

## General Session

### FORAGE IMPROVEMENT IN A BIOTECHNOLOGY ERA

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*Author note: Some sections of the following article were taken directly from the paper “What is on the Horizon for Alfalfa & Biotech” by Joe Bouton. It was published in the Proceedings, National Alfalfa Symposium, 13-15 December 2004, San Diego, CA, UC Cooperative Extension, University of California, Davis 95616. (See <http://alfalfa.ucdavis.edu> for the complete article).*

The future of cultivar development in forages will surely include the new and evolving areas of genomic and transgenic technologies. Genomics research received great publicity with the successful completion of the project to completely sequence the DNA of the human genome. Plant species soon followed suit with an annual relative of cultivated alfalfa, *Medicago truncatula*, being sequenced and used as the reference species for all legumes, and rice being sequenced as the reference species for the grasses. These sequencing data for the reference species, combined with high throughput machinery and data analysis (e.g. bioinformatics), allows determinations of gene expression here to fore not possible. From this understanding, new and innovative methods for improving cultivated forages will evolve.

Transgenics involve the movement of specific and useful genes into the crop of choice and is sometimes referred to as genetic engineering. Scientists using this approach have already shown success in introducing genes which make many important row crops resistant to insects, viruses, and herbicides. It is also been very useful in creating unique plants that allow basic research to be conducted on physiological and biochemical pathways. As will be discussed elsewhere in this symposium, the introduction of genes for resistance to the herbicide Roundup may likely be the first of these transgenes to be introduced into alfalfa.

Although the use of genomics for basic research purposes is not controversial, there has been great controversy surrounding the use of transgenes for crop improvement, especially when transferring genes between two unrelated organisms. This has created a very costly regulatory climate, to go along with inherent costs of obtaining freedom to operate for using the gene and the enabling technologies in order to bring a transgene into the forage seed market.

### WHAT IS HAPPENING TODAY IN FORAGE BIOTECHNOLOGY

The North American Alfalfa Improvement Conference (NAAIC) meets every two years and publishes a “Use of Biotechnology Research in Alfalfa Improvement” report (Brummer et al. 2004). The report is summarized for this presentation in Table 1 and demonstrates the high level of activity currently underway in biotechnology research and its application in alfalfa and related

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species.

Biotechnology research in all 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 Fourth International Symposium on Molecular Breeding of Forage and Turf held in May 2003 in Dallas, Texas, there were approximately 200 scientists in attendance from 19 countries. Research talks were many and varied on every aspect of basic biotechnology in grasses and legumes (Hopkins et al. 2003). This symposium, and many others like it, is direct proof that research in this area is intense and growing. Ironically, it is not basic research that has not moved forward, but the application of that research to solve on-farm problems.

Genomics has a costly investment upfront, but has less controversy for actual use in the cultivar development process. However, the cost of using transgenics in cultivar development for both forage legumes and grasses is two-fold; the patenting issues enabling the use of a specific technology and regulatory trials with the resulting germplasm. The patenting issues are simply a business decision of whether obtaining freedom to operate from the patent holder(s) is a justified cost. The regulatory costs are more problematic. First, there has not been a new crop de-regulated in several years when one defines a “new crop” as one that has never been in commercial production while containing a biotech trait. Since all forage crops would represent a “new crop” by that definition, then there is concern that even doing all requisite safety trials may not be enough to insure de-regulation. Second, two species, creeping bentgrass and alfalfa, are currently being assessed by USDA-APHIS for de-regulation for the Roundup Ready gene, a 1980s technology that is currently found in millions of acres of corn, soybean, and cotton. Many are therefore watching very intently the final disposition of these two applications. The fact that the creeping bentgrass application has now been in the process longer than any crop to date is not encouraging.

## **FORAGE IMPROVEMENT AND BIOTECHNOLOGY MODEL**

In its most simplistic form, the cultivar development process can be outlined as five distinct steps: 1) establishment of objectives and goals, 2) collection and development of parents, 3) selection and breeding to develop elite lines and experimental cultivars, 4) testing to identify the best elite lines and experimental cultivars, and 5) release, dissemination, and commercialization of the best cultivars.

Steps 2 and 3 are probably considered the essence of plant breeding and are the most identifiable steps to agricultural scientists and farmers alike. Although in many ways the most creative steps in the process, these steps are simply the breeder asking the questions do I have enough natural genetic variation for the trait or traits and can I enhance this variation into an acceptable germplasm? It is actually step 4 which requires the most resources in terms of time, labor, supplies, and money. Finally, these steps are very time consuming and can take the breeder up to 12 years to complete them for each cultivar.

As shown in Figure 1, transgenics and genomics have great potential to positively affect the parental development (can lead to very unique traits), breeding and selection (no problems with reproductive barriers) and even testing (simply look for the gene being present to reduce testing

time) steps. However, as the questions posed in the figure, and in the above discussion, highlight, the main questions continue to be: is the trait of such importance that it justifies the use of biotechnologies and can we afford to use them?

## THE FUTURE

The Samuel Roberts Noble Foundation, a non-profit research and outreach organization located in southern Oklahoma, is a good example of the approach needed for forage improvement in this new technology driven climate. The Foundation's mission is to assist farmers and ranchers in the Southern Great Plains region of the USA with their agricultural needs. There are currently three operating divisions at Noble: Plant Biology, a research group emphasizing basic research in plant molecular biology; Agriculture, a consulting and outreach group working directly with farmers in the region and conducting on-farm research to support the outreach mission; Forage Improvement, the forage cultivar development and technology application arm of the Foundation. Although each division works within its own area, the real strengths result when they work across divisions and leverage the abilities of each. For example, genes and technologies discovered by Plant Biology are passed to Forage Improvement for conventional breeding and application of biotechnology to produce a cultivar which is then passed to Agriculture for direct on-farm demonstration and farmer use.

The challenges faced by southern Great Plains agriculture can be broadly classified into concerns with dependability, cost, and ease of use, nutritive quality, and environmental desirability of production. Of these, dependability is the critical characteristic needed in this harsh environment. Therefore, the main target species for the Foundation are perennial forages such as tall fescue, bermudagrass, hardinggrass, western wheatgrass, alfalfa, and red and white clover. The target traits are ones that directly impact dependability and survival and include drought and heat tolerance and pest resistance. However, poor nutritive quality, and less often, low levels of animal toxins, are often found in these species. So, other target traits include improving their rate of digestion by ruminant animals through decreasing lignification, and removing naturally occurring toxic alkaloids.

The Foundation's Forage Improvement Division approaches the incorporation of useful genes that govern these traits almost exclusively with conventional selection and breeding techniques (Figure 1). In this approach, as much of the known plant germplasm, often from the USDA plant germplasm system, is collected and screened for the target traits. Sometimes, the traits are very complex and not contained in a species' primary germplasm. For this scenario, biotechnology approaches are an option for trait incorporation.

The Forage Improvement Division, therefore, has the option of turning to the new biotechnologies to help with incorporation of difficult traits into our cultivars. For a genomics approach, there has been a concentrated effort to produce genetic sequences of genes expressed by *Medicago truncatula*. These are called ESTs and have been useful for producing molecular markers to locate and follow important genes for aluminum tolerance, drought tolerance, and disease resistance in alfalfa. Similarly, the Division's grass genomic and small grain breeding program is focused on development and applications of these types of molecular marks for genetic improvement of forage grass and small grain species. Studies are currently underway to

associate the markers with multiple genes having direct bearing on important traits such as drought resistance. Once these associations are confirmed, then the markers will be used to better select plants with a high frequency of these important genes.

Due to the cost and controversy of using transgenics, it is our option of last resort. In the Division's Transformation Project, transgenes that improve forage quality, drought tolerance and phosphate uptake are the main ones being transferred to the target species. However, although transgenic biotechnologies provide very powerful and useful alternatives to not having the trait altogether, the main question is this: Is the trait of such value and impact that it will justify a transformation approach (Figure 1)? The answer to this question for alfalfa involved an additional component to the Foundation's existing operational model. This component was achieved through the Consortium to Improve Alfalfa.

The Consortium to Improve Alfalfa is made up of researchers from Noble Foundation, the U.S. Dairy Forage Research Center (USDFRC) in Madison, WI, and Forage Genetics International (FGI), a commercial alfalfa research and seed company. The purpose of the consortium is to improve important characteristics of 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. Therefore, the consortium 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. It also brings to bear additional resources to leverage with those of the existing Noble model: commercialization with enabling technologies from FGI and abilities in animal and dairy nutrition including facilities for controlled assessment of the forage with USDFRC. The Noble Foundation, and especially its partnership in the Consortium to Improve Alfalfa, is therefore a future model of what may need to be done to justify the costs when using biotechnology for alfalfa improvement.

## **CONCLUSIONS**

The irony is not whether basic biotechnology research is increasing in forages, because it is, but whether we will be able to deliver useful biotechnology traits directly to the farmer. Consortia of various partners like those described for the Consortium to Improve Alfalfa will also be important to bring the fruits of these new technologies to researchers and farmers alike. However, it is hoped that more funding will be available to help the regulatory agencies in assessing the question of real versus perceived risks. At the end of the day, these agencies will need to make decisions on what are the real risks, establish a rigorous regulatory process to assess these risks, oversee the regulatory process in a fair manner, and make a decision! We can all then move forward based strictly on the value of the traits to the well-being of the environment, the farmer, American agriculture, and all citizens of this country.

## **REFERENCES**

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Table 1. Summary of alfalfa biotechnology research as reported by the North American Alfalfa Improvement Conference (Brummer et al. 2004).

USA	
Arizona State Univ.	Over-expression of salt tolerance genes
Forage Genetics Int'l, West Salem, WI	Commercialization of Roundup Ready gene and down regulation of lignin genes to increase digestibility
Iowa State Univ.	Identifying alfalfa genes controlling yield and winter-hardiness
New Mexico State Univ.	Identifying genes controlling salt and drought stress
Purdue Univ.	Cloning genes for vegetative storage proteins
Samuel Roberts Noble Foundation, Ardmore, OK	Developing molecular markers, studying down regulation of lignin genes, insertion of genes for condensed tannins, identifying and introgression of drought and aluminum tolerance genes
USDA-ARS, Beltsville, MD	Developing molecular markers and using markers for identifying genes for yield and winter survival.
USDA-ARS, Madison, WI	Characterize genes controlling post-harvest proteolysis.
USDA-ARS, St. Paul, MN	Insertion of genes to allow remediation of atrazine and genes to control pectin in cell walls.
USDA-ARS, Prosser, WA	Using molecular techniques for quick identification of disease pathogens.
Univ. of California, LA	Expression of plant genes controlling nodulation and nitrogen fixation.
Washington State Univ. and USDA-ARS	Molecular markers to characterize diversity among alfalfa accessions.
Canada	
Agriculture and Agri-Food Canada. Saskatoon	Alter expression of condensed tannin genes to reduce bloat, greenhouse gases, and protein bypass
Agriculture and Agri-Food Canada. Ste-Foy	Identification of genes for improved persistence, yield, and cold tolerance.
Medicago Inc., Ste-Foy	Using alfalfa as factory to produce pharmaceutical products
Univ. of Guelph	Genetic engineering to modify winter-hardiness.
Mexico	
National Univ. of Mexico	Using anti-sense and over-expression approaches to study nodulation and nitrogen fixation
Europe	
AgroBioInstitute, Bulgaria	In vitro selection to increase tolerance to abiotic stresses
INRA, Toulouse, France	Genomic approaches for nitrogen fixation, disease resistance, and abiotic stress tolerance.
BAP, Toulouse, France	Induction of plant defenses during disease infection via genetic and cellular approaches.
INRA, Lusignan, France	Developing molecular markers for candidate genes for aerial morphogenesis and genetic mapping.
Univ. of Perugia, Italy	Study of aluminum tolerance genes and examining methodology to remove antibiotic resistance during selection of transgenes.



# Biotechnology and Cultivar Development

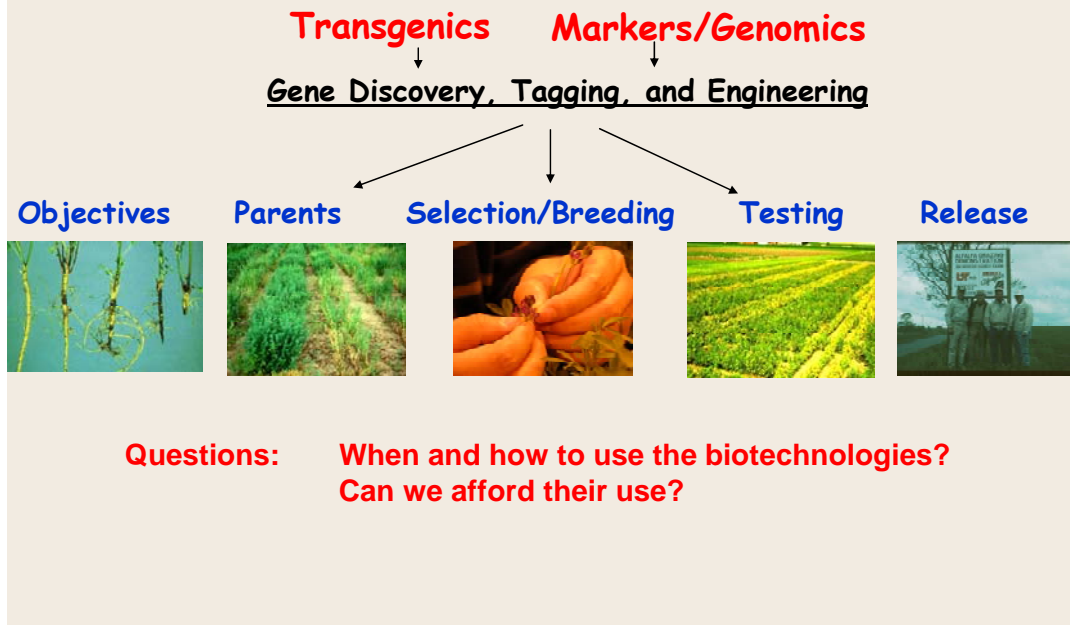


Figure 1. Current model for forage cultivar development and how genomic and transgenic biotechnologies can potentially interact with this model.