NEW STRATEGIES FOR FORAGE QUALITY IMPROVEMENT IN ALFALFA

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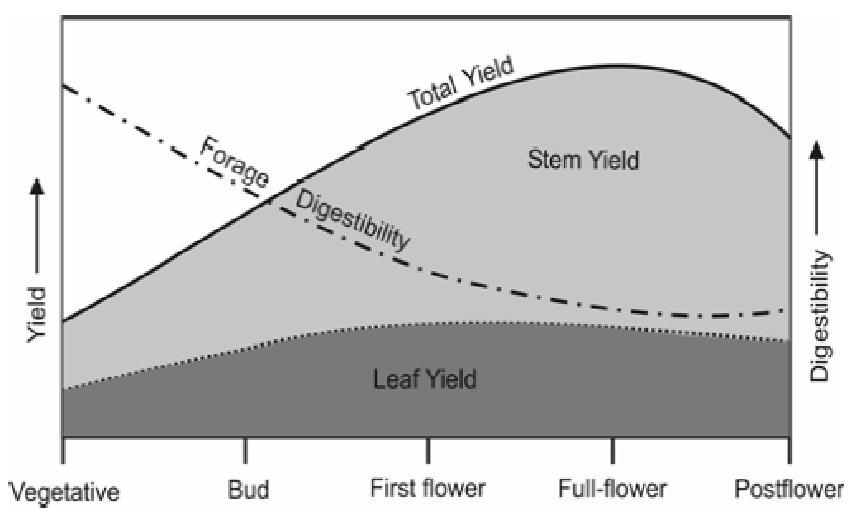
INTRODUCTION

The research presented here is the result of a collaboration of government, industry and private non-profit organizations that formed in 2002 as the Consortium for Alfalfa Improvement. Scientists from the U.S. Dairy Forage Research Center/ARS-USDA, Forage Genetics International, Pioneer HiBred and The Samuel Roberts Noble Foundation, Inc., have been conducting a focused biotech research effort on improving alfalfa forage quality through improvements in fiber digestibility and efficiency of protein utilization.

BACKGROUND

Reduced Lignin Alfalfa. As alfalfa plants mature from vegetative to bloom stage, cell wall content (NDF) increases and the digestibility of cell walls (NDFD) decreases (figure 1). The decrease in NDFD can be primarily attributed to an increase in stem to leaf ratio and consequent increase in lignin content. Lignin is indigestible per se, and cross-links with the cellulose and hemicellulose which also decreases the digestibility of these cell wall components.

Figure 1. Relative forage yield and quality at different alfalfa growth stages.



Source: Balancing Yield, Quality and Persistence. Steve Orloff and Dan Putnam 2004 Proceedings CA Alfalfa Symposium

There are several steps in the process of lignin synthesis in alfalfa. This lignin biosynthetic pathway involves twelve different enzymes, each required for a specific step in the pathway

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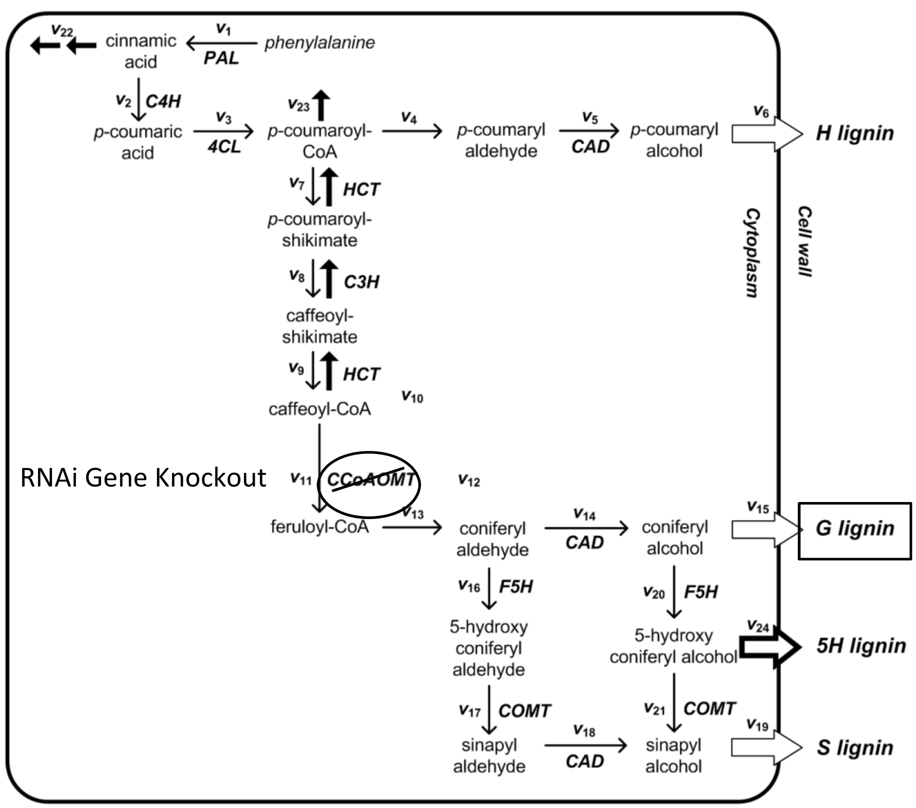
[1] Forage Genetics International – Prior Lake, MN (MM), Nampa, ID (PR), and Davis, CA (JH). **In**: Proceedings, 2014 California Alfalfa, Forage, and Grain Symposium, Long Beach, CA, 10-12 December, 2014. UC Cooperative Extension, Plant Sciences Department, University of California, Davis, CA 95616. (See http://alfalfa.ucdavis.edu for this and other Alfalfa Symposium Proceedings.)

(figure 2). Noble Foundation scientists used gene suppression technology to “knock out” specific genes that code for specific pathway enzymes. FGI scientists took the lead in evaluating elite transgenic RL plants in laboratory, greenhouse and field trial for lignin content, lignin composition, NDFD and agronomic characteristics. Of the several knock outs tested, there was only one specific knockout that resulted in the desired product concept – a decrease in lignin content and increase in NDFD without affecting key agronomic traits such as forage yield, multiple pest resistance, persistence or lodging. FGI produced hundreds of transgenic plants (i.e. events) designed to suppress this specific lignin pathway gene, and identified a single event that best captured the desired RL product concept. In addition to meeting all of the necessary agronomic requirements the single event also met the strict metrics required for regulatory approval. From a single transformed plant carrying this elite RL event FGI breeders developed RL populations designed for use in regulatory trials and also began a process of integrating the RL trait into multiple proprietary breeding lines.

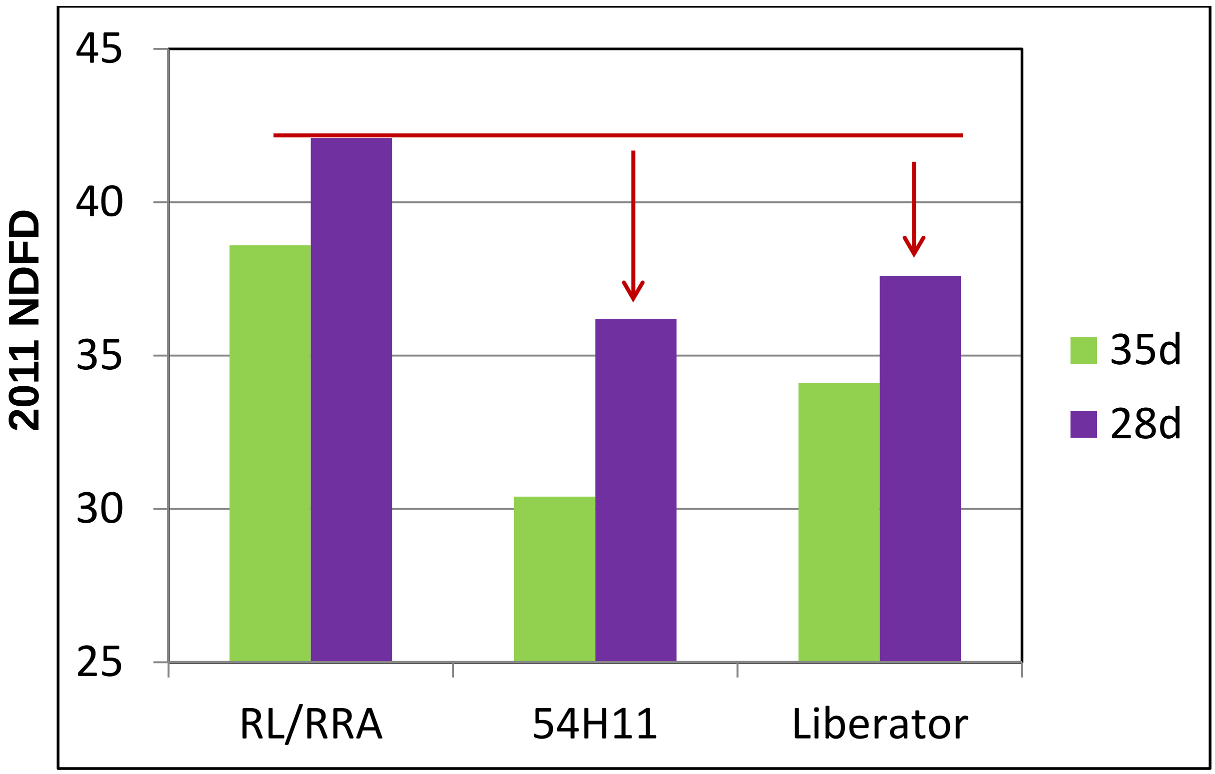
Figure 2. RNAi gene suppression of caffeoyl-CoA O-methyltransferase (CCoAOMT) enzyme in lignin biosynthetic pathway.



Adapted from: Yun Lee, et al, 2011.

During RL trait development FGI scientists demonstrated that plants containing the commercial RL event (RL alfalfa) had a 15-20% decrease in lignin content and a 10-15% increase in NDFD and Relative Forage Quality (RFQ) when compared to related lines without the RL event. They also learned that the rate of change in quality with advancing maturity in RL alfalfa was significantly different than for conventional alfalfa. This difference allows more flexibility in harvest management with a broader harvest window for the production of high quality alfalfa hay/haylage. In various tests designed to better understand this phenomenon, the FGI trait development team learned that RL alfalfa with harvest delayed by seven days had about the same NDFD as conventional alfalfa harvested a week earlier. This was substantiated in cutting management trials comparing RL and conventional alfalfa harvested at 28 day (~late bud) vs 35 day (~10% bloom) cutting intervals (Figure 3).

Figure 3. NDFD in RL Alfalfa vs Commercial checks in 3 vs 4 cut management West Salem, WI (established 2010, harvested in 2011 and 2012)



Multiple university- conducted cutting management trials with conventional alfalfa have consistently demonstrated that while early and more frequent harvest is often required for the production of high quality hay suitable for feeding to high producing dairy cows, forage yield and stand persistence is improved when harvest is delayed until the 10% bloom stage (Blank, et al, 2001, Undersander et al, 2011). For example, trials conducted at the University of Wisconsin have shown a 15-20% forage yield advantage for a three cut vs four cut management system over a four year rotation (Undersander, personal communication). However forage quality of conventional alfalfa in the UW three cut treatment was significantly lower than in the four cut treatment. This “forage yield vs forage quality tradeoff” defines the dilemma for most alfalfa forage producers in designing their cutting management strategy. The potential for delayed harvest of reduced lignin alfalfa without sacrificing forage quality provides a potential "high yield/high quality" solution to this historic dilemma and a potential for fewer cuts/season and lower harvest costs.

In October 2014, the CFIA (Canadian Food Inspection Agency) deregulated the reduced lignin trait in Canada. On 10 November, 2014 the United States Department of Agriculture (USDA) deregulated reduced lignin alfalfa in the U.S. T the coming years the FGI reduced lignin trait will be marketed as HarvXtra™ alfalfa.

The product concept for HarvXtra™ alfalfa is

1) a ≥12% increase in RFQ compared to conventional varieties harvested at the same time; or

2) a ≥ 7 day delay in harvest with the same or better RFQ as a conventional commercial check harvested without the delay.

Furthermore we expect that forage yield potential per se; persistence, multiple pest resistance, and lodging tolerance will be similar to conventional varieties harvested on the same cutting schedule.

In preliminary trials of experimental varieties of HarvXtra Alfalfa, these varieties are showing a 15-20% decrease in lignin content and a 12-15% increase in NDFD and RFQ compared with conventional commercial checks harvested at the same time. This can translate into a ~30 point RFQ advantage and a $30/T price premium based on current Midwest hay pricing standards. HarvXtra varieties with fall dormancy 3-9 are now currently being tested at more than ten locations in the U.S.

Tannin Alfalfa

Condensed tannins (CT) are a class of phenolic compounds found in many plants. Tannins bind with proteins and slow the rate of protein degradation in the rumen. Reductions in rumen protein breakdown could benefit the dairy industry by reducing; the need for supplemental proteins (often the most costly portion of dairy feed), nitrogen and methane emissions attributed to rapid protein breakdown in rumen and reticulum (Dave?), and bloat when grazed (Getachew, 2006). By elevating the amount of CT's in alfalfa it should be possible to increase the amount of protein entering the hindgut of livestock translating into increased milk production and weight gain. Tannin containing forages such as birdsfoot trefoil (*Lotus corniculatus)* and sanfoin (*Onobrychis viciifolia)* have more bypass protein and are non-bloating when grazed by ruminants. Alfalfa produces condensed tannins, but only in the seed coat. Various biotech strategies have been explored for production of condensed tannins in leaves and stems of alfalfa.

Through overexpression of a key transcription factor, TaMYB14) isolated from rabbit's foot clover (*Trifolium arvense*) scientists from AgResearch in New Zealand and Forage Genetics International have successfully developed alfalfa plants with expression of condensed tannins in the leaves (0.9-1.6%) that are comparable to birdsfoot trefoil checks (1.0%) (Hancock 2014). This exciting discovery has led to a sharp increase in proof-of-concept and trait development research to move this technology into potential commercial development.

The U.S. Dairy Forage Research Center estimates that tannin alfalfa could decrease protein feed supplement costs for dairy by 60%, leading to a 12% increase in net return for dairies - and significantly decrease N losses to the environment.

Conclusion

U.S. alfalfa hay production acres have been in gradual decline for the last decade. Biotechnology (applied genomics and genetic engineering) and the potential these tools offer to alfalfa improvement are critical for keeping the crop competitive with alternative GE crops. Genetic engineering offers potential breakthroughs in improving the efficiency of alfalfa forage production, and improving forage quality in ways not possible with traditional plant breeding methods. Specifically, new traits that improve efficiency of nutrient utilization in alfalfa should lead to improved animal performance and decreased loss of nutrients to the environment.

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