# NEW TECHNOLOGY FOR ALFALFA

**Mark McCaslin and Peter Reisen**[1]

Reduced Lignin

Forage Genetics, the Samuel Roberts Noble Foundation and the U.S. Dairy Forage Research Center began working together in 2000 to produce transgenic alfalfa plants with reduced lignin content and improved fiber digestibility. This team of ~ 12 scientists collaborated in using gene silencing technology to systematically "knock out" each of the twelve genes in the lignin biosynthetic pathway and to compare the effect of these individual gene knockouts on alfalfa forage composition, fiber digestibility (NDFD) and agronomic performance. We were able to develop a gene knockout that gave the desired improvement in forage quality, without any negative impact on forage yield and standability. Multiple transgenic events were created containing this commercial gene silencing construct, and in 2009 a single commercial event was selected after extensive field and laboratory testing.

This commercial transgenic event has been introgressed into a wide variety of FGI germplasm to produce Reduced Lignin (RL) alfalfa. RL alfalfa has now been tested in multiple genetic backgrounds for multiple years and in multiple locations. When compared both to the non-transgenic control and to appropriate commercial check cultivars, RL alfalfa has consistently shown a ~10% reduction in lignin content and a ~10% increase in NDFD (both on a whole plant basis). In current trials forage yield potential of current RL alfalfa breeding populations is equal or better than appropriate commercial check cultivars. There is no difference in incidence of lodging of RL alfalfa compared to the non-transgenic control.

In cutting management trials the decreased lignin content of RL alfalfa has resulted in increased flexibility in harvest timing. A 2011 trial (Figure 1) compared performance of a RL alfalfa breeding population to two commercial check cultivars, under two harvest treatments: 3 cuts/yr (harvest interval ~38 days) and 4 cuts/yr (harvest interval ~31 days). In this trial, and in similar trials designed to look at changes in forage quality associated with increased physiological maturity, NDFD in RL alfalfa was equal to or higher than NDFD in conventional alfalfa harvested 7-10 days earlier.

It appears that RL alfalfa may benefit forage producers in two ways:

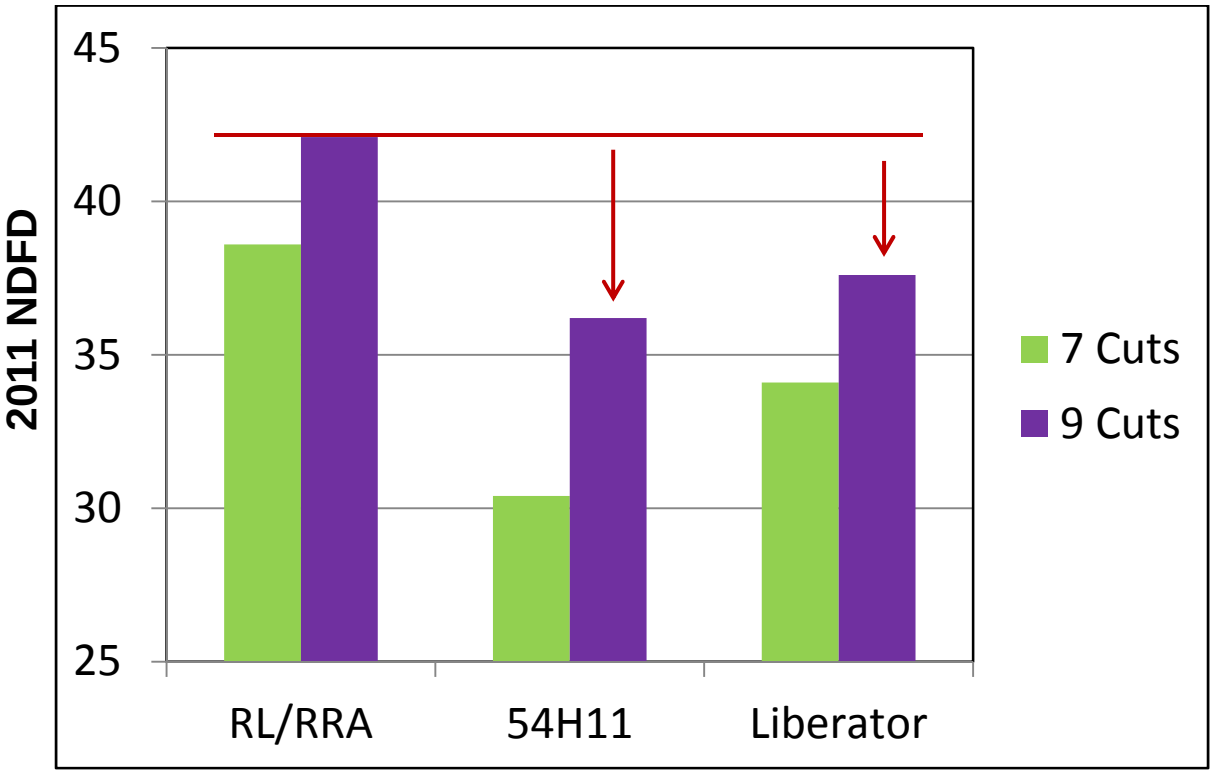
1) Increase the likelihood of harvesting alfalfa hay/haylage with high forage quality.

2) Improve flexibility in alfalfa harvest management by extending the time period in which high quality hay/haylage can be harvested

[1] Mark McCaslin, President, Forage Genetics International. P.O. Box 415, Prior Lake, MN 55372; mccaslin@foragegenetics.com. Peter Reisen, Forage Genetics International, 8770 Hwy 20/26, Nampa, ID 83687; preisen@foragegenetics.com; **In**: Proceedings, 2012 California Alfalfa and Grains Symposium, Sacramento, CA, 10-12 December, 2012. UC Cooperative Extension, Plant Sciences Department, University of California, Davis, CA 95616. (See http://alfalfa.ucdavis.edu for this and other alfalfa symposium Proceedings.)

This project is on a commercial track. An application for deregulation of Reduced Lignin alfalfa will be made in 2012. Product development is also well underway. Reduced Lignin alfalfa will be sold in a trait stack with Genuity Roundup Ready alfalfa, and is expected to be available for U.S. commercial release ~ 2016.

Figure 1. NDFD in RL Alfalfa vs. Commercial checks in 3 vs. 4 cut management
West Salem, WI (established 2010, harvested in 2011 and 2012)




Tannin Alfalfa

Condensed tannins are a class of phenolic compounds found in many plants. Tannins bind with proteins and slow the rate of protein degradation in the rumen. Tannin containing forages (e.g. birdsfoot trefoil and sanfoin) have more bypass protein and are non-bloating when grazed by ruminants. Alfalfa produces condensed tannins, but only in the seedcoat. Various biotech strategies are being explored for production of condensed tannins in leaves and stems of alfalfa. Earlier this year FGI scientists identified transgenic alfalfa plants containing condensed tannins in leaves and stems.
The U.S. Dairy Forage Research Center estimates that tannin alfalfa could decrease protein feed supplement costs for dairy by 60% and significantly decrease N losses to the environment. In addition, UC Davis scientists are exploring strategies for production of hydrolysable tannins in alfalfa, unrelated compounds with positive attributes similar to condensed tannins.

Drought Tolerance and Salt Tolerance

More than half of the alfalfa grown in the U.S. is produced under irrigation or under dryland conditions where moisture commonly limits productivity. Several biotech companies are currently exploring and testing transgenes that increase drought tolerance and salinity tolerance when expressed in crop plants. Several of these gene candidates are now being expressed in alfalfa. Since 2007 Forage Genetics in collaboration with Monsanto has been testing several new potential drought resistant transgenic alfalfa plants. FGI is also evaluating native genes for improved salt tolerance in various greenhouse/growth chamber and field studies.

Marker Assisted Selection

The past ten years has seen technological advances in the speed and cost of deciphering animal and plant genomes. These new tools enable plant breeders to identify and select within a plants native DNA for genes to improve persistence and yield. FGI is making a significant investment in molecular marker platforms and other applied genomics approaches to take advantage of novel non-GE avenues for alfalfa improvement.