When breeding tomorrow’s alfalfa varieties, most groups employ a model of combining traditional plant breeding with biotechnology tools in order to incorporate useful traits. In this model, the conventional variety development process will be the method of choice for most traits where breeders have traditionally made progress such as adaptation, heading date, disease and insect resistance, general persistence conditions such as grazing and traffic tolerance, and even yield. It has been very successful in adding economic value to the forage and livestock operations of many producers (Bouton 2007). These traditionally developed varieties will also be used as the germplasm platform on which to add or incorporate the new and exciting traits to be discussed in this presentation. Finally, traditional breeding will also be the method of choice where the sales and on-farm value of the species do not justify the use of biotechnology (Bouton, 2007).

Some traits are complex to locate and manipulate, or possibly not even contained in a species’ primary germplasm. When this happens, molecular tools are an option for trait incorporation and/or validation through more efficient gene discovery, tagging, and even genetic engineering. These tools are coming mainly from research in new scientific research areas called genomics and transgenics.

In genomics, DNA sequencing data, combined with high throughput machinery and data analysis (e.g. bioinformatics), allows more accurate determinations of species relationships and gene expression. Genomics-based gene discovery programs are also finding thousands of candidate genes that control numerous value-added traits. From this understanding, new and innovative methods for improving crops are evolving such as marker assisted breeding.
Transgenics involve the movement of specific and useful genes into the crop of choice and is sometimes referred to as genetic engineering. Scientists using the transgenic approach have already shown success in introducing genes (many found through the above mentioned gene discovery programs) which have already made many important row crops resistant to insects, viruses, and herbicides. There are literally now millions of acres planed to new corn and soybean varieties with these types of biotech traits. Transgenics is also useful in creating unique plants that allow basic research to be conducted on physiological and biochemical pathways.

Although the use of genomics is not controversial, there has been controversy surrounding the use of transgenes for crop improvement; especially when transferring genes between two unrelated organisms. Although the Roundup Ready® gene was successfully deployed as the first biotech trait in alfalfa, it was re-regulated by court injunction in order for an Environmental Impact Statement (EIS) to be developed to address sensitive hay markets (e.g. primarily organic and export). This EIS is now open for public comment. However, this episode is a good example of both the rigor and cost of the regulatory process for deploying transgenic traits in alfalfa. When coupled with the inherent costs of obtaining freedom to operate for using the gene and the enabling technologies, it also explains the high cost for bringing a transgene into the alfalfa seed market. This in turn makes the price of the final product high to the grower, so each trait must be of such impact that the grower is willing to pay these high prices.

Current Research

Biotechnology research in forage crops, especially to study and/or incorporate complex traits, is in a time of increased emphasis and success throughout the world. For example, at the past International Symposia on Molecular Breeding of Forage and Turf held in Hamilton, Victoria, Australia in 2001 (Spangenberg, 2001), Dallas, Texas in 2003 (Hopkins et al. 2003), Aberystwyth, Wales in 2005 (Humphreys 2005), and in Sapporo, Japan in 2007 (Yamada and Spangenberg, 2008), there were hundreds of scientists in attendance at each meeting from many countries. Research talks at these
symposia as found in these proceedings were mostly on the many aspects of basic biotechnology in forage grasses and legumes including alfalfa.

The North American Alfalfa Improvement Conference (NAAIC) likewise meets every two years and publishes a “Use of Biotechnology Research in Alfalfa Improvement” report in conjunction with that meeting (Brummer et al. 2006). This research report, and many others like it, is proof that research in this area is intense and growing for alfalfa.

From all of these reports, one can conclude that research areas receiving emphasis are accurate genomics techniques to more rapidly identify and manipulate important genes (molecular markers and marker assisted selection breeding), breeding for animal, human and environmental welfare, transgenics, bioinformatics, population genetics, genomics of the model legume \textit{M. truncatula}, and field testing and risk assessment as well as intellectual property rights.

**New Traits for Tomorrow’s Alfalfa**

From these same conference and symposia reports, the traits receiving emphasis are herbicide tolerance (especially expression of the Roundup Ready gene in alfalfa), drought tolerance, resistance to disease and insect pests, tolerance to acid, aluminum toxic and/or saline soils, tolerance to cold or freezing injury, expression of plant genes controlling nodulation and nitrogen fixation, increasing nutritional quality of alfalfa via down regulation of lignin genes, flowering control, increased biomass yield, and reducing bloat and bypass protein via incorporation of genes to express condensed tannins in legumes.

Again, the traits being investigated are ones that breeders have made little progress for improvement through conventional breeding. Another aspect is the potential high impact for farmers of the traits under investigation. This impact will be necessary in order to justify the patent, development, and regulatory costs of the final cultivar.

**Biotech Traits to be Incorporated into Alfalfa**

The Consortium for Alfalfa Improvement (CAI) is made up of researchers from The Samuel Roberts Noble Foundation, the U.S. Dairy Forage Research Center (USDFRC) in Madison, WI, Forage Genetics International (FGI), and Pioneer Hi-Bred International, Inc. The purpose of the consortium is to improve important characteristics in alfalfa such as nutritional content and digestibility. The first two initiatives by the consortium will focus on improving protein utilization and cell wall digestibility via lignin reduction and decreasing bloat and bypass protein problems via insertion of genes to express condensed tannins. Therefore, the consortium’s major goal is to re-design alfalfa as a major forage source. This would be of such impact as to justify use of any
biotechnologies. Below are a few traits being investigated at the Noble Foundation or through its participation in the CAI.

**Lignin Down-Regulation**

Scientists at the Noble Foundation “knocked-out” the main genes that code enzymes required for lignin synthesis in alfalfa. CAI scientists are now in the process of evaluating transgenic plants with reduced expression of one or more lignin biosynthetic genes. Although these transgenic plants contain reduced lignin content, they vary widely in lignin composition. Increased fiber digestibility is also a common feature of these transgenic plants, but agronomic performance varies significantly by transgene. Basically, lignin is reduced by approximately 25% and NDF digestibility increased by 10-12% when compared to conventional alfalfa. Based on multiple lab and field studies initiated since 2000, transgenic plants with reduced expression of two key lignin enzymes, COMT and CCOMT, showed decreased lignin, increased fiber digestibility, and acceptable agronomic performance. Elite alfalfa populations containing the silenced transgenes have now been developed. Hay produced from these populations were used for both CAI sheep and dairy feeding studies designed to confirm improved animal performance of these reduced lignin alfalfa plants. Positive results from these feeding trials were found thereby moving this project into an accelerated development mode. Reduced lignin alfalfa will provide an important new biotech trait for hay producers, providing more flexibility in harvest management and increasing forage quality and/or forage yield. This type of high value hay should, in turn, be very desirable for dairies looking to increase milk production, yet decrease manure output.

**Increased Expression of Condensed Tannins**

Proanthocyanidins (PAs, also known as condensed tannins), a class of flavonoid-derived polymers, reduce pasture boat and improve nitrogen nutrition for ruminant animals when present in forage, but are absent from the leaves and stems of alfalfa. It was found that when introducing PAs into tissues in which they do not naturally occur, it is first necessary to express the anthocyanin pathway responsible for plant pigments from which the precursors of PAs are derived. Additional genes then need to be introduced in order to provide the functions for PA monomer biosynthesis, transport and polymerization. This need to insert all the genes necessary to obtain functioning pathways demonstrates the complex nature of this work. It is also not known how introduction of the PA pathway to alfalfa foliage will affect agronomic performance. However, things are progressing well and some of the early stage experiments have...
demonstrated successful deployment of the pathway and production of condensed tannins.

Water Use Efficiency and Drought Tolerance

A lot of alfalfa grown in the U.S. is produced under irrigation or under dryland conditions where water commonly limits productivity. Several research organizations are currently exploring and testing transgenes that increase drought tolerance and water use efficiency when expressed in crop plants. Gene candidates for drought tolerance are now being expressed in alfalfa. One of these is the WXP1 gene discovered by scientists at the Noble Foundation. This work is in its early stages, but has shown as a “proof of concept” that insertion of transgenes like WXP1 increases alfalfa’s ability to be productive, or even recover more quickly, after periods of limited water.

In a related genomics project (e.g. genetic mapping with molecular markers), the Noble Foundation is collaborating with New Mexico State University to identify genetic mechanisms associated with drought tolerance within cultivated alfalfa itself. Markers
associated with drought tolerance genes would be identified and then used in selecting alfalfa genotypes for the production of new drought tolerant cultivars. This approach brings the power of genomics technologies to bear in the process without the regulatory and public perception problems associated with transgenics. Thus far, genes are being identified that control yield under conditions that are 50% of normal irrigation.

**Biomass and Flowering**

Although genes for increased biomass production or delayed flowering are of little interest for grain crops, they offer exciting potential for alfalfa. Several such genes have been identified in general phenotypic assays of new gene candidates and are now just beginning to be inserted into alfalfa. These new transgenes may offer the best opportunity to significantly increase overall forage yield in alfalfa.

**Alfalfa’s Role and the Biofuels Industry**

The main criteria for any biofuels crop are high yields achieved with low input costs in an environmentally friendly manner. This is why switchgrass is targeted as one of the main dedicated energy crops for cellulosic ethanol production. The requirement of low cost of the delivered feedstock, possibly as low as $50-60 per ton, is the greatest hurdle for alfalfa growers to overcome. At this stage, therefore, it is simply better to sell alfalfa in the high value hay market.

However, alfalfa will have an important role for use in rotation or inter-cropping systems with corn or perennial grasses to off-set production and nitrogen costs of these mainly grass feedstock systems. For alfalfa to be used directly as a biofuel crop, it may also mean dividing the harvested product into components, such as leaves and stems, and using the leaves to produce high value meal and the stems for sale to a biorefinery. This use was very well described at the 2008 Kentucky Alfalfa Conference by Martin (2008). If co-products such as pharmaceuticals are simultaneously extracted from the leaf material, this allows the economics of using alfalfa as a biofuel crop to work even better.

From the CAI lignin project mentioned above, it was also demonstrated that lines containing the lignin down-regulated trait showed a two-fold improvement in enzymatic hydrolysis efficiency. This efficiency could therefore eliminate the requirement for costly chemical pretreatment for sugar production. More ethanol should then be able to be produced per ton of this low lignin alfalfa thereby making it a very efficient biofuel feedstock. This efficiency, in turn, would allow the biorefineries to pay substantially more for the delivered feedstock due to high the alcohol yield per ton along with the concurrent need to store lesser amounts of the feedstock at the biorefinery.
Summary

Biotechnology research in alfalfa, especially to study and/or incorporate complex traits, is in a time of increased emphasis and success throughout the world. The Roundup Ready® gene was the first biotech trait to be successfully commercialized in alfalfa, but new biotech traits continue to be investigated and incorporated. These traits are generally ones that breeders have made little progress for improvement through conventional means. Another aspect is the high impact potential of these new traits to alfalfa growers. Although there are many traits being investigated throughout the world, initial research is concentrating on the following: improving protein utilization and cell wall digestibility via lignin reduction, decreasing bloat and bypass protein problems via insertion of genes to express condensed tannins, and insertion of genes to control water use efficiency and flowering or those to increase biomass production. Consortia of various partners like those described for the Consortium to Improve Alfalfa are also important to bring new biotech traits into alfalfa. Finally, the impact of biotech traits such as reduced lignin, have the potential to increase alfalfa’s value as a feedstock for ethanol production, which in turn should increase its value to the evolving biofuels industry.

References


