fiber Digestibility, NDFD or protein degradability), agronomic performance, and animal performance in controlled feeding trials.

INCREASED FIBER DIGESTIBILTIY

A one unit increase in the in-vitro digestibility of NDF has been shown to be associated with a 0.37 lb/day increase in dry matter intake (DMI) and a 0.55 lb/day increase in 4% fat corrected milk yield per cow (Oba and Allen, 1999). Lignin is an indigestible cell wall component that increases in content with advancing maturity of the alfalfa plant, and is the key factor in limiting cell wall (NDF) digestibility by ruminant animals. Noble Foundation Scientists have been working on biotech manipulation of the lignin pathway since the early 1990's (Dixon and Reddy, 2003). This work has been the foundation of the Reduced Lignin project at the CAI. "Gene knockout" is a biotech process whereby expression of a specific individual gene is greatly reduced. Knocking out a gene coding for a specific lignin biosynthetic enzyme generally results in transgenic plants with significant changes in lignin content and/or lignin composition. The CAI Reduced

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[2] Pioneer HiBred International joined the CAI in 2007, Land O'Lakes/Purina Feed and Monsanto Company have been collaborators in various of the reported CAI projects.

Lignin project has now characterized multiple gene knockouts in the lignin biosynthetic pathway and has identified gene candidates with 10-20% improvement in fiber digestibility, and normal agronomic performance. Agronomic trials have shown that NDFD of Reduced Lignin Alfalfa harvested at 10% bloom is equal to or better than conventional alfalfa harvested seven days earlier at the early bud stage. This potential for delayed harvest increases flexibility in harvest management and potentially reduces the number of harvests per year, without sacrificing forage quality.

Digestibility studies conducted with lactating dairy cattle at Land O'Lakes' Longview Animal Nutrition Center near St. Louis, MO, and with lambs at the US Dairy Forage Research Center in Madison, WI, have confirmed the improvements in NDFD predicted by NIR as a result of down-regulating two key enzymes in the lignin biosynthetic pathway. When compared to its non-modified control, feeding alfalfa containing one of the more promising reduced lignin alfalfa constructs to high producing lactating cows resulting in a 10% improvement in total tract NDF digestibility. Related digestibility work with lambs showed similar benefits in improved total tract NDF digestibility observed with lactating cows. This provides support that lambs can be used as an effective alternative to larger ruminants in validating lab data that suggest improved NDF digestibility as a result of down-regulating lignin biosynthesis. This takes on greater importance due to the limited amounts of Reduced Lignin alfalfa that is available for animal testing.'-

IMPROVED EFFICIENCY OF PROTEIN UTILIZATION

Alfalfa has high protein content, but rapid rumen degradation of alfalfa protein leads to inefficient use. There are two parallel CAI projects designed to improve protein use efficiency: tannin alfalfa and PPO alfalfa. USDFRC scientists predict both approaches will significantly decrease the need for purchased protein supplements and decrease nitrogen losses to the environment on the dairy. Condensed tannins bind with plant proteins, slowing the rate of protein degradation in the rumen. Tannin containing forages (e.g. birdsfoot trefoil) have a slower rate of protein degradation in the rumen, and higher Rumen Undegradable Protein (i.e. bypass protein). Alfalfa produces condensed tannins in seed coats, but not in forage tissue. .Noble Foundation Scientists are working to modify alfalfa to produce tannins in leaves and stems (Tian, et al., 2007). USDFRC models estimate that alfalfa containing condensed tannins will significantly decrease feeding of protein concentrates and reduce N-losses on dairy farms by 40% (Grabber, et al., 2004).

Post-harvest protein proteolysis of alfalfa protein during the making of silage often results in >50% NPN in alfalfa haylage. Red clover, however, has significantly less post-harvest proteolysis during ensiling than alfalfa. USDFRC scientists have identified a key enzyme (polyphenol oxidase, PPO) related to this benefit and have expressed this red clover gene in alfalfa. PPO and specific *o*-diphenol substrates combine to form a quinone, which effectively blocks post harvest proteolysis. (Sullivan and Hatfield, 2006) Further metabolic modifications are underway to replicate the red clover PPO system in alfalfa.

SUMMARY

Plant biotechnology offers exciting new opportunities for crop improvement. In particular we expect new traits in alfalfa will greatly improve the crop as a source of fiber and protein for high producing dairy cows … fundamentally changing, or re-inventing the crop (Martin, et al., 2005). Animal feeding trials will be needed to validate *in vitro* proof of concept, and to design feeding rations that optimize value of these novel forages for dairy producers. The CAI is an example of the type of interdisciplinary research structure required to design and implement the required research, and to eventually commercialize this exciting technology.

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